The Reactivity of Ferricytochrome c with Ionic Ligands*

PHILIP GEORGE, STANLEY C. GLAUSER,† AND A. SCHEJTER‡

From the John Harrison Laboratory of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19140

SUMMARY

The reactions of ferricytochrome c with anionic ligands involve the fission of one of the bonds to basic groups that hold the heme firmly within a crevice in the polypeptide structure. Denoting the group by X, the reactions thus take the form

\[ P^r_\text{Fe}^+X + L^- \rightleftharpoons P^r_\text{Fe}L + X^- \]

where \( L \) is ligand and \( P^r \) is the protein liberated by the incoming ligand. With ferricytochrome c, the \([H^+]\) dependence of the equilibrium constant for complex formation is determined by the ionization equilibrium of the ligand species \( HL \rightleftarrows H^+ + L^- \), the coordinated water molecule \(-Fe^+H_2O \rightleftarrows FeOH + H^+\), and the nitrogenous base group which is liberated \(-2H^+ \rightleftarrows X + H^+\). Studies of the equilibrium constants for the formation of the azide and cyanide complexes, over the pH range 4 to 8, show that there is a change in the \([H^+]\) dependence for both reactions at about pH 5. At 26° and ionic strength of 0.03, the following values were found: \( pK_{XH^+} = 4.98 \) with \( \Delta H = +9.2 \) kcal per mole and \( \Delta S = +8 \) e.u. This indicates that the bond to the Fe, which is broken in complex formation, involves the glyoxalinium nitrogen atom of a histidine residue. The stability constants for the formation of the closed crevice form of ferricytochrome c at 26° and ionic strength of 0.03 are \( \Delta F = -3.5 \) kcal per mole, \( \Delta H = -18.0 \) kcal per mole, and \( \Delta S = -48 \) e.u. At higher ionic strengths, deviations were observed from the pH dependence noted above. An extension of the mathematical analysis to include another group capable of accepting a proton leads to an expression for the equilibrium constant of the same form, and, on this basis, calculation shows that the pK would be about 4.4.

It was shown in a previous publication (1) that the pH dependence of the equilibrium constants for the reaction of ferricytochrome c with cyanide and azide enabled the protein residue liberated upon complex formation to be identified as one of the histidine imidazoles. In this paper, the experimental evidence is presented in detail and it is also shown that under conditions of high ionic strength the pH dependence is significantly different from that at lower salt concentrations. Finally, the thermodynamic factors responsible for the maintenance of the ferrihemochrome structure of ferricytochrome c are discussed in terms of the present results.

EXPERIMENTAL PROCEDURE

Materials and Methods

Cytochrome c was prepared by trichloroacetic acid extraction and precipitation (2) and further purified by chromatography on Amberlite CG-50 (3). Only the fraction eluted with 0.2 M ammonium acetate was used to avoid contamination with cytochrome c dimers and higher polymers (4). All chemicals used were of best analytical grade. Spectrophotometric observations were made on a Beckman DU spectrophotometer, pH values were determined with a glass electrode by means of a Beckman model G potentiometer.

Study of Azide Reaction—This was performed at a wave length of 408 mp. Four spectrophotometric cells were used. Cells 1 and 2 contained 2.5 ml of the buffer; Cells 3 and 4 had 2.4 ml of the buffer plus 0.1 ml of cytochrome c. A spectrophotometric titration was carried out by adding 0.1 ml aliquots of a stock solution of sodium azide to Cells 2 and 4 and measuring the absorbance of all four cells. Because of the small absorbance change accompanying the formation of the complex, two types of readings were made. In the first the absorbance of Cells 2, 3, and 4 was corrected for dilution, and the absorbance increments for the formation of the complex, calculated for each set of data, were usually in agreement to within 0.002 unit.

Because of the hydrolysis of sodium azide, a concentrated buffer solution was needed and an ionic strength of 1.0 was adopted for all the titrations. Sodium cacodylate and hydrochloric acid buffer were used for the pH range 5.5 to 7.0 and acetate buffer for the range 4.0 to 5.3.

