Calorimetric Estimate of the Enthalpy Change for the Substrate-
promoted Conformational Transition of Aspartate Transcarbamoylase from Escherichia coli*

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Two different calorimetric methods are presented for estimating the enthalpy change of the substrate-promoted conformational transition of aspartate transcarbamoylase from Escherichia coli. Method 1, the bisubstrate analog N-(phosphonacetyl)-L-aspartate (PALA) was used to promote the conformational transition of the enzyme from the initial T to final R state at pH 7.0 and 30 °C. Since this transition is essentially complete when only one of the 6 active sites are occupied by PALA (Howlett, G. J., and Schachman, H. K. (1977) Biochemistry 16, 5077–5083), the ΔH value for binding the last 2 eq of PALA is the intrinsic heat of binding PALA to the R state. Thus, the difference between ΔH measurements of PALA binding to the enzyme during and after the T → R conformational transition (monitored by difference sedimentation velocity) allows calculation of the heat of this transition (ΔHΔ); the associated proton uptake was computed from ΔH measurements in 2 mixed KPO4 buffers with different heats of protonation. Values of ΔHΔ actually were calculated from differences between ΔH values for binding the first 2 eq and the third 2 eq of PALA (method 1A) or between ΔH values for binding all 6 eq and the third 2 eq of PALA (method 1B). In method 2, the heats of assembly of the enzyme from catalytic and regulatory subunits were measured in the absence and presence of PALA, yielding ΔH values for the assembly of enzyme in the T state and R state, respectively. These values combined with the heat of binding PALA to the isolated catalytic subunit and the intrinsic heat of binding PALA to the enzyme in the R state (from method 1A) give another estimate of ΔHΔ. The values of ΔHΔ in 40 mM KPO4, at pH 7.0 and 30 °C obtained by methods 1A, 1B, and 2 are −5 ± 3, −14 ± 8, and −14 ± 9 kcal/mol of enzyme, respectively; methods 1A and 1B also provide proton uptake values for the T → R transition of 2 and 6 eq of H+, respectively. Method 1A gives the most reliable estimate for ΔHΔ, −6 ± 3 kcal/mol of enzyme (corrected for buffer deprotonation).

The value of +5.3 kcal/mol of enzyme for ΔHΔ (Howlett, G. J., Blackburn, M. N., Compton, J. G., and Schachman, H. K. (1977) Biochemistry 16, 5091-5099) and the estimate of ΔHΔ from method 1A give ΔSΔ = −31 cal/(deg·mol of enzyme) and −ΔS = +9 kcal/mol of enzyme. Thus, the T → R transition of aspartate transcarbamoylase is entropically controlled at pH 7.0 and 30 °C.

Ultracentrifugal analyses of assembly reactions suggested that the binding of PALA weakens catalytic and regulatory interchain contacts. From the heats of assembly of aspartate transcarbamoylase (C2R3) from catalytic trimers (C) and regulatory dimers (R) in the absence and presence of PALA (−77 ± 3 and −54 ± 7 kcal/mol of enzyme, respectively), the PALA-linked heat of assembly in the absence of assembly is +22 ± 8 kcal/mol of enzyme. This large effect of PALA on the heat of assembly is due to changes in intrasubunit contacts as well as to changes in contacts between catalytic and regulatory chains.

The observation of the sigmoidal dependence of enzyme activity on substrate concentration exhibited by aspartate transcarbamoylase (ATCase; EC 2.1.3.2) from Escherichia coli (1) has led to numerous investigations of the structure and physical properties of the enzyme (2–5). Because the kinetic behavior of ATCase has been interpreted in terms of a substrate-promoted transition in which the oligomeric enzyme is converted from a low affinity T state to a high affinity R state, there have been extensive studies of the gross conformational changes produced by substrates and substrate analogs (6–8). Based on the model of Monod et al. (9), a value for the free energy change for the T → R transition has been inferred from kinetic and physical chemical studies by Howlett et al. (10). However, the corresponding enthalpy change, ΔHΔ, has not been measured. This paper describes attempts to measure by calorimetry the change in enthalpy for the T → R transition of ATCase.

Previous results on the assembly of ATCase from the separate subunits (11) and on the substrate-promoted conformational changes in the enzyme (6–8) provide the bases for different calorimetric approaches for the determination of ΔHΔ. ATCase from E. coli is composed of 2 trimeric catalytic (C) and 3 dimeric regulatory (R) subunits (5, 12–16). Gerhart and Schachman (11) further demonstrated that the enzyme is readily dissociated into separate C and R subunits that can be assembled to form intact enzyme. In addition, the

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bistubrate analog N-(phosphonacetyl)-L-aspartate, synthesized by Collins and Stark (17), binds with high affinity \((-10^8 \text{ M}^{-1})\) to isolated C subunits and to the native enzyme with a stoichiometry of 3 eq of PALA/C subunit and 6 eq of PALA/ATCase (17, 18). PALA promotes the conversion of ATCase from the T to the R state (10). The difference sedimentation studies of Howlett and Schachman (7) show that the change in sedimentation coefficient \((\Delta s/s)\) upon addition of PALA to ATCase is negative and represents the substrate-promoted conformational change of the enzyme; the maximal change in the sedimentation coefficient of ATCase in K-PO4 buffer at pH 7.0 produced by substrates or PALA binding reaches a plateau value of \(\Delta s/s\) to \(-3.5\) with only 4-5 eq of PALA bound/ATCase (7). Thus, the conformational change of ATCase promoted by PALA precedes the binding of PALA to the enzyme (7).

The foregoing aspects of the conformational transition of ATCase permit the calorimetric estimates of \(\Delta H_{T-R}\) presented here. The principles of the methods are described below along with the experimental data for their application. The methods have an inherent limitation in that the calculation of \(\Delta H_{T-R}\) requires subtraction of values obtained from measurements of different processes, resulting in substantial experimental errors. Nonetheless, the approach is novel and the estimates of \(\Delta H_{T,R}\) allow a qualitative characterization of the conformational transition of the enzyme in thermodynamic terms.

**GENERAL CONSIDERATIONS**

The quantity \(\Delta H_{T-R}\) is the change in enthalpy for the conformational transition of ATCase from the initial T state to the final R state. It contains no contribution from the binding of ligand even though PALA is used to promote the conformational change. Method 1 employs differences between measured enthalpy changes for binding PALA to the enzyme at different fractional saturations with PALA. This approach is based on the observation that the gross conformational change in ATCase precedes the binding of PALA to the enzyme, i.e. when only an average of 2 of the 6 active sites on the enzyme are occupied by PALA, approximately one-half of the conformational change has occurred, and the conformational change is essentially complete when only an average of 4 of the active sites are occupied by PALA (7). Method 2 involves measuring the enthalpy of formation of ATCase from C and R subunits in both the absence and presence of PALA (yielding enzyme in the T and R states, respectively) and applying suitable corrections for the enthalpy of binding PALA.

**Method 1A**—On binding the first 2 eq of PALA, the measured heat, \(\Delta H_{2}\), involves contributions from both the intrinsic heat of binding the 2 eq PALA and one-half of the heat for the T \(\rightarrow\) R transition. When the last 2 eq of PALA are bound, only the intrinsic heat of binding PALA contributes to the measured heat, \(\Delta H_{4}\), since the ATCase molecules are already in the R conformation when an average of 4 of the 6 active sites are occupied. Thus, we can write

\[
\Delta H_{2} = \frac{1}{3} \Delta H_{\text{PALA}} + \frac{1}{2} \Delta H_{T-R} \tag{1}
\]

\[
\Delta H_{4} = \frac{1}{3} \Delta H_{\text{PALA}} \tag{2}
\]

where \(\Delta H_{\text{PALA}}\) corresponds to the intrinsic heat of binding 6 eq of PALA to the enzyme in the R state, ATCase. The thermodynamic cycle illustrating these reactions is shown in Fig. 1. Reactions for which the enthalpy changes can be measured directly are shown by full arrows. A hypothetical path from unliganded ATCase (T state) to ATCase saturated with PALA (R state) is given by the dashed arrows. \(\Delta H_{T-R}\)

\[
\Delta H_{T-R} = \Delta H_{4} - \Delta H_{2} \tag{3}
\]

Thus, the desired enthalpy change, \(\Delta H_{T-R}\), can be calculated according to Equation 3 from the two measurable quantities. As seen in Equation 2, \(\Delta H_{\text{PALA}} = 3 \Delta H_{4}\).

