Homotropic Activation via the Subunit Interaction and Allosteric Symmetry Revealed on Analysis of Hybrid Enzymes of L-Lactate Dehydrogenase*

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1-Lactate dehydrogenase from Bifidobacterium longum shows homotropic activation by pyruvate as well as heterotropic activation by fructose 1,6-bisphosphate. Hybrid enzymes were produced from the wild-type subunit and a mutant subunit, whose substrate specificity was altered to that of malate dehydrogenase, and separated to analyze the substrate-induced homotropic activation mechanism. Oxamate, a competitive inhibitor of l-lactate dehydrogenase, was used to mimic the substrate-induced activation of the wild-type subunit as “a regulatory subunit.” The malate dehydrogenase activity of the mutant subunit as “the catalytic subunit” of the hybrid enzymes was measured, and the activity of the mutant subunit was activated on the addition of oxamate. Thus, we directly observed the inter-subunit homotropic activation transmitted from the wild-type to the mutant subunit. Moreover, “isomeric” hybrid enzymes that have different structural subunit arrangements but identical subunit compositions showed identical kinetic natures. This indicates that the enzyme maintains its subunit symmetry during the allosteric transition.

Most oligomeric allosteric proteins do not show hyperbolic Michaelis-type saturation curves, but sigmoidal ones in certain conditions. This phenomenon originates from the activation following an increase in the substrate concentration, and has been called “homotropic activation.” Most kinetic studies on the homotropic activation of allosteric enzymes have focused on their sigmoidal substrate saturation curves. An alternative approach is analysis of the activation with the addition of low concentrations of “inhibitors” with subsaturating levels of substrates (1, 2). This activation indicates that the binding of an inhibitor to an active site affects the catalytic activity of neighboring active sites. With this method, however, complete analysis of the activation is quite complex, because the inhibitor and substrate compete at the active site.

L-Lactate dehydrogenases (LDHs)1 (EC 1.1.1.27) have been purified from a variety of organisms and tissues, and their structures and functions have been studied in detail (3). The active form of LDHs is a tetramer of identical 30–35-kDa subunits. The LDHs from some bacteria are allosteric enzymes exhibiting sigmoidal kinetics for pyruvate (homotropic activation), and are allosterically activated by fructose 1,6-bisphosphate (FBP) (heterotropic activation) (4), unlike non-allosteric vertebrate LDHs. Bifidobacterium longum l-lactate dehydrogenase is an FBP-dependent allosteric LDH (5, 6). Abundant structural information on the enzyme has been obtained from its crystal structures (7, 8). Four subunits of B. longum l-lactate dehydrogenase are related by three molecular 2-fold axes named P, Q, and R, as for vertebrate LDHs (9). The tetramer has four active sites and two FBP-binding sites. The active site of each subunit lies near the Q-axis interface. On the other hand, the FBP-binding site is at the P-axis interface and is composed of residues from two subunits related by the P-axis. B. longum l-lactate dehydrogenase subunits are not in contact along the R-axis. In the crystal structure (7), two tetramers with different allosteric states are packed into the same crystal lattice with the space group of P222. One tetramer is in a no affinity state (T-state) and the other is in a high affinity state (R-state) to the substrate. The P-, Q-, and R-axes correspond to the three crystallographic 2-fold axes of both T- and R-state tetramers. The crystal structure represents the two symmetric allosteric states, and is an ideal example of the concerted model proposed by Monod et al. (10). Therefore, the proposed allosteric model of B. longum l-lactate dehydrogenase is based on the concerted model (7). However, it remains unclear whether the allosteric symmetry is also maintained in solution.