Study of Cyanide Reaction—Since the reactions of ferricytochrome c with HCN and CN⁻ are very slow (5, 6), it was impracticable to titrate a single sample of ferricytochrome c. Instead, several solutions of the hemoprotein at a given pH and ionic strength containing varying amounts of KCN in phosphate or acetate buffers were incubated for 24 hours at 26°. For experiments at 6°, longer equilibration periods of 48 and 72 hours were necessary. Because of the high volatility of HCN,
the samples were either kept in stoppered syringes (5) or in small test tubes stopped with cellophane-wrapped corks so that no gas phase was left in contact with the solution.

Having reached equilibrium, the absorbances of the complex were read at 424 nm against blanks containing similar concentrations of cytochrome c and buffer but no cyanide. This was done in order to accentuate the difference in the extinction coefficients of ferricytochrome c and its cyanide complex (7) which is small even at the optimal wave length chosen. In all of the experiments a cytochrome c concentration of about 20 μM was used, giving an optical density increment of about 0.4 for complete formation of the complex. Under the experimental conditions that gave the highest value of $K_{\text{obs}}$, namely pH 6.80 and 26°C, the concentration of KCN necessary for 50% complex formation was about 550 μM. Even this is far in excess of the total cytochrome c concentration; thus it could be assured throughout that the equilibrium cyanide concentration was identical with the total cyanide concentration.

### Theory

#### Calculation of Equilibrium Constants

The reactions under consideration can be represented as shown in Reactions 1 and 2.

Ferricytochrome c + ligand $\rightleftharpoons$ ferricytochrome c - ligand (1)

with an equilibrium constant

$$K_{\text{obs}} = \frac{[\text{ferricytochrome c} - \text{ligand}]}{[\text{ferricytochrome c}] \times [\text{lig}]} (2)$$

where [lig] stands for the concentration of free ligand at equilibrium.

#### Table I

<table>
<thead>
<tr>
<th>pH</th>
<th>$K_{\text{obs}}$ (°C)</th>
<th>$K_{\text{obs}}$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.80</td>
<td>1.17</td>
<td>1.81</td>
</tr>
<tr>
<td>6.18</td>
<td>0.372</td>
<td>0.93</td>
</tr>
<tr>
<td>5.60</td>
<td>0.142</td>
<td>0.30</td>
</tr>
<tr>
<td>4.74</td>
<td>0.075</td>
<td>0.078</td>
</tr>
<tr>
<td>4.30</td>
<td>0.065</td>
<td>0.067</td>
</tr>
<tr>
<td>4.10</td>
<td>0.063</td>
<td></td>
</tr>
</tbody>
</table>

Reaction 1 implies that only one of the two bonds linking the iron to nitrogenous base groups of the protein is broken, the ligand taking the place of this group to give a structure formally similar to that of the ferrimyoglobin and ferrihemoglobin complexes with cyanide, azide, fluoride, etc. In principle, the second bond could also be broken, giving the complex ferricytochrome c-(ligand)$_2$, and it has been suggested that at very low pH 2 chloride ions combined in this way (8). However, the data reported below for the formation of the cyanide and azide complexes fit Equation 2 within experimental error, indicating that only the formation of a 1:1 complex is important over the pH range of 4 to 7. The reaction of a 2nd ligand molecule will not therefore be considered further in the present paper. In terms of the spectrophotometric readings, $K_{\text{obs}}$ as defined by Equation 2 is given by

$$K_{\text{obs}} = \frac{A' - A_c}{A_w - A'} \times \frac{1}{[\text{lig}]} (3)$$

where $A_c$ is the initial absorbance, with no added ligand; $A_w$ is the absorbance for complete formation of the complex; and $A'$ is the absorbance at an intermediate ligand concentration.

In our experiments, the value of $A' - A_c$ was determined by reading a buffered solution of ferricytochrome c containing 0.1 M KCN against the cytochrome c blank, and, since the value of $A'$ could not be determined accurately, the following equation derived from Equation 3 was used:

$$K_{\text{obs}} = \frac{(A_w - A_c)}{(A_c - A') - (A' - A_c)} \times \frac{1}{[\text{lig}]} (3')$$

which expresses $K_{\text{obs}}$ in terms of the difference between absorbances rather than the absolute quantities. Values of $K_{\text{obs}}$ obtained in this way are listed in Table I.

In the case of the azide complex, the low values of the equilibrium constants made it impossible to determine $A'$ or $(A' - A_c)$ directly, and an extrapolation procedure was employed.