**Method 1B**—Fig. 1 shows that the various experimental quantities can be combined in an alternative fashion to give \(\Delta H_{T,R}\). When ATCase in the T state binds 6 eq of PALA, the measured value, \(\Delta H_{6}\), is the heat of binding 6 eq of PALA to ATCase plus the heat corresponding to the full conformational transition of the unliganded ATCase. This is expressed as

\[
\Delta H_{6} = \Delta H_{\text{PALA}} + \Delta H_{T-R} \tag{4}
\]

Equations 4 and 2 are another pair of simultaneous equations that can be solved for \(\Delta H_{T,R}\), giving

\[
\Delta H_{T-R} = \Delta H_{6} - 3 \Delta H_{4} \tag{5}
\]

Analogous to method 1A, \(\Delta H_{T,R}\) can be computed (by Equation 5) from 2 measurable heats.

In this treatment we have assumed that the fractional extent of the T \(\rightarrow\) R conformational transition was exactly 0.5 when the average composition was ATCase(PALA)6 (regardless of the actual distribution of ligand molecules in the complexes). Similarly we assumed that the T \(\rightarrow\) R conversion was complete for ATCase(PALA)4. These assumptions were made in order to simplify the formulation presented here. For the treatment of the experimental data (see below), the actual values for the fractional extent of the T \(\rightarrow\) R transition were normalized relative to the maximum change in \(\Delta s/s\) and used to calculate a state function \(R\), describing the fractional extent of

\[
\text{Fig. 1. Schematic representation of heats of binding PALA sequentially and of binding 6.0 eq of PALA to ATCase. All reactions are thermodynamically reversible and the corresponding heats of reaction are defined for the direction of the arrows in kcal/mol of ATCase. The superscripts T and R of ATCase indicate that all enzyme molecules are in the initial conformational state or that essentially all enzyme molecules are in the final conformational state, respectively. The subscripts 2 and 4 of ATCase indicate that an average of 2 or 4 of the 6 catalytic sites of ATCase are filled with PALA, respectively. The calorimeter experiments are shown by full arrows. Heats for the sequential binding of 2.0 eq of PALA/ATCase, 0.0 \(\rightarrow\) 2.0, 2.0 \(\rightarrow\) 4.0, and 4.0 \(\rightarrow\) 6.0 eq of PALA/ATCase, are designated \(\Delta H_{2}, \Delta H_{4},\) and \(\Delta H_{6}\), respectively. Heat for binding 6.0 eq of PALA to ATCase is designated \(\Delta H_{6}\). A hypothetical path from unliganded ATCase (T state) to ATCase saturated with PALA (R state) is given by the 2 dashed arrows; initially, the unliganded ATCase undergoes the full conformational change associated with the T \(\rightarrow\) R conversion in the absence of PALA followed by saturation of the enzyme in the final R state with PALA. The corresponding heats of reaction are \(\Delta H_{T,R}\) and \(\Delta H_{\text{PALA}}\), respectively.

The difference sedimentation data of Howlett and Schachman (7) were normalized relative to the maximum change in \(\Delta s/s\) and used to calculate a state function \(R\), describing the fractional extent of the
used. These were determined by difference sedimentation velocity measurements (7).

Method 2—A schematic representation of the heats of assembly of ATCase (CrR) from C and R subunits in the absence and presence of PALA and of heat of binding 3.0 eq of PALA to C subunit. All reactions are thermodynamically reversible and the corresponding heats of reaction are determined for the direction of the arrows. All heats are defined in kcal/mol of ATCase except for $\Delta H_3$, which is defined in kcal/mol of C subunit. The superscripts T and R of ATCase indicate that all ATCase molecules are in the initial or final conformational states, respectively. The calorimetric experiments are shown by full arrows. Heats of assembly in the absence and presence of saturating PALA (giving unliganded ATCase in the T state and fully liganded ATCase in the R state, respectively) are designated $\Delta H_1$ and $\Delta H_3$, respectively. Heat for binding 3.0 eq of PALA to C subunit is designated $\Delta H_2$. A hypothetical path from unliganded ATCase (T state) to ATCase saturated with PALA (R state) is given by the 2 dashed arrows; initially, the unliganded ATCase undergoes the full conformational change associated with the T → R conversion in the absence of PALA followed by saturation of the enzyme in the final R state with PALA. The corresponding heats of reaction are $\Delta H_{T-R}$ and $\Delta H_{R-PALA}$, respectively.

The sum of these reactions gives the heat for the conversion of unliganded ATCase in the T state to unliganded ATCase in the R state:

$$\text{ATCase}_T \leftarrow 2 \text{C} + 3 \text{R} \quad (\Delta H_1)$$
$$2 \text{C} + 6 \text{PALA} \rightarrow 2 \text{C(PALA)}_3 \quad (2 \Delta H_2)$$
$$2 \text{C(PALA)}_3 + 3 \text{R} \rightarrow \text{ATCase}_R^{\text{R}}(\text{PALA})_6 \quad (\Delta H_3)$$

The corresponding heat, $\Delta H_{T-R}$, is calculated from an algebraic sum of the constituent heats according to

$$\Delta H_{T-R} = -\Delta H_1 + 2 \Delta H_2 + \Delta H_3 - \Delta H_{R-PALA} \quad (6)$$

$\Delta H_1$, $\Delta H_2$, and $\Delta H_3$ are directly measured quantities, whereas the value for $\Delta H_{R-PALA}$ is obtained from method 1.

T → R conformational transition at various molar ratios of PALA/ATCase (10). For molar ratios of PALA/ATCase of 0, 2, 4, and 6, values of $\bar{R}$ are 0.0, 0.53, 0.90, and 1.0, respectively (10). In order to simplify the presentation for method 1 (Equations 1–5), we have idealized some $\bar{R}$ values. For molar ratios of 2 and 4 PALA/ATCase, we have approximated 50 and 100% change from the initial to final conformational states of the enzyme, respectively (i.e. $\bar{R} = 0.5$ and $\bar{R} = 1.0$, respectively).

### Table I

**Heats of binding PALA to ATCase**

Each series of calorimetric measurements (with different ATCase preparations) was performed at 30°C in 40 mM K-PO₄ (pH 7.0) or in 40 mM K-PO₄, 100 mM TES/KOH (pH 7.0); both buffers also contained 0.2 mM EDTA and 2 mM 2-mercaptoethanol. Heat measurements were made for the sequential binding of PALA as well as for the saturation of the 6 active sites with PALA. Heats of binding PALA are in kcal/mol of ATCase; the number of heats of binding PALA averaged in each case is given in parentheses. Sample calorimetric data are given in Table II-S of the miniprint supplement.