In the previous paper (11), we reported the mechanism of FBP-dependent heterotropic activation with hybrid enzymes with FBP-sensitive and -insensitive subunits. The hybrid enzymes were obtained by means of an in vivo subunit hybridization system with a plasmid that carries the two B. longum l-lactate dehydrogenase genes tandemly. To separate the mixture of the subunit hybrids by chromatography and electrophoresis, a negatively charged tag was mutationally introduced into one of the pairs of subunits. Kinetic analyses of the hybrid enzymes revealed that the heterotropic activation is transmitted from the FBP-binding site to the active sites of other subunits via the subunit interaction, and thus the results support the validity of the proposed concerted allosteric model (7).

In this study, using the subunit hybridization system constructed previously, we analyzed the mechanism of homotropic activation by the substrate. The mutant B. longum l-lactate dehydrogenase whose Gln-102 residue is replaced with arginine has lost its LDH activity but exhibits malate dehydrogenase (MDH) activity (12), like Bacillus stearothermophilus LDH (13). Using hybrid enzymes comprising the MDH-type Q102R3 subunits. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: LDH, L-lactate dehydrogenase; FBP, fructose 1,6-bisphosphate; MDH, malate dehydrogenase; native PAGE, nondenaturing polyacrylamide gel electrophoresis; L subunit, wild type LDH subunit; M subunit, MDH-type mutant subunit; MES, 2-(N-morpholinoethanesulfonic acid.

2 Residue numbers are given in the N-system (23).

3 Gln-102 was replaced with arginine; similar abbreviations are used as indicated.
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LDH activity was measured using pyruvate as a substrate.

| Hybrid | Vmax (pyruvate) | S0.5 (pyruvate) | nH (pyruvate) | Vmax/S0.5 | Imax S0.5 (pyruvate)
<table>
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<tbody>
<tr>
<td>M4 + FBP</td>
<td>-0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>M3L1 + FBP</td>
<td>200 ± 30</td>
<td>10 ± 3</td>
<td>1.0</td>
<td>20</td>
<td>ND</td>
</tr>
<tr>
<td>M2L2-QR + FBP</td>
<td>590 ± 220</td>
<td>1.6 ± 1.0</td>
<td>1.0 ± 0.3</td>
<td>360</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>M2L2-P + FBP</td>
<td>530 ± 40</td>
<td>1.5 ± 0.2</td>
<td>1.4 ± 0.1</td>
<td>350</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>M1L3</td>
<td>680 ± 9</td>
<td>0.33 ± 0.02</td>
<td>1.7 ± 0.04</td>
<td>2,100</td>
<td>0.42 ± 0.03</td>
</tr>
<tr>
<td>- FBP</td>
<td>84 ± 10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L4 + FBP</td>
<td>1,100 ± 60</td>
<td>0.25 ± 0.05</td>
<td>1.3 ± 0.07</td>
<td>4,400</td>
<td>0.40 ± 0.01</td>
</tr>
<tr>
<td>- FBP</td>
<td>520 ± 10</td>
<td>0.35 ± 0.03</td>
<td>2.4 ± 0.2</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a Values include standard errors on fitting to the Hill equation. 

b Half-saturating concentration of oxamate inhibition, which was determined at 2 mM pyruvate and 0.1 mM FBP.
c ND, not determined.
d Values calculated on fitting to the Michaelis equation.

RESULTS

Hybrid Enzymes Comprising the LDH Subunit and the MDH-type Mutant Subunit—An MDH-type mutant form (M4) of B. longum 1-lactate dehydrogenase whose Gln-102 residue was replaced with arginine lost its LDH activity (Table I), but acquired MDH activity (Table II). Using hybrid enzymes comprising the wild type LDH subunit (L subunit) and the MDH-type mutant subunit (M subunit), we investigated the allosteric interaction between the two types of subunits in one tetramer. In other words, we investigated whether homotropic activation is also transmitted through a quaternary structural change, like the heterotropic activation by FBP (11).

