Cr(III)-Fe(II) hybrid hemoglobins, $\alpha_2(\text{Cr})\beta_2(\text{Fe})$ and $\alpha_2(\text{Fe})\beta_2(\text{Cr})$, in which hemes in either the $\alpha$- or $\beta$-subunits were substituted with chromium(III) protoporphyrin IX (Cr(III)PPIX), were prepared and characterized by oxygen equilibrium measurements. Because Cr(III)PPIX binds neither oxygen molecules nor carbon monoxide, the oxygen equilibrium properties of Fe(II) subunits within these hybrids can be analyzed by a two-step oxygen equilibrium scheme. The oxygen equilibrium constants for both hybrids at the second oxygenation step agree with those for human adult hemoglobin at the last oxygenation step (at pH 6.5–8.4 with and without inositol hexaphosphate at 25°C). The similarity between the effects of the Cr(III)PPIX and each subunits’ oxymeme on the oxygen equilibrium properties of the counterpart Fe(II) subunits within hemoglobin indicate the utility of Cr(III)PPIX as a model for a permanently oxynated heme within the hemoglobin molecule.

We found that Cr(III)-Fe(II) hybrid hemoglobins have several advantages over cyanomet valency hybrid hemoglobins, which have been frequently used as a model system for partially oxynated hemoglobins. In contrast to cyanomet heme, Cr(III)PPIX within hemoglobin is not subject to reduction with dithionite or enzymatic reduction systems. Therefore, we could obtain more accurate and reasonable oxygen equilibrium curves of Cr(III)-Fe(II) hybrids in the presence of an enzymatic reduction system, and we could obtain single crystals of deoxy-$\alpha_2(\text{Cr})\beta_2(\text{Fe})$ when grown in low salt solution in the presence of polyethylene glycol 1000 and 50 mM dithionite.

Human adult hemoglobin (Hb A)$^3$ cooperatively binds four oxygen molecules via a complex sequence of intermediate oxynated states. Information about the intermediate species is required to understand the cooperative mechanism of Hb A, yet little is known about such intermediates because the equilibriums of the intermediates under any conditions are markedly reduced by the cooperativity of Hb.

Cyanomet valency hybrid Hbs have been frequently used for studying the oxygenation intermediates of Hb A (1–12). Structural and functional studies on cyanomet valency hybrids have suggested that cyanide-bound ferric heme mimics natural oxy-heme, thus deoxy-cyanomet hybrid Hbs have been used as models for the intermediate species formed during the cooperative oxygenation process (13–20). However, since conventional met-Hb reducing reagents and enzymatic reduction systems reduce the cyanomet heme, it is very difficult to carry out the experiments using deoxy-cyanomet hybrid Hbs under anaerobic conditions. Thus, there have been no reports on x-ray crystallography of cyanomet valency hybrids because of this difficulty.

During recent years, we have investigated the properties of metal-substituted hybrid Hbs, $\alpha_2(M)\beta_2(\text{Fe})$ and $\alpha_2(\text{Fe})\beta_2(M)$, using the first transition metal ions (M). This metal substitution method is the most suitable modification of Hb A for studying the relationships between the functional states of Hb A and globin-metalloporphyrins interaction. In our systematic investigations, we have observed a wide variation of oxygen affinities in these hybrids, as a result of the variation in the configuration of 3d electrons of the porphyrin metal. With respect to the oxygen affinity of metal-Fe(II) hybrids, we can classify these hybrids into following four groups: (i) hybrids showing oxygen affinities as high as oxy-Hb A, (ii) hybrids showing intermediate oxygen affinities, between oxy-Hb A and deoxy-Hb A, (iii) hybrids showing oxygen affinities as low as deoxy-Hb A, (iv) hybrids showing lower oxygen affinities than deoxy-Hb A. To represent the oxygenation intermediates of Hb A, the hybrids in group (i) and (iii) are particularly important. In our recent series of studies, Ni(II)-Fe(II) hybrid Hbs in group (iii) have been used successfully to investigate the structures and functions of the intermediates appearing in the first half oxygenation of Hb A (21–28). Although the Ni(II)-Fe(II) hybrid system has brought much structural and functional information about the initial-half oxynated intermediates, this approach could not be extended to studies on the latter-half oxygenation of Hb A. Stable hybrids in group (i) were required to model the intermediates appearing in the oxygenation of the last two sites of Hb A.