<table>
<thead>
<tr>
<th>ATCase preparation and buffer used</th>
<th>Heats of binding PALA at following initial → final eq of PALA bound/ATCase values:</th>
<th>kcal/mol ATCase</th>
</tr>
</thead>
<tbody>
<tr>
<td>I in K-PO₄</td>
<td>-15.1 (3)</td>
<td>-13.8 (3)</td>
</tr>
<tr>
<td>II in K-PO₄</td>
<td>-15.1 (3)</td>
<td>-14.9 (2)</td>
</tr>
<tr>
<td>Average in K-PO₄</td>
<td>-16.1 (6)</td>
<td>-14.9 (2)</td>
</tr>
<tr>
<td>III in K-PO₄-TES</td>
<td>-14.1 (3)</td>
<td>-14.2 (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-14.8 (3)</td>
</tr>
</tbody>
</table>

**RESULTS**

**Heats of Binding PALA to ATCase**

Heats for the sequential binding of PALA and for binding 6.0 eq of PALA to ATCase are given in Table I. Measurements were made at 30°C in 40 mM K-PO₄, buffer at pH 7.0 and in 40 mM K-PO₄, 100 mM TES/KOH buffer at pH 7.0 in order to evaluate proton release or uptake accompanying these binding reactions.

Difference sedimentation measurements were used to determine quantitatively the extent of the conformational transition of ATCase promoted by PALA (7) for the various calorimetric experiments in Table I and the values for the change in sedimentation coefficient ($\Delta s/s$) are summarized in Table I-S (see the miniprint supplement). Similar $\Delta s/s$ data were obtained in both K-PO₄ and K-PO₄-TES buffers.

The fractional change in sedimentation coefficient relative to the maximal change in $\Delta s/s$ gives a measure of the conformational transition, which is expressed as $\bar{R}$ in Table I-S. The change in the sedimentation coefficient of ATCase (using liganded enzyme in the final R state as reference solution) for the different molar ratios of PALA/ATCase given in Table I-S agree with the observed $\Delta s/s$ data of Howlett and Schachman (7) (using unliganded enzyme in the initial T state as reference solution). Furthermore, Howlett et al. (10) determined that $\Delta s/s$ data for the PALA promoted conformational change of ATCase is the same at 30°C as at 20°C. The $R$ values of

3 Portions of this paper (including “Experimental Procedures,” some “Results,” and Tables I-S through IV-S) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20014. Request Document No. 80M-2286, cite author(s), and include a check or money order for $5.20 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

4 For the same reasons given by Howlett and Schachman (7), a 40 mM K-PO₄ buffer at pH 7.0 was used in the studies of this paper. The hydrodynamic behavior of ATCase was not changed by the presence of 100 mM TES/KOH in the 40 mM K-PO₄ buffer of pH 7.0. The concentration dependence of the sedimentation coefficient ($\Delta s/s$) of ATCase in the K-PO₄, TES/KOH buffer at pH 7.0 was determined to be $k = -(1/3\sqrt{3})\Delta (s/s) = 0.007$ ml/mg, which is the same value as that previously published for ATCase in K-PO₄, buffer (7) and corresponds to that expected for a nonassociating globular protein (19, 20). Moreover, the sharpness of sedimentation boundaries was not affected by the addition of PALA to the enzyme and the maximal change in the sedimentation coefficient of ATCase produced by PALA was unchanged when the phosphate buffer contained TES/KOH.
Table I-S are required for calculation of $\Delta H_{T\rightarrow R}$ and $\Delta H_{PALA}$ at 30°C by method I.

In the first series of experiments in Table I (with ATCase preparation I), only heats for binding the first and third aliquots of PALA (1.8 eq of PALA/ATCase$^5$) were measured. For all sequential measurements, large amounts of enzyme were required since the measured heats were small and differed only by ~10% (in method 1A, the difference between $\Delta H_{C2R3}$ and $\Delta H_{R}$ is a measure of $\Delta H_{T\rightarrow R}$; see Equation 3). The heat of dilution of ATCase was the same in the absence and presence of subsaturating PALA. Thus, the corrections for the heats of diluting PALA and diluting enzyme are the same for the sequential binding of PALA within each series of experiments. Sample calorimetric data and calculations are presented in Table II-S for the experiments using ATCase preparation II.

The sequential heats of binding PALA become increasingly less negative in K-PO$_4$ buffer and more negative in K-PO$_4$/TES buffer (Table I). Although the differences between the heats for binding the first and third aliquots of PALA are small, in terms of experimental error these differences are significant (see below). Clearly, the enthalpy changes for binding the first and second aliquots of PALA are similar, whereas that for binding the third is different.

Heats of Assembly of ATCase from C and R Subunits

Heat measurements for the assembly process rely on the stability of the isolated C trimers and R dimers of ATCase and on the rapid assembly of ATCase (C$_3$R$_2$) in high yield from C and R subunits (11, 15, 21, 22). In the present studies, assembly reactions were performed with an excess of R subunits in order to compensate for the extent of assembly being less than 100% at a molar r/c chain ratio of 1.0 because of some instability in R dimers.

Since the sedimentation boundaries of R, C, and C$_3$R$_2$ are well separated in the ultracentrifuge (11), the extent of assembly was determined by this technique (Table III-S). As shown by the data in Table III-S, the calorimetric assembly reactions were 80–100% efficient with respect to the incorporation of C subunits into ATCase. In these studies, the extent of assembly of ATCase from the isolated C and R subunits of ATCase was less in the presence than in the absence of PALA (Table III-S). However, the newly assembled ATCase molecules in the experiments of Table II were stable, with or without PALA present. Because the isolated R subunits showed some instability after dialysis, reconstruction experiments were performed as soon as possible after dialysis of the C and of the R subunits.

Two series of experiments were performed using different preparations of both C and R subunits in 40 mM K-PO$_4$ at pH 7.0. These heats of assembly of ATCase in the absence and presence of saturating PALA at 30°C are given in Table II along with averages for both series of measurements and the corresponding probable errors in these mean values. For the second series, sample calorimetric data and calculations are given in Table IV-S. In Table II, the corresponding pairs of values for the 2 series of assembly experiments are in excellent agreement. The difference between the heats of assembly of ATCase in the presence and absence of PALA is substantial, +22 ± 8 kcal/mol of ATCase.

Heats of Binding PALA to C Subunit

Heats for the sequential binding of PALA and for binding 3.0 eq of PALA to C subunit are given in Table III. Measure-

$^5$ The amount of PALA added to ATCase in the sequential binding experiments of Table I was 10% less than initially intended (1.8 eq instead of 2.0 eq of PALA/ATCase). The stock solution of PALA was recorded as 33 ± 3 mM PALA in water, determined by aspartate analysis after acid hydrolysis at the University of California, Berkeley, CA. However, after the first series of experiments in Table I, spectrophotometric titrations and difference sedimentation velocity data of Table I-S indicated that the actual concentration was consistent with 30 mM PALA; this value for the PALA stock concentration was used to calculate all PALA concentrations reported in this work. In the first series of experiments in order to measure the heat of binding the “last” 2.0 eq of PALA to ATCase, we had lowered the initial molar ratio of PALA bound/ATCase to ensure that protein would not be limiting. Thus, in fact, we measured the heat of binding 1.8 eq of PALA, 3.4 ± 5.2 eq of PALA/ATCase. Subsequently, in order to maintain direct comparability of experimental data, conditions of the first series of calorimetric experiments were duplicated rather than increasing the amount of subsaturating PALA added. The third 1.8 eq of PALA bound/ATCase is taken to mean 3.4 ± 5.2 eq of PALA bound/ATCase. Thus, the third 1.8 eq of PALA bound/ATCase overlaps with the second 1.8 eq of PALA bound/ATCase to the extent of 0.2 eq of PALA/ATCase.

$^6$ The probable error in the mean is calculated for a 99% confidence level.

$^7$ Vickers et al. (23) have computed tentative $\Delta H$ values for assembly of ATCase from free subunits in the absence and presence of PALA and for PALA binding to C subunit from differences in enthalpies of thermal denaturation of C, C(PALA)$_3$, R, ATCase, and ATCase(PALA)$_3$ measured by differential scanning calorimetry. In taking these differences, no consideration was made of the different melting temperatures of these protein species. Thus, the resulting calculated $\Delta H$ values were too negative and cannot be compared with the values of this work in order to determine $\Delta C_p$ values for the reactions considered.