In vivo hybrid formation from the M and L subunits, and separation of the hybrids were carried out by almost the same methods as described previously (11). The negatively charged tag used for hybrid separation was the double replacement of Lys-316 and Arg-317 with glutamic acid and aspartic acid, respectively. The allosteric properties of the charge-tagged enzyme are not affected by these mutations, as reported previously (11). Furthermore, the negatively charged tag causes differences in the total surface charge of the hybrid tetramer with different subunit compositions, and makes it possible to separate hybrid enzymes by anion exchange chromatography and native PAGE (11). The negatively charged tag was introduced into the L subunit, the M and L subunits having mutations Q102R and K316E/R317D, respectively.

Production and Separation of the Hybrid Enzymes—An expression plasmid carrying the genes for the M and L subunits was constructed as described under “Materials and Methods.” The hybrid enzymes produced by E. coli cells were separated into six peaks on anion-exchange chromatography (Fig. 1a), as expected from the previous results (11). The enzymes gave five bands with different mobilities on native PAGE (Fig. 1b, lanes 0). The band intensities were well reflected by the binomial distribution of tetrameric hybrids between the M and L subunits in the molar ratio of 1:9.3 (unlabeled subunit: charge-tagged subunit). This means that random hybridization between the M and L subunits had occurred. The lowest protein bands in lanes 0 in Fig. 1b were not clearly visible because of the lower expression level of the L subunit than that of the M subunit. The five bands at regular intervals arose from five differently charged hybrids. From the profile on chromatography (Fig. 1a) and the mobility on native PAGE (Fig. 1b), the numerical subunit compositions could be determined. Thus, the hybrids in peaks 1, 2, 5, and 6 were named M4, M3L1, M1L3, and L4, respectively (Fig. 1c). The hybrids in peaks 3 and 4 showed the same mobility as that of M2L2 on native PAGE (Fig. 1b, lanes 3 and 4). The ratio of the areas of these peaks on anion-exchange chromatography was about 2:1 (1:8:1 in Fig. 1a). Re-chromatography of each of the hybrids from the two separate peaks only gave a peak at the same position as before. These results of native PAGE and chromatography were almost the same as those for the previously reported hybrid enzymes, D2W2-QR and D2W2-P, similarly obtained by hybridization between the desensitized sub-

MATERIALS AND METHODS

Chemicals—Sodium pyruvate and NADH were purchased from Boehringer Mannheim and Life Technologies Oriental (Tokyo, Japan), respectively. Oxaloacetic acid and sodium oxamate were purchased from Nacalai Tesque (Kyoto, Japan). The enzymes used for DNA manipulation were obtained from Boehringer Mannheim, Takara Shuzo (Kyoto, Japan), and Toyobo Biochemicals (Tokyo, Japan).

Construction of an Expression Vector for in vivo Hybridization—The mutations for K316E/R317D (11) and Q102R (12) in B. longum 1-lactate dehydrogenase were introduced as described previously. The genes for both mutant enzymes were carried by plasmids derived from pUBM9 (14). In the plasmids, a BamHI unique site and a PvuII unique one are present before the promoter and after the terminator, respectively. The expression vector for in vivo hybridization was constructed so as to carry the two B. longum 1-lactate dehydrogenase genes tandemly. The 1.6-kilobase BamHI-PvuII fragment of the charge-tagged K316E/R317D LDH gene was blunt-ended and then inserted into the PvuII site of the expression vector of the Q102R mutant gene.

Production and Separation of the Hybrid Enzymes—The hybrids were produced and separated as described previously (11). At the final step of Mono-Q column chromatography with a linear gradient of 0 to 0.6 M KCl in 60 min, the hybrids were separated on the basis of their charged tags as expected. Nondenaturing polyacrylamide gel electrophoresis (native PAGE) was performed as described previously (11).