Equation 3 can be rearranged to give

$$A' = A_c - (A' - A_c) \times \frac{1}{[\text{lig}]} (4)$$

Hence a plot of $A'$ against $(A' - A_c)/[\text{lig}]$ should be linear with a slope equal to the reciprocal of the equilibrium constant. A typical plot of this kind is shown in Fig. 1 and values of $K_{\text{obs}}$ obtained by this method are given in Table II.

#### Theoretical Equations for $K_{\text{obs}}$

In order to determine the pK of the group in the protein liberated by the incoming ligand, the following reactions have to be considered (9).

1. $Pr-Fe^+(H_2O) \xrightarrow{K_{\text{Fe}}} Pr-Fe^+ X + H_2O$ (5)
2. $Pr-Fe^+(H_2O) \xrightarrow{K_{\text{Fe}}} Pr-FeOH^- X + H^+$ (6)
3. $Pr-Fe^+(H_2O) \xrightarrow{K_{\text{Hx}}} Pr-Fe^+(H_2O) X + H^+$ (7)
4. $Pr-Fe^+(H_2O) \xrightarrow{K_{\text{ML}}} Pr-FeL^- X + H_2O$ (8)
5. $Pr-Fe^+(H_2O) \xrightarrow{K_{\text{ML}}} Pr-FeL^- X + H_2O$ (9)
TABLE II  

<table>
<thead>
<tr>
<th>Temperature</th>
<th>pH</th>
<th>$K_{\text{obs}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>25°C</td>
<td>4.0</td>
<td>76.0</td>
</tr>
<tr>
<td></td>
<td>4.3</td>
<td>37.5</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>25.6</td>
</tr>
<tr>
<td></td>
<td>4.7</td>
<td>13.8</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>5.8</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>4.9</td>
</tr>
</tbody>
</table>

where $P_r$ is the protein group binding position 5 of the iron-porphyrin and $X$ is the crevice-forming protein group. As appears later, these reactions make up the simplest reaction scheme of this type. Reaction 5 represents the equilibrium between the open and closed forms of the crevice, Reaction 6 the ionization of the iron-bound water molecule, and Reaction 7 the ionization of the crevice-forming protein group when it is not bonded to the iron. Reactions 8 and 9 represent the formation of the complex with the ligand $L$ by the open crevice form of the hemoprotein. The crevice-forming group in the protein is in its conjugate base and conjugate acid form, respectively, and it is assumed that the thermodynamic reactivity of the iron atom is unaffected by this ionization, the equilibrium constant in both cases being denoted by $K_{ML}$. The equilibrium concentration of the dissociated form of the ligand, $[L^-]$, is related to the total concentration of the free ligand, $[lig]$, by the equation

$$[L^-] = [lig] \times \frac{K_{HL}}{[H^+] + K_{HL}} \quad (10)$$

where $K_{HL}$ is the ionization constant of $HL$.

On the basis of these reactions and Equation 10, the following expression can be derived for $K_{\text{obs}}$ (16):

$$K_{\text{obs}} = K_{ML} \times K_{HL} \times \frac{[H^+] \times ([H^+] + K_{HL})}{[H^+] + [H^+] + [H^+] + [H^+] + K_{HL}K_{K_H} + K_{HL}K_{K_H}} \quad (11)$$

This derivation does not include any approximations; however, over the pH range of the present experiments, pH 4 to 8, the following considerations show that two of the terms in the denominator quadratic in $[H^+]$ may be neglected, giving a simplified equation that is amenable to graphical analysis.

The data in Tables I and II indicate a change in the pH dependence of $K_{\text{obs}}$ at about pH 5; hence $K_{HX}$ is about $10^{-6}$ M. $K_{pr}$ probably has a value of about $10^{-8}$ M in view of the similar ionizations of ferrimyoglobin and ferrihemoglobin (11, 12), so $K_{pr}K_{HX} = 10^{-14}$ M$^2$. Thus, provided that $K_{cr}$ is greater than about $10^3$,

$$[H^+]^3 < [H^+]K_{HX}K_{cr} < K_{pr}K_{HX} \quad (12)$$

and over the pH range 4 to 8, Equation 11 reduces to

$$K_{\text{obs}} \times \frac{[H^+] + K_{HL}K_{K_H}K_{K_H}}{K_{ML}K_{HL}} = K_{ML} + K_{ML}[H^+] + K_{ML}[H^+]^2 \quad (13)$$

A plot of the function $K_{\text{obs}} \times ([H^+] + K_{HL})/K_{HL}$ against $[H^+]$ should thus give a straight line, allowing $K_{HX}$ to be calculated from the slope and intercept. It is shown later under "Discussion" that this assumption regarding a minimum value of $K_{cr}$ is justified.

