Kinetics of Cyanide Binding to *Chromatium vinosum* Ferricytochrome *c'* †

Marjan Motie,‡ Richard J. Kassner,*,‡ Terrence E. Meyer,§ and Michael A. Cusanovich§

Department of Chemistry, University of Illinois at Chicago, Chicago, Illinois 60680, and Department of Biochemistry, University of Arizona, Tucson, Arizona 85721

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ABSTRACT: The kinetics of the reversible binding of cyanide by the ferric cytochrome *c'* from *Chromatium vinosum* have been studied over the pH range 6.9–9.6. The reaction is extremely slow at neutral pH compared to the reactions of other high-spin ferric heme proteins with cyanide. The observed bimolecular rate constant at pH 7.0 is 2.25×10^{-3} M^{-1} s^{-1}, which is $\sim 10^7$-fold slower than that for peroxidases, $\sim 10^5$-fold slower than those for hemoglobin and myoglobin, and $\sim 10^2$-fold to $\sim 10^3$-fold slower than that recently reported for the *Glycera dibranchiata* hemoglobin, which has anomalously slow cyanide rate constants of 4.91×10^{-1}, 3.02×10^{-1}, and 1.82 M^{-1} s^{-1} for components II, III, and IV, respectively [Mintorovitch, J., & Satterlee, J. D. (1988) *Biochemistry 27*, 8045–8050; Mintorovitch, J., Van Pelt, D., & Satterlee, J. D. (1989) *Biochemistry 28*, 6099–6104]. The unusual ligand binding property of this cytochrome *c'* is proposed to be associated with a severely hindered heme coordination site. Cyanide binding is also characterized by a nonlinear cyanide concentration dependence of the observed rate constant at higher pH values, which is interpreted as involving a change in the rate-determining step associated with the formation of an intermediate complex between the cytochrome *c'* and cyanide prior to coordination. The pH dependence of both the binding constant for the formation of the intermediate complex and the association rate constant for the subsequent coordination to the heme can be attributed to the ionization of HCN, where cyanide ion binding is the predominant process. Furthermore, in the pH range studied, there are no detectable protein ionizations that influence cyanide binding.

The cytochromes *c'* are a unique class of heme proteins found in a variety of photosynthetic and denitrifying bacteria. Most of the cytochromes *c'* isolated to date are dimers of molecular weight 24000–30000 in contrast to the more usual monomeric or tetrameric nature of most hemoproteins. These proteins resemble the low-spin *c*-type cytochromes in their covalent attachment of the heme to the polypeptide chain; however, they are more closely related to the high-spin heme proteins in many of their physical properties including electronic absorption spectra and magnetism. X-ray crystallographic studies (Weber et al., 1981) have established that the heme iron in ferric cytochrome *c'* is pentacoordinate with a histidyl imidazole group providing the single axial ligand to the iron, which is similar to the coordination in horseradish peroxidase (Spiro et al., 1979). By contrast, the low-spin cytochromes *c* are characterized by histidine–methionine or histidine–histidine heme coordination while many high-spin type ferric heme proteins, such as hemoglobin and myoglobin, have histidine–water coordination at neutral pH.

The cytochromes *c'* show unique reversible changes in their optical spectra as a function of pH (Horio & Kamen, 1961; Taniguchi & Kamen, 1963; Imai et al., 1969a; Cusanovich et al., 1970). At pH 7.0, an anomalous quantum mechanical admixed state (with $S = {}^5/_2, {}^3/_2$) is believed to exist for the oxidized protein (Maltempo et al., 1974, 1980; Maltempo, 1974; Maltempo & Moss, 1976) which is converted to a high-spin state at high pH values up to ~11 (Ehrenberg & Kamen, 1965; Emptage et al., 1977; Rawlings et al., 1977).