Steady-state Kinetics—The standard assay for LDH activity was performed at 30 °C in 50 mM MES-NaOH buffer (pH 6.0) containing 0.2 mM NADH, 5 mM pyruvate, and 0 or 0.1 mM FBP. For MDH activity, 2 mM oxaloacetate was used as the substrate instead of pyruvate. The steady-state kinetic of the hybrids were examined with various concentrations of the substrate, and the Hill equation was used to fit the data to the Hill equation.

where Vmax is the maximum velocity of catalysis, S0.5 is the half-saturating concentration of the substrate, and nH is the Hill coefficient. For hyperbolic saturation curves, the Michaelis equation was used (nH of the Hill equation = 1). For oxamate activation curves, A0.5(oxamate) was calculated instead of S0.5.
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MDH activity was measured using oxaloacetate as a substrate.

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>$V_{\text{max}}$ (oxaloacetate)</th>
<th>$S_{0.5}$ (oxaloacetate)</th>
<th>$n_{H}$ (oxaloacetate)</th>
<th>$V_{\text{max}}/S_{0.5}$</th>
<th>$I_{\text{Oxam}0.5}$ (oxaloacetate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M4</td>
<td>530 ± 30</td>
<td>0.051 ± 0.046</td>
<td>3.4 ± 1.0</td>
<td>10,000</td>
<td>41 ± 3</td>
</tr>
<tr>
<td>M3L1</td>
<td>610 ± 30</td>
<td>0.19 ± 0.06</td>
<td>2.3 ± 0.3</td>
<td>3,200</td>
<td>31 ± 3</td>
</tr>
<tr>
<td>M2L2-QR</td>
<td>490 ± 40</td>
<td>0.36 ± 0.12</td>
<td>1.7 ± 0.3</td>
<td>1,400</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>M2L2-P</td>
<td>500 ± 10</td>
<td>0.35 ± 0.03</td>
<td>1.9 ± 0.1</td>
<td>1,400</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>M1L3</td>
<td>420 ± 50</td>
<td>0.81 ± 0.25</td>
<td>1.2 ± 0.2</td>
<td>520</td>
<td>0.25 ± 0.04</td>
</tr>
<tr>
<td>L4</td>
<td>430 ± 90</td>
<td>4.1 ± 1.2</td>
<td>1.0</td>
<td>100</td>
<td>0.13 ± 0.01</td>
</tr>
</tbody>
</table>

$^a$ Values include standard errors on fitting to the Hill equation.
$^b$ Half-saturating concentration of oxamate inhibition, which was determined at 2 mM oxaloacetate and 0.1 mM FBP.
$^c$ Values calculated on fitting to the Michaelis equation.

a unit (D) and the wild-type subunit (W) of B. longum L-lactate dehydrogenase (11). Thus, the hybrids in peaks 3 and 4 were named M2L2-QR and M2L2-P (Fig. 1c), and assigned as “isomers” with different structural arrangements. M2L2-P contained two M subunits along the molecular P-axis, and M2L2-QR was a mixture of two other hybrids with two M subunits along the Q- and R-axes. The structural assignment of D2W2-QR and D2W2-P was carried out on the basis of the FBP sensitivities of the hybrids (11), because the FBP-binding sites are located at the P-axis interface and one site is composed of residues from two subunits related by the P-axis (7).

Kinetic Properties of the MDH-type Mutant and MDH-LDH Hybrid Enzymes—From the saturation curves for both LDH (pyruvate as a substrate) and MDH (oxaloacetate as a substrate) activities, $V_{\text{max}}, S_{0.5}$, and $n_{H}$ (the Hill coefficient) of the hybrid enzymes were calculated, and are summarized in Tables I and II, respectively. $V_{\text{max}}$ (pyruvate), $S_{0.5}$ (pyruvate), and $n_{H}$ (pyruvate) are parameters for LDH activity, and $V_{\text{max}}$ (oxaloacetate), $S_{0.5}$ (oxaloacetate), and $n_{H}$ (oxaloacetate) are for MDH activity. In the absence of FBP, the LDH and MDH activities were both very weak for all hybrids (data not shown) but L4 and M1L3 (Table I). L4 and M1L3 exhibited weak LDH activities, but their $S_{0.5}$ (pyruvate) were large. In the presence of FBP, L4 and M4 exhibited high LDH and MDH activities, which were their canonical activities, respectively. As for non-canonical activity, M4 exhibited no LDH activity, but L4 had considerable MDH activity. $V_{\text{max}}$ (oxaloacetate) of L4 was almost the same as that of M4, but $S_{0.5}$ (oxaloacetate) was about 100 times different between the two enzymes, i.e., 4.1 and 0.051 mM, respectively. These results indicate that pyruvate can bind only to the L subunit, and that oxaloacetate can bind strongly to the M subunit but weakly to the L subunit.