If in addition to Reactions 5 to 9 there is another group in the protein the ionization of which is normally prevented in ferricytochrome c for steric reasons, but which becomes operative when the crevice is opened by complex formation, then Reactions 5 and 7 need elaborating as follows:

$$Pr-Fe^{+}(H_2O) \quad XH^+ \quad C + H_2O \quad (14)$$

$$Pr-Fe^{+}(H_2O) \quad XH^+ \quad CH^+ \quad (15)$$

$$Pr-Fe^{+}(H_2O) \quad XH^+ \quad CH^+ \quad (16)$$

The equation for $K_{\text{obs}}$, corresponding to Equation 13 for the simpler case is now (10):  

$$K_{\text{obs}} \times \frac{[H^+] + K_{HL}K_{K_H}K_{K_H}}{K_{ML}K_{HL}} = a_0 + a_1[H^+] + a_2[H^+]^2 \quad (17)$$

which can be rewritten in the form

$$K_{\text{obs}} \times \frac{([H^+] + K_{HL})}{K_{ML}K_{HL}} = a_0 + a_1[H^+] + a_2[H^+]^2 \quad (18)$$

From a series of experimental data, $a_0$, $a_1$, and $a_2$ can be obtained by the method of least squares: $K_{HX}$ is then given by $a_0/a_2$ and $K_{HC}$ by $a_1/a_2$. It is to be noted that Equations 14 to 16 imply that $K_{HC} > K_{HX}$, whereas these ionization constants might be very similar in magnitude. A more rigorous derivation would require that all the possible pathways for the ionization of

$$Pr-Fe^{+}(H_2O) \quad XH^+ \quad CH^+ \quad (19)$$

be taken into account, specifying the intrinsic ionization constants. However, an analysis of this kind would require independent experimental data that are not available and the only value that can be obtained, with the use of Equation 17, are the macroscopic ionization constants.

Even so, Equation 17 cannot be regarded as a unique solution for the case of an additional ionizing group sequestered in the crevice. In the scheme of Reactions 14 to 16 and 6 to 9, Reaction 14 supposes that the group is sequestered only in the form of its conjugate base, and as a consequence the ionization of the group makes no contribution to the stability of the crevice structure in the range pH $> pK_{HC}$, because in the open structure it is already present almost entirely in its conjugate base form. On the other hand, if it was sequestered only in the form of the conjugate acid, a different equation would result because Reaction 14 would be replaced by
and the formation of the crevice structure would be progressively more favored with decreasing pH.

Furthermore, if the ionizing group could be sequestered both as the conjugate base and as the conjugate acid, although the crevice structure itself would not (by definition) engage directly in an ionization equilibrium, an equilibrium would nevertheless be set up through the mediation of the open structures. Presumably different values for $K_a$ would have to be assigned to the two crevice formation reactions, and the apparent ionization constant for the group in the crevice would be given by $K_a$ multiplied by the ratio of these crevice constants. The corresponding equation for $K_{obs}$ would be much more complicated and analysis for the various constants could not be made by any simple graphical procedure.

These considerations of the role of an ionizing group sequestered in the crevice also necessitate a re-evaluation of the first reaction scheme set out above. It is apparent that even if experimental data conform to Equations 11 and 13 there would in principle be an alternative reaction scheme in which an ionizing group sequestered in the protein as the conjugate base was responsible for the observed pH dependence of $K_{obs}$ instead of the group liberated from the iron atom. This point is taken up below, after presenting the results for the ferrocyanochrome c reactions.

### RESULTS

#### Results at Low Ionic Strength

The data obtained for the reaction with cyanide at low ionic strength (Table I) were plotted according to Equation 13. Fig. 2 shows two plots, for different temperature. Dividing the slopes by the intercepts, the values of $K_{HX}$ were obtained, and are listed in Table III as the $pK$.