### Table II

Heats of assembly of ATCase (C$_3$R$_2$) from C and R subunits in the absence and presence of saturating PALA

All heats of assembly are in kcal/mol of ATCase formed and were measured at 30°C; final buffer composition was 40 mM K-PO$_4$ (pH 7.0) containing 2 mM 2-mercaptoethanol and 15 µM zinc acetate. The number of heats of assembly in each case is given in parentheses. Heats of assembly for each series are given and averages for the two series of measurements are given with the probable error in each mean. Sample calorimetric data and calculations are given in Table IV-S and the extent of assembly of ATCase in each case was determined by ultra centrifugation (Table III-S) (see the miniprint supplement).

<table>
<thead>
<tr>
<th>Subunit preparations</th>
<th>Heat of assembly</th>
</tr>
</thead>
<tbody>
<tr>
<td>C R</td>
<td>2 C + 3 R → ATCase</td>
</tr>
<tr>
<td>C(PALA)$_3$ + 3 R →  ATCase (PALA)$_3$</td>
<td></td>
</tr>
</tbody>
</table>

### Table III

Heats of binding PALA to C subunit

Two series of measurements (with different preparations) were made at 30°C in 40 mM K-PO$_4$ (pH 7.0) containing 0.2 mM K-EDTA and 2 mM 2-mercaptoethanol. Heat measurements were made for the sequential binding of PALA as well as for the saturation of the 3 active sites with PALA. Heats of binding PALA are given in kcal/mol of C subunit; the number of heats of binding PALA averaged in each case is given in parentheses. Approximately 15 mg of C subunit was used for each calorimeter experiment. The average heat for binding saturating PALA to C subunit is given with the probable error in the mean.

<table>
<thead>
<tr>
<th>C preparation</th>
<th>Heats of binding PALA at following initial → final eq of PALA bound/C subunit values:</th>
<th>kcal/mol C subunit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0 → 0.91</td>
<td>0.91 → 1.8</td>
</tr>
<tr>
<td>I</td>
<td>-13.5 (2)</td>
<td>-12.8 (2)</td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Averages</td>
<td>-40.9 (3)</td>
<td>-39.6 (3)</td>
</tr>
</tbody>
</table>

$^8$ The probable error in the mean is calculated for a 99% confidence level.

$^9$ Vickers et al. (23) have computed tentative $\Delta H$ values for assembly of ATCase from free subunits in the absence and presence of PALA and for PALA binding to C subunit from differences in enthalpies of thermal denaturation of C, C(PALA)$_3$, R, ATCase, and ATCase(PALA)$_3$ measured by differential scanning calorimetry. In taking these differences, no consideration was made of the different melting temperatures of these protein species. Thus, the resulting calculated $\Delta H$ values were too negative and cannot be compared with the values of this work in order to determine $\Delta C_p$ values for the reactions considered.
\[ \Delta H^\circ_{\text{III}} = \Delta H_{\text{PALA}} + \Delta H_{\text{T-R}} \]  
(4)

\[ \Delta H_{\text{all}} = \Delta H_{\text{PALA}} + 0.23 \Delta H_{\text{T-R}} \]  
(8)

Solving these simultaneous equations for \( \Delta H_{\text{T-R}} \) and \( \Delta H_{\text{PALA}} \) gives

\[ \Delta H_{\text{T-R}} = 4.29 \Delta H_{\text{all}} - 14.3 \Delta H_{\text{all+2}} \]  
(11)

\[ \Delta H_{\text{PALA}} = 14.3 \Delta H_{\text{all+2}} - 3.29 \Delta H_{\text{all}} \]  
(12)

Values for \( \Delta H_{\text{T-R}} \) and \( \Delta H_{\text{PALA}} \) for each experiment of Table I were calculated using Equations 11 and 12 (method 1B). The average values for \( \Delta H_{\text{T-R}} \) and \( \Delta H_{\text{PALA}} \) and corresponding probable errors in the mean values in K-PO₄ and K-PO₄-TES buffers are given in Table IV. As in method 1A, proton release or uptake and corrected \( \Delta H \) values are presented for method 1B.

Calculated values for \( \Delta H_{9.6} \) for methods 1A and 1B were determined from Equation 4 using appropriate \( \Delta H_{\text{T-R}} \), and \( \Delta H_{\text{PALA}} \) values of Table IV. The probable error in \( \Delta H_{9.6} \) for method 1A was calculated from the probable errors in the corresponding independent \( \Delta H_{\text{T-R}} \) and \( \Delta H_{\text{PALA}} \) values. Since Equation 4 is one of the simultaneous equations used to determine \( \Delta H_{\text{T-R}} \) and \( \Delta H_{\text{PALA}} \) for method 1B, the calculated and measured \( \Delta H_{9.6} \) values must be equal (Table IV).

Values of \( \Delta H_{\text{T-R}} \) for methods 1A and 1B in the same buffer are in accord with experimental error (Table IV). Both are small and negative in K-PO₄ buffer and small and positive in K-PO₄-TES buffer indicating that a proton uptake accompanies the conformational transition. Similarly, the values of \( \Delta H_{\text{PALA}} \) for methods 1A and 1B in the same buffer are also in agreement within experimental error (Table IV). Both values are negative in K-PO₄ buffer and more negative in K-PO₄-TES buffer, indicating a proton release for the local effect of PALA (Equation 4) to ATCase and the heat for binding the third aliquot of PALA (Equation 8).
binding PALA. In addition, the calculated values of 
$\Delta H_{\text{obs}}$ for method 1A and the directly measured heats in the same buffer are in accord within experimental error (Table IV). The $\Delta H_{\text{obs}}$ values in K-PO$_4$ and K-PO$_4$-TES buffers are the same within experimental error, indicating a negligible proton uptake for the overall effect of binding PALA (local effect + conformational effect). Furthermore, all $\Delta H$ values corrected for proton effects are the same as those in K-PO$_4$ buffer within experimental error (Table IV).

Method 2—$\Delta H_{T-R}$ can be calculated from Equation 6 with the experimentally measured heats of assembly of ATCase in the absence and presence of PALA ($\Delta H_T$ and $\Delta H_{T-R}$, respectively; Table II), the heat of binding 3.0 eq of PALA to C subunit ($\Delta H_{T-PALA}$, Table III), and $\Delta H_{PALA}$. The value of $\Delta H_{T-PALA}$ used to compute $\Delta H_T-R$ is that from method 1A because it is more accurate than that from method 1B (compare experimental errors in Table IV and see below). The resultant value of $\Delta H_T-R = -(77) + (2-40) + (-54) - (-43) = -14$ kcal/mol of ATCase (Table IV).

$\Delta H_{T-R}$ can be calculated by solving Equations 4 and 6 for $\Delta H_{T-R}$ in terms of $\Delta H_T$, $\Delta H_{T-PALA}$, and $\Delta H_{PALA}$, giving

$$\Delta H_{T-R} = -\Delta H_T + 2 \Delta H_{T-PALA} + \Delta H_{PALA} \quad (13)$$

This computed value of $\Delta H_{T-R}$ is presented in Table IV (method 2) with the probable error.

Heats of assembly and the heat of binding 3.0 eq of PALA to C subunit were measured in K-PO$_4$ buffer only. Thus, no proton release or uptake data and no corrected $\Delta H_T$ and $\Delta H_{T-R}$ values from method 2 are available. However, the heat of protonation of the K-PO$_4$ buffer is small ($-0.66$ kcal/mol of H$^+$) and, therefore, has little effect on the heats used in method 2 to calculate $\Delta H_T-R$ and $\Delta H_{T-R}$: these values are in accord within experimental error with corresponding values from methods 1A and 1B in K-PO$_4$ buffer.