Oxamate, which is a non-reactive analog of pyruvate, strongly inhibited L4 ($I_{\text{Oxam0.5}0.5}$ (pyruvate) = 0.4 mM and $I_{\text{Oxam0.5}0.5}$ (oxaloacetate) = 0.13 mM), but only slightly inhibited the MDH activity of M4 ($I_{\text{Oxam0.5}0.5}$ (oxaloacetate) = 41 mM), indicating that oxamate can bind to the active site of the L subunit, but not to that of the M subunit.

$n_{H}$ (oxaloacetate) of M4 was 3.4, exhibiting strong cooperativity as a tetramer. As the number of the M subunits decreased, $n_{H}$ (oxaloacetate) decreased, approaching 1. However, $n_{H}$ (pyruvate) of L4 was 1.3. The allosteric equilibrium of M4 may have shifted greatly toward the inactive T-state compared with L4, probably because of the mutation in the active site.

Inter-subunit Homotropic Activation—In the absence of FBP, the MDH activities (at 2 mM oxaloacetate) of the hybrid enzymes were measured with the addition of oxamate (Fig. 2a), which can only bind to the L subunit. In this measurement, the L and M subunits act as “a regulatory subunit” and “a catalytic subunit,” respectively. The L subunit possibly has MDH activity in this condition, but, as shown in Table II, $S_{0.5}$ (oxaloacetate) of L4 (4.1 mM) was sufficiently larger than $I_{\text{Oxam0.5}0.5}$ (oxaloacetate) of L4 (0.13 mM). Therefore, the MDH activity of the L subunits seemed to be ignored owing to the inhibition by oxamate. The hybrids, which consisted of both M and L subunits (M3L1, M2L2-QR, M2L2-P, and M1L3), were...
activated on the addition of oxamate. On the other hand, M4 and L4 were not activated. These results indicate that the homotropic activation mimicked by oxamate bind to the L subunit is transmitted to the M subunit in an inter-subunit manner.

Table III shows the parameters characterizing these oxamate activation curves (Fig. 2a), which were obtained using the Hill equation (15). M3L1 gave a hyperbolic Michaelis-type curve \( n_{H}(\text{oxamate}) = 1 \) since it had only one oxamate-binding L subunit. As the number of the L subunits in the hybrids increased, \( n_{H}(\text{oxamate}) \) increased (1.2 for M2L2-QR and M2L2-P, and 2.1 for M1L3), and the enzymes gave sigmoidal activation curves. Conversely, \( A_{0.5}(\text{oxamate}) \) decreased as the number of the L subunits increased. \( V_{\text{max}}(\text{oxamate}) \) should be proportional to the number of the M subunits. \( V_{\text{max}}(\text{oxamate}) \) of M2L2-QR and M2L2-P were about two times as large as that of M3L1, but M3L1 showed lower \( V_{\text{max}}(\text{oxamate}) \) than those of the two M2L2 hybrids. This contradiction seems to be a consequence of oxamate inhibition of the M subunit with high concentrations of oxamate. Because \( A_{0.5}(\text{oxamate}) \) of M3L1 was larger than that of the other hybrids, over 40 mM oxamate was needed for saturation, at which the M subunit was inhibited, showing only about half the maximum activity \( (I_{\text{oxam} 0.5}(\text{oxaloacetate}) \) of M4 = 41 mM in Table II).