In contrast to most high-spin ferric heme proteins such as hemoglobin, myoglobin, and the peroxidases which bind a variety of anionic ligands, earlier studies have indicated that no anionic ligands bind to ferric cytochromes *c'* (Taniguchi & Kamen, 1963). However, a recent report from our laboratory (Kassner et al., 1985) demonstrated cyanide binding to the ferric cytochrome *c'* from *Chromatium vinosum* with an equilibrium constant of 2.1×10^4 M^{-1}, which is $\sim 10^4$-fold smaller than those for horse metmyoglobin and human methemoglobin. It was suggested that the difference between these results and earlier observations may be due to the greater cyanide concentrations and longer equilibrium times needed for complex formation.

In this study, we report the kinetics of cyanide binding to the cytochrome *c'* from the purple photosynthetic bacterium *C. vinosum* to gain further insight into the nature of the active site in these proteins. Rate constants were measured at neutral and higher pH values in order to understand the structural basis for the low equilibrium constant and to examine the mechanism of the reaction with respect to the differential binding of CN^- and HCN. This approach of studying the kinetics of ligand binding as a function of pH has been used for a variety of heme proteins in order to obtain information on the number and nature of the so-called heme-linked ionizable groups (Ver Ploeg et al., 1968; Dolman et al., 1968; Erman, 1974a,b; Job et al., 1980; Vega-Catalan et al., 1986; Ikeda-Saito, 1987).

Materials and Methods

Cytochrome *c'* was isolated according to the method of Cusanovich (1967). Cary 14R and 17D spectrophotometers were used to follow absorbance changes during kinetic experiments. The cell compartments of the spectrophotometers were maintained at a constant temperature of 25 °C, with a Lauda K4R circulating temperature bath during all kinetic runs. Bottle-type cuvettes were constructed by fusing a Kontes glass septum holder to the open end of a rectangular glass cell.

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* Address correspondence to this author.
‡ University of Illinois at Chicago.
§ University of Arizona.

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The cuvettes were sealed with 0.25-in. blue septa purchased from Altech Associates. pH measurements were made with a Radiometer pHM64 pH meter equipped with an Ingold combination microelectrode.

The kinetic assays were carried out under pseudo-first-order conditions where a large excess of a KCN solution was injected into each cytochrome c' solution, with a Hamilton gas-tight syringe, after allowing for temperature equilibration. The protein concentration ranged from ~3 to 5 μM with an extinction coefficient of 87 mM^{-1} heme at 400 nm (Bartsch, 1978). Fresh KCN stock solutions of desired pH and concentration were prepared as follows. Several equally weighed KCN samples were placed in 1-dram vials. To each was added an equal volume of a potassium phosphate buffer of the desired pH. A few such prepared KCN solutions were titrated with concentrated HCl, with a Gilmont glass buret, in order to determine the volume of HCl required to neutralize the KCN solution to the desired pH. This predetermined volume of HCl was then placed in an empty 1-dram vial that was then sealed with a rubber septum. One of the prepared KCN solutions was then removed with a gas-tight syringe and delivered into the vial containing the HCl solution. All KCN solutions and the HCl solutions needed to neutralize them were kept on ice before mixing since neutralization generates heat, which promotes the polymerization of cyanide that appears as a yellow tint in the KCN solution.

The reaction of cytochrome c' with cyanide was followed at 417 nm as a function of time. The pH of the solution was measured after the completion of the reaction. No effort was made to keep the ionic strength constant for all kinetic runs since an initial study showed no ionic strength dependence of the rate constant over the ionic strength range of 0.3–2.0 M at 0.1 M cyanide concentration and pH 7.0 (±0.1). The ionic strength of the kinetic assays varied from 0.27 to 0.6 M. The pK_a for the dissociation of hydrocyanic acid is 9.21 at 25 °C and 0 ionic strength but varies slightly with increasing ionic strength (Izatt et al., 1962). The acid ionization constant for HCN at 25 °C was determined for our experimental conditions with the method of Albert and Serjeant (1984) for the determination of ionization constants. The pK_a was determined to be equal to 9.15 (±0.05), showing very little change, within experimental error, with ionic strength from 0.27 to 0.6 M.