A Comparison of Errors in $\Delta H_{T-R}$, $\Delta H_{T-PALA}$, and $\Delta H_{PALA}$

Values from Methods 1A, 1B, and 2

The only direct measure of the total error in $\Delta H_{T-R}$ and $\Delta H_{T-PALA}$ values (in K-PO$_4$) from methods 1A and 2 involves comparing the corresponding computed $\Delta H_{T-PALA}$ values, $-48 \pm 5$ and $-57 \pm 8$ kcal/mol of ATCase, respectively, with the experimentally determined $\Delta H_{T-PALA}$ value, $-50 \pm 2$ kcal/mol of ATCase (see Table IV). Both calculated values agree with the experimental value within experimental error. The value from method 1A is nearer the experimental value and its error is smaller, indicating that the sum of $\Delta H_T-R$ and $\Delta H_{T-PALA}$ from method 1A is more accurate than that from method 2. The computed $\Delta H_{T-PALA}$ of method 1B cannot be used as a measure of the error in corresponding $\Delta H_T-R$ and $\Delta H_{T-PALA}$ values. The computed and experimental values of $\Delta H_{T-PALA}$ from method 1B must be equal since the experimental value was used in Equation 4 in determining $\Delta H_T-R$ and $\Delta H_{T-PALA}$.

The different values for both $\Delta H_T-R$ and $\Delta H_{T-PALA}$ are all internally consistent within experimental error (Table IV). However, the errors in $\Delta H_T-R$ and $\Delta H_{T-PALA}$ values from method 1A are approximately one-half those from methods 1B and 2, further suggesting that method 1A gives the most accurate values for $\Delta H_T-R$ and $\Delta H_{T-PALA}$.

The greater accuracy of method 1A is due to the form of the equation used to calculate $\Delta H_T-R$ (Equation 9) and to the way in which the heats of sequentially binding PALA to ATCase were measured. In measuring $\Delta H_{T-PALA}$ and $\Delta H_{PALA}$ for PALA binding to ATCase, within each series of experiments, the same protein and PALA solutions were used. Thus, the heat of dilution corrections were the same for each uncorrected heat (e.g. see Table II-S). In directly subtracting $\Delta H_{T-PALA}$ from $\Delta H_{T-PALA}$ in Equation 9, the heat of dilution corrections cancel and thereby introduce no error into this $(\Delta H)$ value. Furthermore, published $\dot{R}$ data for $\Delta H_{T-PALA}$ (7, 10) give an equation for $\Delta H_{T-PALA}$ almost identical with that for $\Delta H_{T-PALA}$ (Equation 7), predicting that $\Delta H_{T-PALA} \approx \Delta H_{T-PALA}$. This is corroborated by data of Table I and uncorrected heats of Table II-S, further attesting to the accuracy of the sequential heats of binding. In method 1B, since an unequally weighted difference is taken (Equation 11) and different protein and PALA solutions were used for the sequential and saturating measurements, corrected heats (Table I), which contain additional error due to the heat of dilution corrections, must be used. In method 2, an algebraic sum of four heats (Equation 6), each corrected for heats of dilution, must be taken. Thus, $\Delta H_T-R$ from method 1A should be the most accurate estimate of the value for the PALA-promoted conformational change of ATCase.

DISCUSSION

Heats of Binding PALA to ATCase and C Subunit—The heats of binding 6.0 eq of PALA to ATCase and 3.0 eq of PALA to C subunit in K-PO$_4$ buffer at pH 7.0 and 30°C are $-50 \pm 2$ kcal/mol of ATCase and $-40 \pm 1$ kcal/mol of C subunit, respectively. Because the heat of protonation of K-PO$_4$ is small ($-0.66$ kcal/mol of H$^+$) and the proton effect on binding PALA is small (1 eq of H$^+$ uptake/ATCase), the corrected values and uncorrected values in K-PO$_4$ buffer are essentially the same. Knier and Allewell (24) have performed calorimetric titrations of ATCase and of C subunit with PALA in the absence of $P_i$ At pH 8.3 and 25°C. They report heats of $-51 \pm 6$ kcal/mol of ATCase and $-34 \pm 4$ kcal/mol of C subunit after correcting for relatively large proton effects. Thus, the heats reported here and those of Knier and Allewell (24) are in reasonable agreement.

$P_i$ is assumed to bind competitively with PALA to C subunit and to ATCase since $P_i$ is competitive with carbamoyl phosphate binding to C subunit with $K_i = 1.5$ mm (25). Thus, in the presence of 40 mm $P_i$ (conditions of all experiments reported here), all “unliganded” ATCase and C subunit were saturated with $P_i$ and the binding of PALA required displacement of bound $P_i$. The agreement between the values reported here for binding PALA to ATCase and to C subunit with those of Knier and Allewell (24) may be due to fortuitous cancellation of opposing effects or may indicate that the heats of binding are not very dependent on pH or temperature and that the heat of binding $P_i$ to both ATCase and C subunit is about zero.

The striking difference between the heat of binding PALA to isolated C subunits (Table III) and the intrinsic heat of binding PALA to intact ATCase (Table IV), $-13 \text{ versus } -7$ kcal/eq of PALA bound, is in accord with evidence from enzyme kinetics (26) that free C subunit does not constitute an appropriate model for the R state of ATCase. The variation in the apparent heat of binding PALA sequentially to C subunit, although slight, is significant. There may be a conformational change in the C subunit upon binding the first PALA molecule which affects the apparent heat of binding subsequent PALA molecules to the other two chains. In this regard, it should be noted that PALA causes a strengthening of the interchain interactions within the C subunit (27). Proton effects in binding PALA to the isolated C subunit eventually
should be evaluated for the conditions used in this study.

Heats of Assembly of ATCase—As seen in Table II, there is a large difference between the heats of assembly of ATCase in the presence and absence of PALA (−54.5 ± 7.1 versus −76.8 ± 2.6 kcal/mol of ATCase). This large PALA-promoted change in the enthalpy of assembly, ΔHf − ΔHf0 = +22 ± 8 kcal/mol of ATCase, doubtless reflects in part the effect of PALA on the c−r chain interaction enthalpy. If the observed enthalpies of assembly are totally attributed to bonding domain formation between c and r chains (six in ATCase), the heats of formation of these bonds would be −9 and −13 kcal/mol of c−r bond in the presence and absence of PALA, respectively. However, the value of ΔHf − ΔHf0 probably also contains contributions from changes in interchain and intrachain contacts within C trimers and R dimers.

The difference between the enthalpies of assembly for ATCase (PALA) and unliganded ATCase from the corresponding subunits is the difference between the enthalpy for binding 6 eq of PALA to ATCase and twice that for binding 3 eq of PALA to C subunit, i.e. ΔHf − ΔHf0 = ΔHfPALA − ΔHf0 (see Equation 13). Our value for ΔHfPALA − 2 ΔHf0 is +30 ± 3 kcal/mol and Nieri and Alievell (24) report a value of +17 kcal/mol for this dimer at pH 8.3. Both of these estimates in agreement with the value for the PALA-promoted change in the enthalpy of assembly of ATCase. Because C subunit is not a good model for the R state of ATCase, twice the heat of binding PALA to isolated C subunits (2 ΔHf0) cannot be used as a measure of ΔHfPALA for ATCase. Therefore, the effect of PALA on the enthalpy of assembly of ATCase is not a measure of ΔHfPALA (see Equation 6).