This paper reports the preparation and oxygen equilibrium properties of Cr(III)-Fe(II) hybrid Hbs. It also shows that these Cr(III)-Fe(II) hybrids are an excellent model for the intermediates appearing in the oxygenation of the last two sites of Hb A. The influence of Cr(III)PPIX on the oxygen equilibrium properties of ferrous subunits within Cr(III)-Fe(II) hybrid Hbs results in quantitative similarities with oxynated heme under various conditions. This view is reinforced by other structural results, namely (i) similar porphyrin geometry and metal-globin bonds between Cr(III)PPIX and natural oxymeme,
and (ii) the occupation of the sixth coordination position of Cr(III)-Fe(II) hybrid Hbs and (ii) the occupation of the sixth coordination position of a globin. For the preparation of CrHb, apo-Hb was used. The apo-Hb was prepared from native Hb A as described by Shibayama et al. (22). The apo-Hb was combined with an equimolar amount of Cr(III)PPIX, and the reconstituted CrHb was purified by the purification procedure described above. All of the preparative works and subsequent manipulations were carried out at 4°C. All of the samples containing Cr(III)PPIX were treated in the dark, because of their light sensitivity. The purities of all the Hbs were checked by isoelectric-focusing electrophoresis (Pharmacia).

### Experimental Procedures

Preparation of Cr(III)-Fe(II) Hybrid Hbs and CrHb—Cr(III)-Fe(II) hybrid Hbs were prepared as described by Hori et al. (29), Hb A and its isolated chains, semihemoglobin a and semihemoglobin b, were prepared in carbon monoxide forms as described by Fujii et al. (30). The preparation method for Cr(III)-Fe(II) hybrid Hbs was essentially similar to that for Ni(II)-Fe(II) hybrid Hbs (22). The preparation of Cr(III)-Fe(II) hybrid Hbs was carried out as follows. Bound CO was removed from semihemoglobin by the method of Kilmartin and Rossi-Bernardi (32). The spectrophotometric titration of semihemoglobin b with Cr(III)PPIX at 440 nm gave a well defined inflection point, from which the molecular stoichiometry of 1.1 was estimated. The solution of semihemoglobin in oxy form (500 mg) was mixed with an equimolar amount of Cr(III)PPIX, which was dissolved in a minimal amount of N,N-dimethylformamide. The mixture was stirred at 4°C for 2 h and then concentrated by ultrafiltration and passed through a Sephadex G-25 (Pharmacia Biotech Inc.) column equilibrated with 10 mM phosphate buffer, pH 6.85. The sample was applied to a column of CM52 cellulose (Whatman) equilibrated with the same buffer. The column was eluted by a linear gradient of 10 mM phosphate buffer, pH 7.10, and 15 mM phosphate buffer, pH 7.45. A main peak corresponding to $\alpha_2$Cr(III)$\beta_2$(Fe$^2+$-O$_2$) was collected and concentrated by ultrafiltration. The concentrated sample was passed through a column of Sephadex G-25 equilibrated with 20 mM Tris-HCl buffer, pH 8.2 and stored in liquid nitrogen (yield, 150 mg). Preparation of $\alpha_2$(Fe$^2+$-O$_2$)$\beta_2$(Cr) was carried out by the same procedure described above, using corresponding constituents, Cr(III)PPIX, and semihemoglobin a. For the preparation of CrHb, apo-Hb was used. The apo-Hb was prepared from native Hb A as described by Shibayama et al. (22). The apo-Hb was combined with an equimolar amount of Cr(III)PPIX, and the reconstituted CrHb was purified by the purification procedure described above. All of the preparative works and subsequent manipulations were carried out at 4°C. All of the samples containing Cr(III)PPIX were treated in the dark, because of their light sensitivity. The purities of all the Hbs were checked by isoelectric-focusing electrophoresis (Pharmacia).