Results

The reaction of cyanide with *Chromatium vinosum* cytochrome c' was followed at 417 nm where an increase in absorbance is observed upon binding to cyanide (Kassner et al., 1985). The reaction was found to be pseudo first order (Figure 1), and the observed rate constant (k_{obs}) increased with increasing cyanide concentration. Plots of k_{obs} as a function of cyanide concentration were obtained in the pH range 6.9–9.6. Although these plots appear to be linear at lower pH values over a large concentration range, such as shown in Figure 2 for data obtained at pH 7.0, they are distinctly nonlinear at higher pH values. The nonlinear cyanide concentration dependence of k_{obs} indicates a change in the rate-determining step which can be explained in terms of the formation of an intermediate complex between the cytochrome c' and cyanide as given by eq 1. Assuming $k_{-1} \gg k_2$, k_{obs} is given by eq 2,

$$\text{cyt } c' + \text{CN} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{cyt } c'\cdots\text{CN} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{cyt } c'\text{-CN} \quad (1)$$

$$k_{obs} = \frac{k_2[\text{CN}]K_1}{1 + [\text{CN}]K_1} + k_{-2} \quad (2)$$

where $K_1 = k_1/k_{-1}$. According to eq 2, the observed rate constant will be a linear function of cyanide concentration at low cyanide concentrations with a slope equal to k_2K_1 and an intercept equal to k_{-2}. At higher cyanide concentrations, the observed rate constant reaches a plateau with the limiting rate constant equal to $k_2 + k_{-2}$. The pH 7.0 data (Figure 2) were used to obtain the apparent bimolecular association rate constant for cyanide binding, corresponding to k_2K_1, from the slope and the apparent dissociation rate constant, k_{-2}, from the intercept. Furthermore, the plots of k_{obs} versus the con-

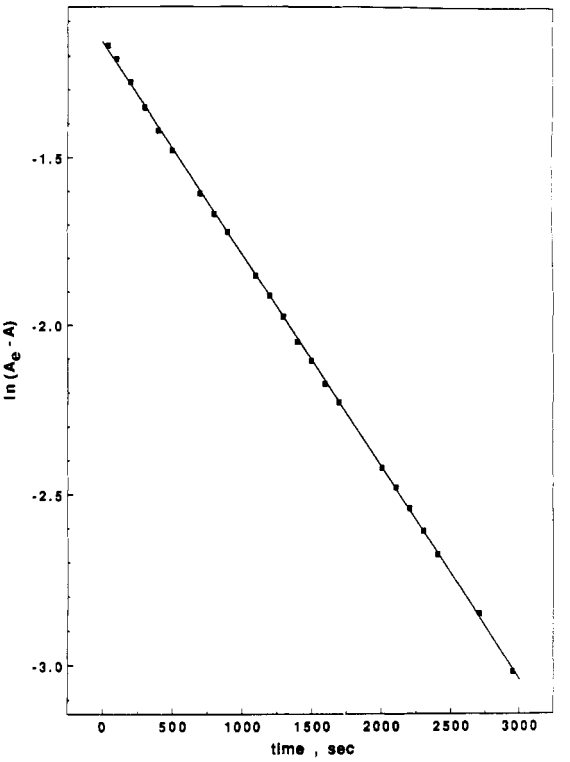


FIGURE 1: Pseudo-first-order plot of ln ($A_e - A$) vs t (s) for cyanide binding to cytochrome c' at pH 7.0, 0.225 M KCN, 25 °C. A was measured at 417 nm.