In our assembly experiments at fixed τ/c chain ratios, the extent of C trimer incorporated into ATCase was less in the presence than in the absence of PALA (Table III). This indicates that the free energy of assembly is less negative in the presence of PALA than in its absence, suggesting that PALA produces some weakening of intersubunit bonding domains. Subramani et al. (28) in studies of the disproportionation of ATCase molecules lacking 1 R subunit showed that PALA causes a 300-fold increase in the rate of rupture of the bonding domain between c and r chains. The estimate of Subramani et al. (28) for the difference in the free energies of formation of 6 c−r bonds in the presence and absence of PALA, Δ(ΔGf), and our corresponding value for the change in the enthalpy of assembly, Δ(ΔHf), indicate that Δ(ΔGf) is positive (~ +40 cal/degree-mol) for the PALA-produced change in the entropy of assembly.

Protein surface interactions are reflected in enthalpies of subunit association. Lord et al. (29) obtained a van’t Hoff ΔH of −17.2 kcal/mol for the dimerization of insulin at pH 2. In view of the crystallographic structure for the protein, they concluded that the enthalpy of association is not governed by the extent of surface contact but rather by the types of specific interactions involved.

From detailed studies on the thermodynamics of hemoglobin assembly, Ackers and associates (30–33) have concluded that hydrophobic interactions play an increased role within the dimer-dimer (αββα) contact region upon oxygenation or that hydrogen bonds and ion pair interactions participate to a lesser extent on oxygenation. For αββα dimer association to αββα tetramer, Ip and Ackers (30) measured van’t Hoff ΔH values of −28.9 and +3.8 kcal/mol (tetramer) in the absence and presence of oxygen, respectively. Thus, the oxygen-linked change in enthalpy of αβ βα dimer association is +32.7 kcal/mol of αββα. The PALA-linked change in enthalpy of assembly of ATCase is positive also; however, for the assembly of ATCase from C and R subunits in the absence and presence of PALA, enthalpy changes are both negative (Table II).

The principal goal of our studies has been to obtain a measurement of the heat for the substrate-promoted conformational change of ATCase from an initial T state to a final R state (ΔHfT,R). With the exception of related studies on hemoglobin (30–35), we know of no previous determination of ΔHfT,R for a conformational transition of an allosteric protein. Using normal hemoglobin A and a α chain mutant hemoglobin that presumably has the quaternary T → R transition blocked, Gaud et al. (34) measured ΔHfT,R = +9.0 ± 2.5 kcal/mol at pH 7.4. This value is about the same as that estimated by Imai and Tyuma (35) and is lower than that predicted by Ackers and co-workers (30–33). The determination by Ip and Ackers (30) of the oxygen-linked change in enthalpy of dimer association equals ΔHfT,R for hemoglobin since recent studies (32, 33) show that the intrinsic enthalpy change for binding oxygen is the same for the αβ dimer and αββα tetramer.

ΔHfT,R with Respect to Molecular Models—The methods used to calculate ΔHfT,R for the substrate-promoted conformational transition in ATCase are model-independent. Here, we will use the simplest model, the 2-state model of Monod et al. (9), since it has been used successfully to account for conformational changes in ATCase (10). However, it should be noted that our considerations apply equally well to the sequential model (36) if it is assumed that the intrinsic heat of binding ligand is the same for each conformational species.

In this case, methods 1A, 1B, and 2 still yield ΔHfT,R which represents the heat for the complete conformational transition from the initial T state to the final R state (with an unspecified number of intermediate states).

The 2-state model (9) involves an equilibrium between a constrained or low affinity (T) state and a relaxed or high affinity (R) form of the enzyme and the perturbation of the equilibrium by the addition of various ligands. In the absence of any ligand, the equilibrium constant for ATCase, $[T]/[R]$, is 250 (10). The affinity of ATCase in the R state for PALA is at least 20 times greater than that of the enzyme in the T state (10). In applying the 2-state model to our calorimetric data, it is not necessary to assume that the intrinsic heat of binding PALA to ATCase is the same for the enzyme in the T and R states.9

Observable Heat and Proton Effect of Binding PALA to ATCase—Values for ΔHfT,R and ΔHfPALA from method 1A,

9 After binding the third 1.6 eq or all 6.0 eq of PALA/ATCase, $R$ is 0.77 or 1.00, respectively (Table I); in these cases, essentially all ATCase with any PALA bound is in the R state. Thus, Equations 8 and 4 are correct as written. However, after binding the first 1.8 eq of PALA/ATCase, $R$ is 0.43 (Table I-S); in this case, not all ATCase with PALA bound is in the R state; 86% of the catalytic sites filled with PALA are in R state molecules and 14% are in T state molecules (see below). Thus, Equation 7 is not rigorously correct since $ΔHfPALA$ is assumed to be the intrinsic heat of binding PALA to the R state. In order to correct Equation 7, the 0.30 $ΔHfPALA$ term should be replaced by 0.26 $ΔHfPALA + 0.04 ΔHfPALA$ where $ΔHfPALA$ is the intrinsic heat of binding PALA to the T state. The indicated modification of Equation 7 affects the estimate of $ΔHfR$ only for method 1A. Assuming large differences between $ΔHfPALA$ and $ΔHfPALA$ does not substantially change the estimate for $ΔHfR$ by method 1A. The fraction of catalytic sites filled with PALA that are in R state and in T state molecules were obtained by solving and evaluating Equation 1 of Monod et al. (9) for $a$ in terms of $R$, $L$, and $c$; the expressions for $R_L$ and $T_L$ (9) were solved in terms of $a$, $L$, $c$, and $T_L$ and evaluated as multiples of $T_0$ (i.e. $R_L = a(L) T_0$ and $T_L = g(a, L, c) T_0$). The fraction of catalytic sites filled with PALA that are in molecules in the R state is the ratio $\frac{\sum_{n=1}^{6} n R_n}{\sum_{n=1}^{6} n (R_n + T_n)}$ and the fraction of catalytic sites filled with PALA that are in molecules in the T state is 1.00 minus this ratio. The following values were used: $L = 250$, $c = 1/20$, and $R = 0.49$.
-6.2 and -42 kcal/mol of ATCase, respectively, are the best estimates due to the greater accuracy of method 1A (see "Results"). Knier and Allewell (24) report linearity within experimental error in the thermal titrations of ATCase with PALA, concluding that any enthalpic effect associated with the T → R conformational transition is small. More recently, Hofmann et al. (37) have estimated upper and lower limits of +12 and -14 kcal/mol of ATCase, respectively, for ΔH_{T-R} at pH 8.3 using the difference between the calorimetric heat for binding 6.0 eq of PALA to ATCase and the van't Hoff heat from the temperature dependence of K_r for PALA.

The contributions of the ΔH_{T-R} and ΔH_{PALA} values (method 1A) to the observable heat for binding PALA are calculated and plotted in Fig. 3 (--- and ----, respectively) as a function of eq of PALA added/ATCase; the sum of these two components, plotted in Fig. 3 (——), is the observable heat. The ΔH_{T-R} contribution is small and varies as \( R \times \Delta H_{T-R} \), which is nonlinear with respect to PALA bound. In contrast, the ΔH_{PALA} contribution is large and varies linearly as \( Y \times \Delta H_{PALA} \) where Y is the fractional saturation of ATCase with PALA. As a result, the observable heat is almost completely linear with respect to PALA bound and this explains why Knier and Allewell (24) did not detect any nonlinearity in flow calorimetric titrations of ATCase with PALA. The nonlinearity in observable heat as a function of PALA bound/ATCase is only detectable by \( \Delta(\Delta H) \) measurements such as those used in this study.

ΔH_{T-R} and ΔH_{PALA} values from method 1B predict the greatest nonlinearity in the observable heat. However, a plot similar to that of Fig. 3 of the data from method 1B shows only a slight increase in curvature of the observable heat in the subsaturating PALA region; the nonlinearity remains barely discernible.

Under conditions of experiments reported here, a proton uptake of 2-6 eq of H'/ATCase accompanies the T → R conformational transition and a proton release of 2-5 eq of H'/ATCase due to local effects accompanies the binding of 6.0 eq of PALA with displacement of P_i. The net (observed) effect is an almost negligible proton uptake on binding 6.0 eq of PALA (0-1 eq of H'/ATCase) and on binding subsaturating PALA.