These results were different from those of the former study on hybrids of the MDH-type \( B. \ longum \) l-lactate dehydrogenase (12). In that study, the M2L2 hybrid could not be separated into two peaks (M2L2-QR and M2L2-P), and the M3L1 hybrid was not activated on the addition of oxamate. The separation tags (two charged residues) added at the C-terminals of both the M and L subunits seem to have caused a structural change, affecting the allosteric transition. Therefore, in our previous study (11), the tag position was chosen so as not to affect the allosteric properties of \( B. \ longum \) l-lactate dehydrogenase, and accompanying steric effects enabled M2L2 isomers to be separated.

**Identical Allosteric Properties of the Isomeric Hybrids—**

M2L2-QR and M2L2-P have the same subunit composition, but their structural arrangements are different, as illustrated in Fig. 1c. Interestingly, however, their kinetic properties were identical in every condition we examined here. Not only with various oxamate concentrations and a constant oxaloacetate concentration (Fig. 2a), but also with various oxaloacetate concentrations and constant (10 and 50 mM) oxamate concentrations (Fig. 2b), M2L2-QR and M2L2-P showed the same activity, within the limit of experimental error at every measured point. The parameters for the inter-subunit homotropic activation (Table III) as well as those for the substrate saturation curves of the LDH and MDH activities (Tables I and II) were all very similar between M2L2-QR and M2L2-P. This finding that hybrid enzymes with different structural arrangements were kinetically identical strongly suggests the symmetric allosteric activation of the enzyme.

**DISCUSSION**

To analyze the homotropic activation using hybrid enzymes, oxamate, a substrate analog and an inhibitor of LDH, was used as an activator in this study, because it can only bind to the L subunit in the R-state. In the absence of FBP and pyruvate (or oxamate), practically all molecules of \( B. \ longum \) l-lactate dehydrogenase are in the T-state (7). Oxamate bound to the L subunit shifts the allosteric equilibrium toward the R-state, and thus activates the L subunit. The results of this study demonstrated that the activation was transmitted to the M subunit from the L subunit via subunit interaction. In our previous paper (11), the heterotropic activation by FBP was shown to be transmitted via inter-subunit communication, using hybrid enzymes with FBP-sensitive and -desensitized subunits. Here, we showed a similar activation mechanism for the homotropic activation by substrate analog.

Detailed quantitative analyses including determination of the allosteric constant were not possible because the strong substrate inhibition of \( B. \ longum \) l-lactate dehydrogenase (11) prevented exact curve fitting. Comparison of the kinetic parameters among the hybrid enzymes, however, provided strong support for the concerted allosteric model of \( B. \ longum \) l-lactate dehydrogenase. As the number of the L subunits in the hybrids increased, \( n_{H}(\text{oxamate}) \) increased and \( A_{0.5}(\text{oxamate}) \) decreased (Table III). The change in \( n_{H}(\text{oxamate}) \) indicates the greater cooperativity of the hybrids with more L subunits which can bind oxamate. It is apparent from \( A_{0.5}(\text{oxamate}) \) that a smaller amount of oxamate can saturate the hybrid enzymes with more L subunits, because the hybrid enzymes with more
L subunits can bind more oxamate and the cooperativity of the active sites promotes oxamate saturation. These tendencies of the kinetic parameters agree with the concerted allosteric model (10).

In the FBP-induced heterotropic activation (11), we could not observe the P-axis related interaction because the FBP-binding site is located at the P-axis interface. In this study, however, the use of an active-site mutant made it possible to reveal the inter-subunit communication at the P- and Q-axis interfaces. Because the enzyme has no R-axis related interaction, the activation of M2L2-P represents the interaction at the Q-axis interface. Similarly, the activation of M2L2-QR corresponds to the sum of the interactions at the P- and Q-axis interfaces. The structural features of the P- and Q-axis interfaces are different (7), but their contributions to the homotropic activation were concluded to be the same, because if either the P-or Q-axis related dimer was the strongly linked cooperative unit, the homotropic activation of M2L2-P and M2L2-QR would not be identical. Comparison between hybrids M2L2-P and M2L2-QR with different structural arrangements confirmed the concerted activation of the enzyme. Because the structural “isomers,” M2L2-QR and M2L2-P, showed identical activation, the cooperative unit of the enzyme seems to be a whole tetramer, and the allosteric symmetry of the tetramer should be maintained during a shift of the equilibrium. Thus, the allosteric state of the enzyme should be either the T-state tetramer or the R-state tetramer.