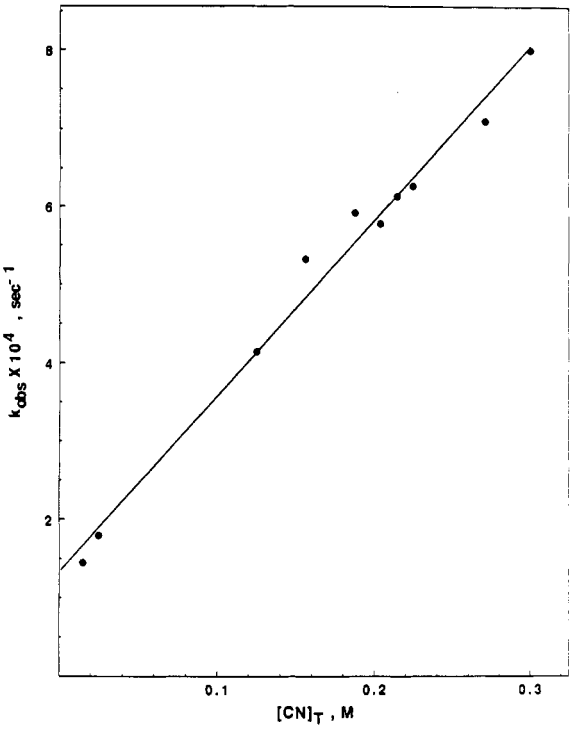


FIGURE 2: Plot of k_{obs} vs total cyanide concentration at pH 7.0.

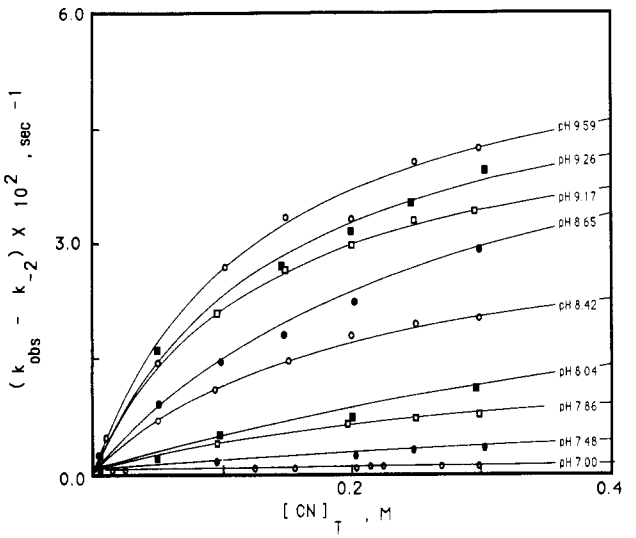


FIGURE 3: Plots of $(k_{obs} - k_{-2})$ vs total cyanide concentration at several pH values.

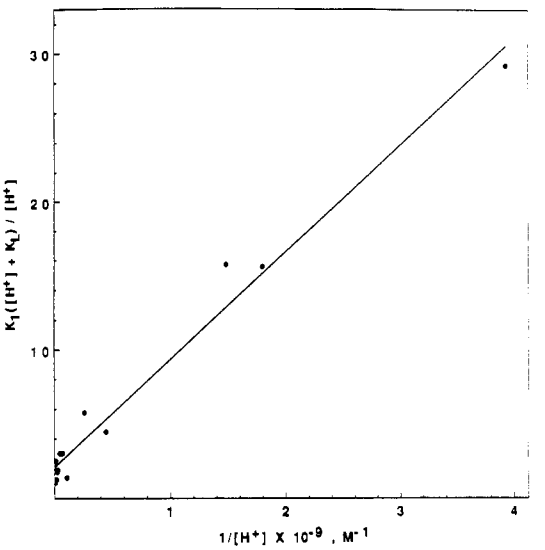
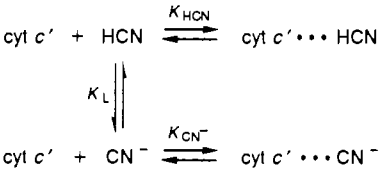


FIGURE 4: Plot of $K_1([H^+] + K_L)/[H^+]$ vs $1/[H^+]$.

Scheme I



centration of cyanide (not shown) appear to approach the same value at very low cyanide concentrations, suggesting that the value of the dissociation rate constant is pH independent. The apparent dissociation rate constant obtained at pH 7.0 was used as an estimate of the value of k_{-2} in order to fit the cyanide concentration dependence of k_{obs} to eq 2 with a rectangular hyperbola fitting routine. Figure 3 shows the fitted curves corresponding to the observed cyanide concentration dependence of $k_{obs} - k_{-2}$ obtained at several pH values. The computed values of K_1 and k_2 increase as the pH increases. Since cyanide is present as a mixture of HCN and CN^- depending on the pH, the observed pH dependence of the kinetic parameters, K_1 and k_2, is not surprising. Scheme I presents the simplest mechanism for describing the pH dependence of K_1. The observed binding constant, K_1, for Scheme I is given by eq 3. Therefore, a plot of $K_1([H^+] + K_L)$ vs $[H^+]$ is