The proton uptake on binding 6 eq of PALA to ATCase at pH 7.0 and 25 °C in the absence of P_i was reported (24) as 3.8 eq of H'/ATCase and within experimental error was linear with respect to eq of PALA bound/ATCase (38). These results are compatible with ours and indicate that the binding of 6 eq of P_i/ATCase has an attendant proton uptake of ~3 eq of H'/ATCase. Thus, in the absence of P_i the observable proton effect almost exclusively derives from the local effect of binding PALA, which is linear.

Thermodynamic Parameters for the T → R Transition—

The 2-state model gives a value of +3.3 kcal/mol of ATCase at pH 7.0 and 20 or 30 °C (10) for the free energy change of the T → R conformational transition (ΔG_{T-R}^0). ΔG_{T-R}^0 and the best estimate for ΔH_{T-R}, -6 kcal/mol of ATCase, give a value of ~31 cal/(degree·mol of ATCase) for ΔS_{T-R} and +9 kcal/mol of ATCase for −ΔH_{T-R} at 30 °C. Thus, the T → R transition is entropically controlled at pH 7.0 and 30 °C. Since all measurements used to determine ΔG_{T-R} and ΔH_{T-R} were in KPO_4 buffer at pH 7.0, these thermodynamic parameters are for enzyme species that have bound P_i.

The small negative value of ΔH_{T-R} suggests that the principal effect in the overall conformational transition of ATCase is disruption of hydrophobic interactions (39, 40). The extent of breakup of hydrogen bonds and ion pairs is probably small although this positive contribution to ΔH_{T-R} is opposed by the negative contribution due to proton uptake by the enzyme. This bond breakup is a net effect and does not preclude substantial disruption of hydrogen bonds and ion pairs followed by subsequent formation of almost equal numbers of each type of interaction in the final conformational state. The final R state of ATCase is a more swollen, open structure than the initial conformational state (6-8, 49, 50). However, ΔS_{T-R} is negative and small. This further implicates a net breakup of hydrophobic linkages (40).

Calorimetric measurements of this study indicate that the heat for the substrate-promoted conformational T → R transition in ATCase is small and negative, ~ -6 kcal/mol of ATCase, and that the proton uptake effect associated with the T → R transition is ~2 eq of H'/ATCase. A net disruption of hydrophobic interactions in the final conformational state is suggested.

Acknowledgment—We are very much indebted to Ying R. Yang at the University of California, Berkeley, CA, for purifying the ATCase and for preparing the catalytic and regulatory subunits of ATCase used in this work. We also thank her for performing activity assays of ATCase and C subunits before and after calorimetric studies.

REFERENCES

FIG. 3. Observable heat of binding PALA to ATCase at pH 7.0 and 30 °C and contributions from ΔH_{T-R} and ΔH_{PALA}. The observable heat of binding PALA to ATCase in kcal/mol of ATCase is calculated and plotted as a function of eq of PALA added/ATCase (——). The contributions from the heat of the conformational transition (---) and from the intrinsic heat of binding PALA (----) to the observable heat are presented separately, each in kcal/mol of ATCase. The observable heat is the sum of these contributions: \( R \times \Delta H_{T-R} + Y \times \Delta H_{PALA} \), where R is the fractional saturation of ATCase with PALA, and \( \Delta H_{PALA} \) is the intrinsic heat of binding 6.0 eq of PALA to ATCase in the R state. At saturating levels of ligand, the observable heat is \( \Delta H_{PALA} \) and equals \( \Delta H_{T-R} + \Delta H_{PALA} \) (Equation 4). Values of \( \Delta H_{T-R} \) and \( \Delta H_{PALA} \) used are those from method 1A, corrected for proton effects (Table IV). R values, used to calculate the \( R \times \Delta H_{T-R} \) term, are from Table I-S.
ΔH° for Aspartate Transcarbamoylase Conformational Change

24. Knier, B. L., and Allew, N. M. (1978) Biochemistry 17, 784–790
SUPPLEMENTARY MATERIAL TO

CALORIMETRIC ESTIMATE OF THE ENTHALPY CHANGE FOR THE SUBSTRATE-PROMOTED CONFORMATIONAL TRANSITION OF ASPARTATE TRACERAMBOYLCASE FROM ESCHERICHIA COLI

Andrew Shraeke, Ann Ginsburg, and H.K. Schachman

This supplement contains the entire "Experimental Procedures" and provides in "Results" sample calorimetric data (Tables II-S and IV-S) and results from sedimentation velocity experiments performed to monitor conformational transitions (Tables I-S and III-S).

EXPERIMENTAL PROCEDURES

Materials -- ATCase, purified by the method of Gerhart and Holoduk (42) and catalytic (C) and regulatory (R) subunits of ATCase, isolated by the method of Yang et al. (42), were prepared by T.R. Yang at the University of California, Berkeley, California and shipped at 0°C in ammonium sulfate. Upon receipt, ATCase and subunit preparations were assigned preparations numbers and were dialyzed at 4°C against three changes of buffer over an approximately 20 h period. The appropriate final dialysate was used for preparation of buffer and PALA solutions for calorimetry.

For measurement of the heat of binding PALA, the dialyze buffer for preparations I and II of ATCase and preparations I and II of C subunit was 40 mM K-Pi, pH 7.0 at 30°C containing 0.2 mM K-EDTA and 2 mM 1-mercaptoethanol. The buffer for preparation III of ATCase was the same except for the additional presence of 100 mM Tris/KOH.

For the assembly experiments, C subunit (preparations III and IV) was dialyzed against 40 mM K-Pi, pH 7.0 at 30°C containing 2 mM 1-mercaptoethanol, whereas the D subunit (preparations I and II) was dialyzed against the same buffer also containing 20 mM zinc acetate. The final buffer composition after assembly was 40 mM K-Pi, pH 7.0 containing 2 mM 1-mercaptoethanol and 20 mM zinc acetate.

Melbach et al. (21) showed that Zn²⁺ (4 mg/ATCase) has an essential role in the quaternary structure of ATCase and that the presence of Zn²⁺ with a subunit is required for the assembly of ATCase from free subunits. A similar analysis of the dialyzed R subunit (as performed 8) in the course of a study (42) in the laboratory of the Catholic University of America indicates that with 20 mM zinc acetate at pH 7.0 present, 4% of Zn²⁺ are bound to regulatory polyphosphate chains. B-(Phosphorylcytidylyl)²⁻-ATCase (PALA) was a generous gift from Dr. C.R. Stark. The stock solution was 30% ATCase in water. Since ATCase and C subunit bind PALA with high affinity (17,18), essentially all PALA added at saturating levels is bound when either protein is present in the molar concentration range (the conditions of all experiments reported in this paper).

Spectrophotometric measurements -- Protein concentrations were determined from the turbidity of the stock solution (43). 0.39 for ATCase, 0.71 for C subunit, and 2.0 for R subunit. Protein difference spectra were used to measure the extent of PALA binding to ATCase and to C subunit. The extent of PALA binding was measured for each ATCase and for each C subunit preparation, these values agreed with known stoichiomterics (17,18) within 10%.

The absorbance values for peak-trough absorption differences at 289-285 nm agreed within experimental error with that estimated from the difference spectrum for ATCase (18) or with that for C subunit (18).

Calorimetric measurements -- Heats of binding PALA to ATCase and to C subunit and heats of assembly of ATCase were measured at 30.0 ± 0.1°C using an IBM Bench microcalorimeter 1070 equipped with gold cells. Recording and measuring the heats of reaction, calibrating the calorimeter, loading the calimeter cells, and running separate experiments to determine heats of dilution were all as described previously (44).