### Determination of Oxygen Equilibrium Properties—Oxygen equilibrium curves were measured as described by Imai et al. (33, 34). Our automatic oxygenation apparatus was interfaced to a microcomputer (Nippon Electric Co., Tokyo) for on-line data acquisition, storage, and analysis. Light-path length of the cell was variable in this apparatus and was set at 15 mm in the case of 60 mM and 12 mM (on a metal basis) or at 8 mm in the case of 240 mM (on a metal basis). The concentration of CO was removed from semihemoglobin by the method of Fujii et al. (30). The preparation of Cr(III)-Fe(II) hybrid Hbs was essentially similar to that for Ni(II)-Fe(II) hybrid Hbs (22). The preparation of Cr(III)-Fe(II) hybrid Hbs was carried out as follows. Bound CO was removed from semihemoglobin by the method of Kilmartin and Rossi-Bernardi (32). The spectrophotometric titration of semihemoglobin b with Cr(III)PPIX at 440 nm gave a well defined inflection point, from which the molecular stoichiometry of 1.1 was estimated. The solution of semihemoglobin in oxy form (500 mg) was mixed with an equimolar amount of Cr(III)PPIX, which was dissolved in a minimal amount of N,N-dimethylformamide. The mixture was stirred at 4°C for 2 h and then concentrated by ultrafiltration and passed through a Sephadex G-25 (Pharmacia Biotech Inc.) column equilibrated with 10 mM phosphate buffer, pH 6.85. The sample was applied to a column of CM52 cellulose (Whatman) equilibrated with the same buffer. The column was eluted by a linear gradient of 10 mM phosphate buffer, pH 7.10, and 15 mM phosphate buffer, pH 7.45. A main peak corresponding to $\alpha_2$Cr(III)$\beta_2$(Fe$^2+$-O$_2$) was collected and concentrated by ultrafiltration. The concentrated sample was passed through a column of Sephadex G-25 equilibrated with 20 mM Tris-HCl buffer, pH 8.2 and stored in liquid nitrogen (yield, 150 mg). Preparation of $\alpha_2$(Fe$^2+$-O$_2$)$\beta_2$(Cr) was carried out by the same procedure described above, using corresponding constituents, Cr(III)PPIX, and semihemoglobin a. For the preparation of CrHb, apo-Hb was used. The apo-Hb was prepared from native Hb A as described by Shibayama et al. (22). The apo-Hb was combined with an equimolar amount of Cr(III)PPIX, and the reconstituted CrHb was purified by the purification procedure described above. All of the preparative works and subsequent manipulations were carried out at 4°C. All of the samples containing Cr(III)PPIX were treated in the dark, because of their light sensitivity. The purities of all the Hbs were checked by isoelectric-focusing electrophoresis (Pharmacia).

### Table I

| pH | IHP | Red | $K_1$ | $K_2$ | $P_{50}$ | $n_{max}$ | met | crg |
|----|-----|-----|------|------|--------|---------|-----|-----|
| 6.5 | -   | -   | 0.88 | 1.66 | 0.85   | 1.2     | 10.6 | -   |
|     | +   | -   | 0.026 | 0.708 | 24     | 1.3     | 8.2  | -   |
|     | +   | (0.030) | [0.082] | [20] | [1.3] | [8.3] | -   | -   |
| 7.4 | -   | -   | 2.2  | 3.48 | 0.37   | 1.1     | 5.7  | -   |
|     | +   | (2.8) | (3.3) | (0.33) | (1.0) | (3.1) | -   | -   |
|     | +   | (1.8) | (2.5) | (0.47) | (1.1) | (1.3) | -   | -   |
| 8.4 | -   | -   | 0.069 | 0.399 | 0.32   | 1.1     | 3.3  | -   |
|     | +   | (0.73) | (2.98) | (0.69) | (1.3) | (4.4) | -   | -   |

| pH | IHP | Red | $K_1$ | $K_2$ | $P_{50}$ | $n_{max}$ | met | crg |
|----|-----|-----|------|------|--------|---------|-----|-----|
| 6.5 | -   | -   | 0.25 | 0.729 | 2.4    | 1.3     | 4.0  | -   |
|     | +   | -   | 0.012 | 0.0399 | 47    | 1.4     | 3.2  | -   |
|     | +   | (0.010) | [0.043] | [48] | [1.4] | [3.0] | -   | -   |
| 7.4 | -   | -   | 1.2  | 2.77 | 0.55   | 1.2     | 2.4  | -   |
|     | +   | (1.1) | (3.1) | (0.55) | (1.2) | (0.7) | -   | -   |
| 8.4 | -   | -   | 1.1  | 2.5  | 0.60   | 1.3     | 0.7  | -   |
|     | +   | 0.018 | 0.209 | 17   | 1.6     | 0.9   | -   | -   |
|     | +   | (0.015) | (0.23) | (12) | (1.6) | (1.2) | -   | -   |
|     | +   | 0.026 | 0.15  | 16   | 1.4     | 0.7   | -   | -   |