$$K_1 = \frac{K_{HCN}[H^+]}{[H^+] + K_L} + \frac{K_{CN}K_L}{[H^+] + K_L} \quad (3)$$

expected to be linear with the slope equal to K_{HCN} and the intercept equal to $K_{CN}K_L$. Also, a plot of $K_1([H^+] + K_L)/[H^+]$ vs $1/[H^+]$ is expected to be linear with a slope equal to $K_{CN}K_L$ and an intercept equal to K_{HCN}. The latter plot shown in Figure 4 gave a higher correlation coefficient and values of K_{CN^-} and K_{HCN} equal to 10.3 M^{-1} and 2.08 M^{-1}, respectively. The relative values of these constants indicate that cyanide anion binds preferentially to the protein to form the intermediate complex.

The simplest mechanism for describing the pH dependence of k_2 is shown in Scheme II, where K_{PL} is given by eq 4. K_{PL}

$$K_{HCN}K_{PL} = K_{CN}K_L \quad (4)$$

was calculated with the values of K_{HCN} and K_{CN^-} obtained from the fitting of the pH dependence of K_1 and the experimentally measured value of K_L. The corresponding pK_{PL} of 8.45 is significantly lower than that of the uncomplexed hydrogen

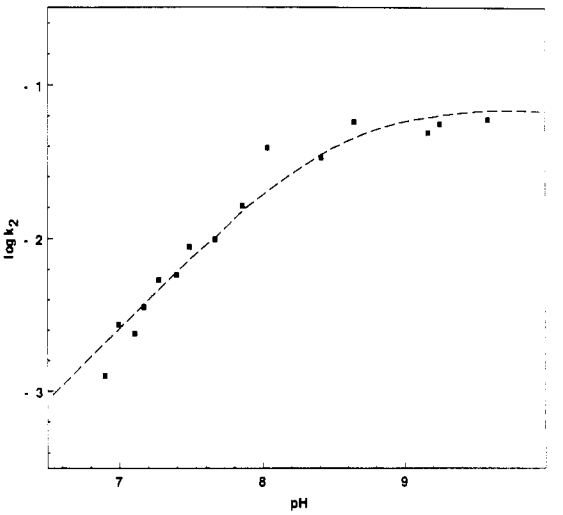
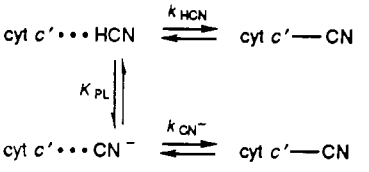


FIGURE 5: Plot of log k_2 vs pH for the binding of cyanide by cytochrome c'. The squares are experimental data, and the dashed line is the calculated curve from the best-fit parameters of Scheme II.

Scheme II



cyanide (pK_L = 9.15). The observed association rate constant, k_2, for the mechanism shown in Scheme II is given by eq 5.

$$k_2 = \frac{k_{HCN}[H^+]}{[H^+] + K_{PL}} + \frac{k_{CN}K_{PL}}{[H^+] + K_{PL}} \quad (5)$$

A plot of $k_2([H^+] + K_{PL})$ vs $[H^+]$ was found to be linear, yielding values of 7.29×10^{-2} s^{-1} and 7.30×10^{-5} s^{-1} for k_{CN^-} and k_{HCN}, respectively. Thus, the rate constant for cyanide ion coordination is much greater than that for hydrogen cyanide.