On the basis of the results obtained on analyses of the hybrid enzymes in this study and the previous one (11), together with the knowledge of the crystal structures (7), we can describe a more detailed allosteric model of B. longum l-lactate dehydrogenase, like the “differential gear-model” of aspartate transcarbamylase (16). In Fig. 3, the P- and Q-axis interfaces are schematically represented as indents with different shapes. The allosteric transition between the T- and R-states involves the rotation of each subunit (7). His-68 causes steric hindrance at the active site of the next subunit in the T-state, but the subunit rotation abolishes the hindrance between the Q-axis related dimers. The allosteric cooperativity originates from the equilibrium between the T- and R-state tetramers, and the allosteric transition mechanism can explain both the homotropic cooperativity and the heterotropic activation, as described for the concerted model (10). For homotropic activation, the substrate bound to the active site shifts the equilibrium toward the R-state. Hence, the substrate saturation curve becomes sigmoidal. For heterotropic activation, FBP bound to the effector site also shifts the equilibrium toward the R-state. The phosphate moiety of FBP neutralizes the positive charge repulsion at the P-axis interface, and stabilizes the R-state in which the subunits at the P-axis interface slightly approaches each other (0.5 Å closer at the FBP-binding site) than in the T-state. The subunit interactions at the P- and Q-axis interfaces link the structural change and maintain the allosteric state of the tetramer symmetric.

Subunit interactions also play an important role in allosteric transitions in the cases of other well studied allosteric proteins (16). In particular, the communication between the catalytic subunits of hybrid aspartate transcarbamylase has been demonstrated: the effects of ligand binding are propagated to chemically introduced chromophores on the other catalytic subunits via the regulatory subunits (17). In the case of B. longum l-lactate dehydrogenase, each of the four subunits rotates as a rigid unit on the allosteric transition, and this is a rare and the most simple example of the concerted model in terms of the structural change when compared with well studied tetrameric allosteric proteins (18). In tetrameric allosteric proteins like hemoglobin (16), phosphofructokinase (16), and fructose-1,6-bisphosphatase (19), the transition is based on the rotation of one dimer with respect to the other, in such a way that the intersubunit contacts within each of the two rotating bodies are preserved. In studies involving hybrid formation systems of partially ligated hemoglobin subunits, cooperative free energy for the specific “microstate” isomers has been dissected, and cooperativity between the α and β hemes of each dimeric half-molecule has been revealed (20).

It is an interesting question as to how the symmetry of the allosteric transition is general among various allosteric proteins. In the crystal structures of allosteric pyruvate kinase (21), the tetramer exhibits 222 subunit symmetry, and rotation of the domains in the subunit controls the activity. However, the symmetry of the allosteric transition in solution is unknown. The cooperative unit of the insulin hexamer is a trimer, and the two trimers undergo changes in their allosteric states independently (T6-T3R3-R6) (22). Therefore, the protein exhibits unique negative homotropic cooperativity. Like the B. longum l-lactate dehydrogenase studies reported here and in Ref. 11, the mutational subunit hybridization technique can also provide information on the allosteric symmetry in solution for other proteins, if a proper tag mutation is introduced so that the structural isomers can be separated.

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Homotropic Activation and Symmetry of l-Lactate Dehydrogenase

Homotropic Activation via the Subunit Interaction and Allosteric Symmetry Revealed on Analysis of Hybrid Enzymes of l-Lactate Dehydrogenase
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