### Notes

1. Experimental conditions are as follows: temperature, 25°C; buffer conditions, 50 mM bis-Tris or Tris with 100 mM chloride; protein concentration, the data sets are based on 60 mM, and numbers in parentheses are based on 240 mM, and numbers in square brackets are based on 12 mM (on a metal basis); wavelength of detection light, 560 nm in the case of 240 mM and 60 mM (on a metal basis), or 430 nm in the case of 12 mM (on a metal basis).

2. We found that Cr(III)-Fe(II) hybrid Hbs that had been irradiated with a 100-watt incandescent bulb for 30 min for removal of bound CO showed very high affinity for oxygen with little Bohr effect or little IHP effect. In addition, it was found that when Cr(III)-Fe(II) hybrid Hbs and CrHb were exposed to strong light during isoelectric focusing, these samples gave broad bands. We have not found out the exact cause of such light-induced damage to Cr(III)-Fe(II) hybrid Hbs, but it is likely that some globin moiety of Cr(III)-Fe(II) hybrid Hbs were damaged by $O_2$ radical produced by interaction between strong light and Cr(III)PPIX. Since we found this problem, we have prepared Cr(III)-Fe(II) hybrid Hbs in $O_2$ form, and we have taken care that Cr(III)-Fe(II) hybrid Hbs are not exposed to strong light.
RESULTS

Fig. 1 presents the isoelectric focusing of Cr(III)-Fe(II) hybrid Hbs in CO form and Hb A in CO form and CrHb. α_{2}(Mn^{3+})β_{2}(Fe–CO) (42) and α chain are shown as controls. Each hybrid Hb appears as a nearly single band. CrHb and the hybrid Hbs migrate toward the higher pH region compared with Hb A, due to the presence of trivalent Cr(III)PPIX.

The pH dependence of the absorption spectrum of CrHb is presented in Fig. 2A. Upon raising pH from 6.5 to 8.4, the Soret peak shifted from 445 to 439 nm, and a visible peak at 764 nm shifted to 752 nm. In the range of pH 6.5–8.4, the isosbestic points were reproducibly observed at 748, 720, 441, and 407 nm.

The absorption spectrum of CrHb was not affected by the presence of either 50 mM dithionite under anaerobic condition or the enzymatic reduction system of Hayashi et al. (37), indicating that Cr(III)PPIX in CrHb was not reduced to Cr(II)PPIX by these reductants. Thus, the oxygen equilibrium curves of Cr(III)-Fe(II) hybrid Hbs could be measured with enzymatic reduction system in order to reduce the met-heme contents to a minimal level. Moreover, absorption spectra of deoxygenated Cr(III)-Fe(II) hybrid Hbs under anaerobic condition in the presence of 50 mM dithionite did not change remarkably for 96 h at 20°C except decreasing of dithionite absorption (Fig. 3). After these measurements, CO could reasonably bind to the ferrous subunits of Cr(III)-Fe(II) hybrid Hbs (Fig. 3). Autooxidation rate of ferrous subunits of α_{2}(Cr)β_{2}(Fe–O_{2}) and α_{2}(Fe–O_{2})β_{2}(Cr) were measured by detecting 410-nm absorbance change in air equilibrated buffer, 37°C condition (Fig. 4). Precipitate did not appear. The autooxidation rates of Cr(III)-Fe(II) hybrid Hbs were comparable with that of Hb A under the same condition (Fig. 4), meaning that ferrous subunits of Cr(III)-Fe(II) hybrid Hbs were as stable as those of Hb A against autooxidation. Thus, we conclude that Cr(III)-Fe(II) hybrid Hbs have enough stability for our oxygen equilibrium and crystallization experiments. The sum of the absorption spectra of α_{2}(Cr)β_{2}(Fe–O_{2}) and α_{2}(Fe–O_{2})β_{2}(Cr) was almost identical to that of oxy-Hb A and CrHb. (Fig. 2, B and C). Oxy-deoxy difference spectrum of each Cr(III)-Fe(II) hybrid Hb agrees closely with that of isolated α or β chain. These findings mean that Cr(III) subunits do not bind oxygen molecules and that the absorption spectra of Cr(III) subunits are not affected by the ligation state of the corresponding ferrous subunits within Cr(III)-Fe(II) hybrid Hbs.