The fitted values of k_{HCN} and k_{CN^-} were used to calculate the theoretical values of k_2 given by eq 5 at pH values between 6.5 and 10. Figure 5 shows a comparison of the theoretical fit with the experimental values of k_2, in a log k_2 vs pH plot,

Table I: Rate Constants for Cyanide Complex Formation for Cytochrome c' and Other Ferric Heme Proteins[a]

protein	temp (°C)	pH	k (M^{-1} s^{-1})
cytochrome c'[b]	25	7.0	2.25×10^{-3}
cytochrome c[c]	24.6	7.0	1.08×10^{-1}
G. dibranchiata hemoglobin (II)[d]	20	7.0	4.91×10^{-1}
G. dibranchiata hemoglobin (III)[e]	20	7.0	3.02×10^{-1}
G. dibranchiata hemoglobin (IV)[e]	20	7.0	1.82×10^{0}
hemoglobin[f]	20	6.05	1.05×10^{2}
myoglobin[f]	21–23	7.0	1.7×10^{2}
horseradish peroxidase[g]	25	7.0	1.06×10^{5}
cytochrome c peroxidase[h]	25	7.0	1.1×10^{5}

[a] Rate constants correspond to total cyanide concentration. [b] This paper. [c] George and Tsou (1952). [d] Mintorovitch and Satterlee (1988). [e] Mintorovitch et al. (1989). [f] Antonini and Brunori (1971). [g] Dunford et al. (1978). [h] Loo and Erman (1975).

which indicates that the proposed scheme is consistent with the observed data.

Discussion

The results indicate that cytochrome c' has an extremely slow (total) cyanide association rate constant in comparison with other high-spin ferric hemoproteins, as is shown in Table I. The rate constant at pH 7.0 is also $\sim 10^2$-fold smaller than that for cytochrome c in spite of the fact that ligand binding in cytochrome c corresponds to the substitution of the bound methionine in contrast to ligand addition in cytochrome c'. The rate constant is likewise $\sim 10^2$-fold to $\sim 10^3$-fold smaller than that recently reported for monomeric *Glycera dibranchiata* methemoglobin component II (Mintorovitch & Satterlee, 1988) and *G. dibranchiata* methemoglobin components III and IV (Mintorovitch et al., 1989) whose rate constants are $\sim 10^2$-fold to $\sim 10^3$-fold smaller than those of other ferric hemoglobins and myoglobin. In comparison to the five-coordinated horseradish peroxidase, where ligand addition also takes place, the cyanide binding rate constant is more than 10^7-fold smaller in cytochrome c'. The strikingly slow rate constant agrees with the very small equilibrium constant for cytochrome c' binding to cyanide relative to other heme proteins (Kassner et al., 1985). The cyanide equilibrium binding constant for cytochrome c' is $\sim 10^4$-fold smaller than that for horse metmyoglobin and human methemoglobin, which is reflected in the greater than 10^4-fold difference in their association rate constants. Thus, the much smaller affinity for cyanide in cytochrome c' can be accounted for by the much smaller rate of association, which suggests that cyanide binding to cytochrome c' is characterized by severe steric hindrance. This suggestion is supported by X-ray studies of *Rhodospirillum molischianum* ferric cytochrome c', which indicate that the distal heme surface is surrounded by aromatic or other hydrophobic amino acid residues that are directed so as to restrict access of exogenous ligands to the sixth heme iron coordination site (Weber et al., 1980, 1981). According to these X-ray studies, in *Rs. molischianum* cytochrome c', Met-16, Leu-19, and Trp-86 pack about the sixth coordination site with the Met S lying 3.75 Å from the heme iron such that some relocation of the residues must accompany ligand binding. In *Chromatium vinosum*, the amino acids corresponding to these residues are Tyr-16, Met-19, and Ala-86 (Amber et al., 1981) such that, in the absence of large structural differences in the proteins, similar steric interactions would be expected. The unusual formation of the intermediate complex prior to coordination may be related to the large steric effect.

The formation of the intermediate complex is influenced by the ionization of HCN such that the binding constant for CN^- is significantly greater than that for HCN. Since this binding constant does not appear to be influenced by any ionizations on the protein, the formation of this intermediate complex could not involve binding to an ionizable group which has an ionization constant in the pH range of this study. Hydrogen bonding to a group either on the exterior of the protein or in the heme pocket, such as Tyr-16 on the distal side of the heme ion, may be involved. This type of bonding would be expected to be stronger for CN^- than for HCN and thus consistent with the greater CN^- binding constant for the intermediate complex formation.