The protein calorimetric experiments described in this work, the electrical calibration heaters were checked by measuring the precision of the heat of dilution of sucrose (45).

For experiments measuring the heats of binding PALA to ATCase and to C subunit, ATCase final dialyze and C subunit final dialyze, respectively, were used in the reference cell. For all experiments to determine heats of assembly of ATCase, C subunit final dialyze was used in the reference cell.

Ultracentrifugation -- Sedimentation experiments were performed at 20°C with a Beckman model E analytical ultracentrifuge (equipped with rotor temperature and control unit) using schlieren optics (with phase plate). Instrument calibration, schlieren photography, and density and viscosity measurements were as described previously (46). Difference sedimentation velocity measurements (47) were at 52,000 rpm for ATCase and at 56,000 rpm for C subunit employing rotor and two cells containing 12 mm, 4° single sector Keel F cuvettes, one of the two cells had a 1° positive wedge upper quartz window for pattern displacement and was used for the reference pride the rotor and two cells containing 12 mm, 4° single sector Keel F cuvettes, one of the two cells had a 1° positive wedge upper quartz window for pattern displacement and was used for the reference pride the rotor and two cells containing 12 mm, 4° single sector Keel F cuvettes, one of the two cells had a 1° positive wedge upper quartz window for pattern displacement and was used for the reference.

Solutions of ATCase from the PALA binding experiments in the calorimeter were diluted with dialyze to a concentration of 1.9 mg/ml for ATCase (Table I-S). The C subunit (in the absence and presence of saturating PALA) was run at 2.8 mg/ml.

A Nikon (model E-6) two-dimensional microphotometer at 20-fold magnification was used to measure the radial position of the meniscus peak as a function of time. Plates were aligned by manual and the rotor reference hole. With the same run, the measurement was in sedimentation coefficient (47) was calculated by the procedure of Howlett and Schachman (7) with a precision of 0.1% in the negative values of x/a values reported earlier (7) relative to that of the liganded form of ATCase.

These values agree (within experimental error) with the curve of Howlett and Schachman (7) for the fractional change in sedimentation coefficient in terms of the maximal change as a function of the molar ratio of PALA to ATCase (determined under nearly identical conditions to those used here).

ΔΗ for Aspartate Transcarbamoylase Conformational Change

Table I-S gives the change in sedimentation coefficient upon PALA addition to ATCase, which is used to determine the extent of the transition. Table II-S gives sample experimental data for heats of binding PALA sequentially and of binding 6 eq of PALA to ATCase. Table III-S shows ultracentrifugacal measurements of the extent of the incorporation of C subunit into ATCase in two-dimensional experiments in the presence and absence of PALA, and in the presence of saturating PALA in the calorimeter. Table IV-S gives representative experimental data for heats of assembly of ATCase and C subunit in the absence and presence of saturating PALA. The calculations are sufficiently detailed to describe the determination of values presented in the main text of this paper.

Table I-S: Change in sedimentation coefficient upon PALA binding to ATCase

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<thead>
<tr>
<th>Subunit</th>
<th>ΔΗ (Cal/mole)</th>
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<tbody>
<tr>
<td>C subunit</td>
<td>-20.0</td>
</tr>
<tr>
<td>ATCase</td>
<td>-18.0</td>
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Molar ratio of PALA bound to ATCase

<table>
<thead>
<tr>
<th>Molar ratio</th>
<th>ΔΗ (Cal/mole)</th>
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</thead>
<tbody>
<tr>
<td>0.0</td>
<td>-20.0</td>
</tr>
<tr>
<td>1.0</td>
<td>-18.0</td>
</tr>
</tbody>
</table>

Table II-S: ΔΗ for Aspartate Transcarbamoylase Conformational Change

<table>
<thead>
<tr>
<th>Sector 1</th>
<th>Sector 2</th>
<th>(Reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.0</td>
<td>100</td>
</tr>
<tr>
<td>0.0</td>
<td>1.0</td>
<td>90 (extent of T-R transition)</td>
</tr>
</tbody>
</table>
\begin{table}
\centering
\caption{Sample experimental data for heats of binding PALA to ATCase.}
\label{table:binding-heats}
\begin{tabular}{lcccc}
\hline
 & Initial \rightarrow Final eq of PALA bound/ATCase & \\
\hline
Avg uncorr'd heat of reaction, mcal & 0.0 & -1.8 & 1.8 & -3.6 & 3.6 & -5.2 & 0.0 & -4.0 \\
Avg heat of PALA dilution, mcal & -2.62 (3) & -2.60 (2) & -2.49 (3) & -3.04 (3) \\
Heat of enzyme dilution, mcal & -0.03 (3) & -0.04 (3) \\
Corr'd heat of reaction, mcal & -2.56 & -2.54 & -2.43 & -2.98 \\
Heat of reaction, kcal/mol of ATCase & -15.1 & -14.9 & -14.3 & -15.3 \\
\hline
\end{tabular}
\end{table}

\begin{table}
\centering
\caption{Ultracentrifugal measurement of the extent of C subunit incorporation into ATCase.}
\label{table:ultracentrifuge}
\begin{tabular}{lcccc}
\hline
Submit & Initial conc & Molar chain ratio & Percent incorporation of C into ATCase & \\
preparations & of C & & (PALA absent) & (PALA present) & \\
\hline
C & R & & & \\
III & I & 1.28 & 1.3 & 100$^a$ & 100 \\
& II & 1.07 & 1.3 & 100 & 96 \\
IV & II & 1.07 & 1.3 & 100 & 96 \\
\hline
\end{tabular}
\end{table}

\begin{table}
\centering
\caption{Sample experimental data for heats of assembly of ATCase from C and R subunits in absence and presence of PALA.}
\label{table:assembly-heats}
\begin{tabular}{lcccc}
\hline
 & 2 C + 3 R & \rightarrow & ATCase & 2 C(PALA)$_2$ + 3 R & \rightarrow & ATCase(PALA)$_4$ & \\
Experiment & & & & & & \\
\hline
Uncorr'd heat of assembly (Run 1; Run 2), mcal & -2.37; -2.43 & -1.56; -1.86 \\
Avg heat of C subunit dilution into R-buffer, mcal & -0.54 (4) & -0.54 (4) \\
Avg heat of R subunit dilution into C-buffer, mcal & +0.31 (2) & +0.31 (2) \\
Mixing C- and R-buffers, mcal & -0.28 (3) & -0.28 (3) \\
Heat of assembly corr'd for dilutions (Run 1; Run 2), mcal & -2.42; -2.48 & -1.61; -1.91 \\
Fraction of C subunit incorp'd into ATCase (Run 1; Run 2) & 1.00; 1.00 & 0.96; 0.94 \\
Avg heat of assembly, kcal/mol of ATCase & -75.3 & -37.0 \\
\hline
\end{tabular}
\end{table}

$^a$ after 4 days at 4°C, the area of the 11 S component was the same upon re-running in the ultracentrifuge.

$^b$ Instability of R subunit preparation 1 (with either 0.20 or 0.020 mM zinc acetate in dialysates) was observed. This calorimeter reaction was performed 5 days after dialysis of the R subunit. Other assembly reactions with saturating concentrations of PALA were performed within 48 hr after dialysis of R subunit against 40 mM K-P0$_4$ (pH 7.0) containing 2 mM 2-mercaptoethanol and 0.020 mM zinc acetate. Without PALA present, assembly reactions were carried out within 72 hr after dialysis of the R and C subunits. After storing these R and C subunit preparations for 10 days at 4°C, only 40% of C subunit was incorporated into ATCase with the same molar r/c chain ratio in the absence of PALA.
Calorimetric estimate of the enthalpy change for the substrate-promoted conformational transition of aspartate transcarbamoylase from Escherichia coli.
A Shراك, A Ginsburg and H.K. Schachman


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