Oxygen Equilibrium Parameters—K_{i} and K_{2} values (the equilibrium constants for the first and second oxygen molecule to bind to hybrid Hbs, respectively). P_{50} values (the oxygen pressure at half-saturation), n_{max} values (the maximal slopes of the Hill plots), and met-heme contents after measurements (the percent of the met-hemes in the total hemes) are listed in Table I. The met-heme contents are markedly reduced by adding the enzymatic reduction system, but the oxygen equilibrium parameters are little affected by the same. We also found that the oxygen affinities of the hybrids are not significantly dependent on the protein concentrations in the range from 60 to 240 μM (on a metal basis) at pH 7.4, and in the range from 12 to 60 μM (on a metal basis) at pH 6.5.

In the absence of IHP, Cr(III)-Fe(II) hybrid Hbs showed very high affinity for oxygen molecules. At pH 8.4, both hybrids bound oxygen noncooperatively (n_{max} = 1.0–1.1) with very high affinity comparable with that of isolated α or β chain, while they exhibited n_{max} values significantly higher than unity (n_{max} = 1.2–1.3) at pH 6.5. In both hybrids, Hill coefficients became larger as pH decreased. There were slight differences between the cooperativity of α_{2}(Cr)β_{2}(Fe) and that of α_{2}(Fe)β_{2}(Cr). The n_{max} values of α_{2}(Cr)β_{2}(Fe) (n_{max} = 1.1–1.2) were slightly smaller than those of α_{2}(Fe)β_{2}(Cr) (n_{max} = 1.1–1.3) at all pH values examined, and the latter hybrid exhibited a slightly larger Bohr effect than the former. The oxygen equi-
Oxygen Equilibrium Properties of Cr-Fe Hybrid Hbs

The addition of IHP significantly affected the librium properties of both hybrids. The oxygen affinities were reduced, and the cooperativity and the number of released Bohr protons was increased (Fig. 5). It is important to note that the extent of the IHP effect on the $K_2$ values of both hybrids was very similar to those on the $K_2$ value of native Hb A (43) (see Fig. 5). These results suggest that Cr(III)PPIX behaves like a permanent oxy-heme.

We succeeded in obtaining single crystals of deoxy-$\alpha_2\beta_2$(Fe) from solution of 2% protein, in the presence of 27–28% polyethylene glycol 1000 and 50 mM dithionite under anaerobic condition (Fig. 6). Crystals were examined by precession photography. The deoxy-$\alpha_2\beta_2$(Fe) crystals were found to be isomorphous to the native deoxy-Hb A crystals, which belong to space group P21212.

3 K. Imai and K. Imaizumi, unpublished observations.
IHP(2mM) Data for HbA with IHP(2mM) are from K. Imai (Footnote 3). Conditions for hybrid Hbs are as follows: protein concentration, 60 µM (on a metal basis); temperature, 25°C; buffer condition, 50 mM bis-Tris or Tris buffer with 100 mM chloride; wavelength of detection light, 560 nm. Protein concentration of HbA is 60 µM (on a metal basis), and other solution conditions are the same as those for hybrid Hbs.

Fig. 5. pH dependence of the equilibrium constant for the last oxygen molecule to bind to Hb. $K_4$ (torr$^{-1}$) of $\alpha_{2}(Cr)\beta_{2}(Fe)$ (C); $K_2$ (torr$^{-1}$) of $\alpha_{2}(Fe)\beta_{2}(Cr)$ (C); and $K_4$ (torr$^{-1}$) of HbA (C) (Ref. 43). Log $K_2$ and log $K_4$ values are plotted. Filled symbols indicate the presence of IHP (2mM). Data for Hb A with IHP (2mM) are from K. Imai (Footnote 3). Conditions for hybrid Hbs are as follows: protein concentration, 60 µM (on a metal basis); temperature, 25°C; buffer condition, 50 mM bis-Tris or Tris buffer with 100 mM chloride; wavelength of detection light, 560 nm. Protein concentration of HbA is 60 µM (on a metal basis), and other solution conditions are the same as those for hybrid Hbs.