The pH dependence of cyanide coordination to the heme, i.e., the second step in eq 1, can also be accounted for by the ionization of bound HCN, which would have a lower pK_a value than the uncomplexed HCN on the basis of the greater binding constant for CN^- compared to HCN for the formation of the intermediate complex. The ratio of the rate constants k_{CN^-}/k_{HCN} is ~ 1000, indicating that CN^- is the predominant reactive form at neutral and higher pH values. Previous studies have shown that the sole product of cyanide ligation to heme proteins is protein–CN (and not protein–HCN), regardless of whether CN^- or HCN is the reacting species (George & Hanania, 1955). ^{15}N NMR and IR studies more recently have also provided evidence for cyanide binding as a metal cyanide (Fe–CN) and not as a metal–HCN in a nitrile-type bonding (Fe—N≡C—H) (Morishima & Inubushi, 1978; Yoshikawa et al., 1985; Behere et al., 1986). Thus, for HCN to bind, a proton must be released. Studies of cyanide binding to metmyoglobin have shown that CN^- and HCN bind with similar rate constants at pH 7.0 (Chance, 1952; Ver Ploeg et al., 1971). It has been suggested that the distal histidine acts as a hydrogen acceptor in HCN binding to metmyoglobin (Mintorovitch et al., 1989). Similarly, the distal histidine in spleen myeloperoxidase has been suggested to most likely trap the proton released from HCN upon binding (Ikeda-Saito, 1987). The absence of a proton acceptor in cytochrome c' may explain the very small rate constant obtained for HCN binding. Similar high ratios of k_{CN^-}/k_{HCN} were observed for *G. dibranchiata* hemoglobin components, which also lack a distal histidine (Mintorovitch & Satterlee, 1988; Mintorovitch et al., 1989). The much more rapid rate of coordination to cyanide anion in cytochrome c' may also be caused by the electrostatic interaction between the ferric heme ion and the negatively charged cyanide as compared to the un-ionized hydrogen cyanide.

The present paper provides further insight into the unusual ligand binding properties of cytochrome c' relative to those of other high-spin heme proteins. The lowered cyanide binding affinity of these proteins appears to be largely a consequence of the extremely slow rate constants for binding, which may be associated with severe steric hindrance. The proposed mechanism of cyanide binding by *Chromatium vinosum* cytochrome c' is also unusual in having an intermediate complex prior to coordination. The pH-dependent kinetics of the intermediate complex formation and the subsequent coordination indicate that cyanide anion is the major reactive form for binding to *C. vinosum* cytochrome c'.

Registry No. CN^-, 57-12-5; heme, 14875-96-8; cytochrome c', 9035-41-0.

References

Albert, A., & Serjeant, E. P. (1984) in *The Determination of Ionization Constants*, 3rd ed., Chapman and Hall, New York.

Ambler, R. P., Bartsch, R. G., Daniel, M., Kamen, M. D., McLellan, L., Meyer, T. E., & Van Beeumen, J. (1981)