Assuming that the Van't Hoff isochore equation is applicable, the standard enthalpy of ionization is found to be 9.2 kcal per mole. Provided that the extrapolation of $pK$ to zero ionic strength involves only a small increment, from the value of the $pK$ adjusted for 25° an approximate value of 7.0 kcal per mole is obtained for the standard free energy of ionization with the use of the equation $\Delta F^o = -RT \ln K$. An approximate value for the standard entropy of ionization of 8 e.u. then follows from the relationship $\Delta F^o = \Delta H^o - T \Delta S^o$.

#### Results at High Ionic Strength

In this case, it was necessary to know the value of $K_{HN}$, at $\Gamma/2 = 1.0$, and this was estimated by measuring the pH of half neutralized solutions of sodium azide; the values obtained are listed in Table IV. Assuming that the Van’t Hoff isochore equation is applicable, the standard enthalpy of ionization is found to be 9.2 kcal per mole. Provided that the extrapolation of $pK$ to zero ionic strength involves only a small increment, from the value of the $pK$ adjusted for 25° an approximate value of 7.0 kcal per mole is obtained for the standard free energy of ionization with the use of the equation $\Delta F^o = -RT \ln K$. An approximate value for the standard entropy of ionization of 8 e.u. then follows from the relationship $\Delta F^o = \Delta H^o - T \Delta S^o$.

#### Table V

<table>
<thead>
<tr>
<th>$a_0$</th>
<th>$a_1$</th>
<th>$a_2$</th>
<th>$pK_{HX}$</th>
<th>$pK_{HC}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.588 X 10^-3</td>
<td>2.035 X 10^3</td>
<td>4.908 X 10^3</td>
<td>4.62</td>
<td>4.36</td>
</tr>
<tr>
<td>3.879</td>
<td>5.857 X 10^6</td>
<td>1.764 X 10^-10</td>
<td>5.18</td>
<td>4.48</td>
</tr>
</tbody>
</table>

### Table III

<table>
<thead>
<tr>
<th>Ionization constants for Fe-linked nitrogenous group of ferrocyanochrome c at ionic strength of 0.03</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temperature</strong></td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>6°C</td>
</tr>
<tr>
<td>25°C</td>
</tr>
</tbody>
</table>

### Table IV

<table>
<thead>
<tr>
<th>pH values of half-neutralized solutions of NaN₃ at ionic strength of 1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temperature</strong></td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>2°C</td>
</tr>
<tr>
<td>25°C</td>
</tr>
</tbody>
</table>
TABLE VI
Values for pK of imidazole group in molecules of increasing basicity

<table>
<thead>
<tr>
<th>Compound</th>
<th>pK of imidazole group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidazole</td>
<td>7.0</td>
</tr>
<tr>
<td>Histidine</td>
<td>6.0</td>
</tr>
<tr>
<td>Histidylglycine</td>
<td>5.8</td>
</tr>
<tr>
<td>Histidylhistidine</td>
<td>5.6</td>
</tr>
</tbody>
</table>

FIG. 4. Observed values for the formation constant of the cyanide complex of ferricytochrome c (K_{obs}) and a theoretical curve with the use of Equation 18 and the values of a_0, a_1, and a_2 listed in Table V. Ionic strength, 1.0; temperature, 25°.

FIG. 5. Observed values for the function K_{obs} × [H^+] / K_{HL} for the formation of the azide complex of ferricytochrome c, with the use of Equation 18 and the values of a_0, a_1, and a_2 listed in Table V. Ionic strength, 1.0; temperature, 25°.

K_{obs} × [H^+] = a_0 K_{HL} + a_1 K_{HL} [H^+] + a_2 K_{HL} [H^+]^2 (18')

The new coefficients all contain K_{HL} as a common term, and the ratios which give K_{HX} and K_{HC} are therefore unaffected by its magnitude. The values obtained for K_{HL} and K_{HC} from the cyanide reaction data at Γ/2 = 1.0, as can be seen in Table V, are in satisfactory agreement with those calculated from the azide reaction data.