**DISCUSSION**

The pH-dependent spectra of CrHb show well defined isosbestic points (Fig. 2A), indicating that CrHb exists in a pH-dependent equilibrium between two alternate states. Since Cr(III) complexes are almost universally hexacoordinated (44), and Cr(III)PPIX prefers counter anion due to an extra positive charge, it is reasonable to consider that the proximal histidine coordinates to the Cr(III) ion and that the remaining coordination position is occupied by a water molecule or a hydroxyl ion, as in the case of aquomHb. Thus, observed pH-dependent spectral changes may result from a coordination equilibrium between H$_2$O and OH$^-$ in CrHb. Previously, Fiechtner reported that hemichrome structure is formed in CrHb (45); however, their published absorption spectrum is quite different from ours. Their OD$_{578}$/OD$_{280}$ ratio is 1.3, whereas ours is 3.6 at pH 7.4.

The dimer-tetramer association equilibrium constants of $\alpha_{2}(Cr)\beta_{2}(Fe-CO)$ and $\alpha_{2}(Fe-CO)\beta_{2}(Cr)$, which were obtained by gel-filtration experiments, are about $5 \times 10^5$ M$^{-1}$ and $1 \times 10^6$ M$^{-1}$, respectively. Although part of the Cr(III)-Fe(II) hybrids dissociated into αβ dimers under our experimental conditions for oxygen equilibrium experiments, the Hill plots of both Cr(III)-Fe(II) hybrids exhibited little protein concentration dependence (Table I). We calculated the protein concentration dependence of the Hill plots of Cr(III)-Fe(II) hybrids using estimates of dimerization from dimer-tetramer association equilibrium constants and the assumption that a heme-globin dimer containing Cr(III)PPIX and Fe(II)PPIX exhibits high oxygen affinity, comparable with that of isolated chains. The calculations revealed that the Hill plots were only slightly influenced by dimerization in the concentration range of above 10 µM (on a metal basis). Because the experimental Hb concentrations which we measured oxygen equilibrium curves were 12, 60, and 240 µM (on a metal basis), the theoretical consideration was consistent with the experimental results.

$K_4$ values of both Cr(III)-Fe(II) hybrid Hbs agreed with $K_4$ values of Hb A, including both pH and IHP dependence (Fig. 5). In recent years, both association and dissociation rate constants for the last step ($\alpha$- and $\beta$-subunit, respectively) in oxygen binding to Hb A were determined kinetically, and the Adair constants ($K_4$ for $\alpha$ subunit and $K_4$ for $\beta$ subunit, respectively) were calculated from these rate constants (46–49). In Table I, we compared the $K_4$ values of the Cr(III)-Fe(II) hybrid Hbs with the kinetically determined $K_4$ values of Hb A. The $K_4$ values of the Cr(III)-Fe(II) hybrids were comparable with the $K_4$ values of Hb A. These findings indicate that the influence of Cr(III)PPIX on the oxygen equilibrium properties of the counterpart ferrous subunits is similar to that of oxyheme. Stereoelectrical theory of Hb allostery (50) indicates that the oxygen affinity of Hb is regulated by the equilibrium position of the central metal with respect to the porphyrin ring, so that the

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* K. Kajitani and H. Morimoto, unpublished observations.
position of the proximal histidine relative to the heme plane is a key determinant of oxygen affinity of Hb. In this regard, Cr(III)PPIX can be an adequate model for an oxyhemoglobin for several reasons. (i) The Cr(III) ion has an ionic radius of 0.62 Å (51), which is almost equal to that of low spin Fe(II). (ii) The Cr(III) ion is also expected to lie in the mean porphyrin plane (52). (iii) Cr(III) complexes are almost universally hexacoordinated (44), and (iv) Cr(III) porphyrin binds ligands so tightly (52) that the Cr(III)-histidine bond is expected to be as short as Fe(II)-histidine bond in oxy-Hb. Thus, both the proximal and the distal environments of Cr(III)PPIX in Hb may be similar to those of oxy-heme.

Cyanomet valency hybrid Hbs have been widely used as models for understanding the nature of the intermediate species formed during the cooperative oxygenation process. There are several structural and functional reasons for using cyanide-bound ferric heme as an oxyhemoglobin model. (i) The crystal structure of cyanomet-Hb (10) is closely similar to that of oxy-Hb A including pH effect (11), 5

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