Proc. Natl. Acad. Sci U.S.A. 78, 6854–6857.
Antonini, E., & Brunori, M. (1971) in *Hemoglobin and Myoglobin in Their Reactions with Ligands*, North-Holland, Amsterdam.
Bartsch, R. G. (1978) in *The Photosynthetic Bacteria* (Clayton, R. K., & Sistrom, W. R., Eds.) Plenum Press, New York.
Beher, D. V., Gonzales-Vergara, E., & Goff, H. M. (1985) *Biochim. Biophys. Acta 832*, 319–325.
Chance, B. (1952) *J. Biol. Chem. 194*, 483–487.
Cusanovich, M. A. (1967) Ph.D. Thesis, University of California at San Diego.
Cusanovich, M. A., Tedro, S. M., & Kamen, M. D. (1970) *Arch. Biochem. Biophys. 141*, 557–570.
Dolman, D., Dunford, H. B., Chowdhury, D. M., & Morrison, M. (1968) *Biochemistry 7*, 3991–3996.
Dunford, H. B., Hewson, W. D., & Steiner, H. (1978) *Can. J. Chem. 56*, 2844–2851.
Ehrenberg, A., & Kamen, M. D. (1965) *Biochim. Biophys. Acta 102*, 333–340.
Emptage, M. H., Zimmerman, R., Que, L., Jr., Munck, E., Hamilton, W. D., & Orme-Johnson, W. H. (1977) *Biochim. Biophys. Acta 495*, 12–23.
Erman, J. E. (1974a) *Biochemistry 13*, 34–39.
Erman, J. E. (1974b) *Biochemistry, 13*, 39–44.
George, P., & Tsou, C. L. (1952) *Biochem. J. 50*, 440–448.
George, P., & Hanania, G. I. H. (1955) *Discuss. Faraday Soc. 20*, 216–224.
Horio, T., & Kamen, M. D. (1961) *Biochim. Biophys. Acta 48*, 266–286.
Ikeda-Saito, M. (1987) *Biochemistry 26*, 4344–4349.
Imai, Y., Imai, K., Sato, R., & Horio, T. (1969a) *J. Biochem. (Tokyo) 65*, 225–237.
Imai, Y., Imai, K., Ikeda, K., Hamaguchi, K., & Horio, T. (1969b) *J. Biochem. (Tokyo) 65*, 629–637.
Izatt, R. M., Christensen, J. J., Pack, R. T., & Bench, R. (1962) *Inorg. Chem. 1*, 828–831.
Job, D., Zeba, B., Puppo, A., & Rigand, J. (1980) *J. Biochem. (Tokyo) 107*, 491–500.
Kassner, R. J., Kykta, M. G., & Cusanovich, M. A. (1985) *Biochim. Biophys. Acta 831*, 155–158.
Loo, S., & Erman, J. E. (1975) *Biochemistry, 14*, 3467–3470.
Maltempo, M. M. (1974) *J. Chem. Phys. 61*, 2540–2547.
Maltempo, M. M., & Moss, T. H. (1976) *Q. Rev. Biophys. 9*, 181–215.
Maltempo, M. M., Moss, T. H., & Cusanovich, M. A. (1974) *Biochim. Biophys. Acta 342*, 290–305.
Maltempo. M. M., Moss, T. H., & Spartalian, K. (1980) *J. Chem. Phys. 73*, 2100–2106.
Mintorovitch, J., & Satterlee, J. D. (1988) *Biochemistry 27*, 8045–8050.
Mintorovitch, J., Van Pelt, D., & Satterlee, J. D. (1989) *Biochemistry 28*, 6099–6104.
Morishima, I., & Inubushi, T. (1978) *J. Am. Chem. Soc. 100*, 3568–3574.
Rawlings, J., Stephens, P. J., Nafie, L. A., & Kamen, M. D. (1977) *Biochemistry 16*, 1725–1729.
Spiro, T. G., Strong, J. D., & Stein, P. (1979) *J. Am. Chem. Soc. 101*, 2648–2655.
Taniguchi, S., & Kamen, M. D. (1963) *Biochim. Biophys. Acta 74*, 438–455.
Vega-Catalan, F. J., Odeyemi, O. J., & Okonjo, K. O. (1986) *J. Biol. Chem. 261*, 10576–10581.
Ver Ploeg, D. A., & Alberty, R. A. (1968) *J. Biol. Chem. 243*, 435–440.
Ver Ploeg, D. A., Cordes, H., & Gurd, F. R. N. (1971) *J. Biol. Chem. 246*, 2725–2733.
Weber, P. C., Bartsch, R. G., Cusanovich, M. A., Hamlin, R. C., Howard, A., Jordan, S. R., Kamen, M. D., Meyer, T. E., Weatherford, D. W., Xuong, N. H., & Salemme, F. R. (1980) *Nature (London) 286*, 302–304.
Weber, P. C., Howard, A., Xuong, Ng. H., & Salemme, F. R. (1981) *J. Mol. Biol. 153*, 399–424.
Yoshikawa, S., O'Keefee, D. H., & Caughey, W. S. (1985) *J. Biol. Chem. 260*, 3518–3528.