DISCUSSION

Cytochrome c has a closed crevice structure in which the iron atom is octahedrally coordinated to the 4 nitrogen atoms of the porphyrin ring and two basic groups on the polypeptide chain (13). As yet, there is no direct evidence for the identification of the amino acid residues involved, but spectrophotometric and acidimetric titrations have been interpreted as indicating that both groups are histidine imidazoles (14) or one imidazole and one ε-amino group of lysine (15). The reaction of ferricytochrome c with ligands such as cyanide and azide in which the 1:1 complex is formed requires the cleavage of one of these bonds, and in the above analysis of the equilibrium constant data it has been tacitly assumed that the nitrogenous base group liberated is free to accept a proton in an approximate pH region and not sequestered once again within the protein structure.

The values of pK_{HX} and the standard enthalpy and entropy of ionization listed in Tables III and V can be attributed to the dissociation of a histidine imidazole since this is the only nitrogenous base group among the amino acid side chains that has ionization characteristics of this kind. The pK value is rather lower than might have been expected, but this could be attributed to the influence of its immediate structural environment. In Table VI, the pK for the ionization of the imidazole group is listed for a series of compounds in order of increasing net positive charge on the conjugate acid (16-18). The pK is lower the greater the positive charge. Cytochrome c is a very basic molecule, with an isoelectric point of 10.65 (19), and it is not surprising therefore that the imidazole group in question has a pK lower than normal.

Recent studies on the carboxymethylation of ferric cytochrome c and its cyanide complex appear to indicate that histidine imidazoles are not made available to alkylation when the iron-protein bonds of native cytochrome c are broken by complex formation (20). It is possible, however, for an imidazole group to be available for protonation while the access of an alkylating agent is sterically hindered.

Equation 13, derived on the assumption that the ionization of HX is the only pH-dependent variable in the range of acidity investigated, was not satisfied by the results obtained at high ionic strength. However, Equation 17, which takes into account the possibility of an additional ionization operating in K_{obs}, was fully satisfied by both the azide and cyanide data. The agreement between the experimental results and the theoretical curves calculated on the basis of Equation 17 is shown in Figs. 4 and 5. The value of pK_{HC}, which could only be obtained at the high ionic strength of 1.0, is only about 0.5 pH unit lower than the pK_{HC}. However, since the thermodynamic parameters for an ionization can be appreciably altered at ionic strength as high as 1.0, an attempt to identify the group on the basis of known data can not be made because these data almost invariably refer to much lower ionic strength conditions.

The physical meaning of the equations from which Equation 17 was derived is that the reactivity of the iron is affected by the ionization of a group not involved in iron coordination. Long range effects of this kind are known to exist in another hemo-

TABLE VII
Thermodynamic parameters for stability constant for crevice-closed form of ferricytochrome c at 25°

<table>
<thead>
<tr>
<th>∆F</th>
<th>∆H</th>
<th>∆S</th>
</tr>
</thead>
<tbody>
<tr>
<td>kcal/mole</td>
<td>kcal/mole</td>
<td>e.u.</td>
</tr>
<tr>
<td>-3.5</td>
<td>-18.0</td>
<td>-48</td>
</tr>
</tbody>
</table>

Downloaded from http://www.jbc.org/
protein; thus, in ferric hemoglobin, a single amino acid substitution resulting in a change of charge at a point located 30 A away from the iron atom significantly affects the reactivity of the latter (21). The appearance of this effect at high ionic strength may be a result of changes in the conformation of the protein chain brought about by high salt concentrations. In relation to that, it is to be noted that the reactivity of the cytochrome c iron is markedly dependent on the ionic strength, as shown by the effect of salt concentration on the rates of enzymic (22) and nonenzymic (23) oxidation of ferrocytochrome c.

In an earlier paper (24), the thermodynamic parameters for similar reactions of cytochrome c and other hemoproteins with ionic ligands were compared, and it was shown that coordination of the cytochrome c iron with cyanide was favored by a large positive entropy change. 2.

The unfavorable $\Delta S$ term must arise from the constraints imposed on the protein conformation by the rigidity of a closed crevice structure. Thus the degrees of freedom lost when the crevice is closed are recovered when complex formation causes its breakage, as evidenced by the favorable $\Delta S$ that accompanies the latter process.

REFERENCES

The Reactivity of Ferricytochrome c with Ionic Ligands
Philip George, Stanley C. Glauser and A. Schejter


Access the most updated version of this article at http://www.jbc.org/content/242/8/1690

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/242/8/1690.full.html#ref-list-1