Ligand Binding and Autoxidation of a Tetra Sulphonated Phthalocyanine Iron(II)–Apomyoglobin Complex*

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Abstract

Changes in the UV–Vis spectrum of (tspc)-Fe(III)–apoMb by the addition of KCN indicates the changes in spin states of iron. The rate constant, k_{obs}, of autoxidation of (tspc)Fe(II)–apoMb was measured to be $2.93 \pm 0.05 \times 10^{-5}$ s^{-1} at 25 °C and pH 7.6. The dependence of k_{obs} on $[H^+]$ was suggested by the following rate equation:

$$k_{obs} = k_1[H^+] + k_{-1}$$

The values of k_1 and k_{-1} were calculated to be $2.24 \pm 0.02 \times 10^2$ M^{-1} s^{-1} and $3.01 \pm 0.04 \times 10^{-5}$ s^{-1}, respectively. The equilibrium binding constant ($k_d = k_{-1}/k_1$) for the distal histidine in (tspc)Fe(II)–apoMb was found to be 74.4 nM at 25 °C. Thermodynamic parameters for autoxidation of (tspc)Fe-(II)–apoMb were calculated by Eyring equation and found to be $\Delta H^* = 19.1 \pm 0.2$ kcal/mol and $\Delta S^* = -13.7 \pm 0.7$ e.u.

Introduction

Derivatives of myoblogin and hemoglobin, with substituted phthalocyanine metal complexes, binds reversibly with molecular oxygen [1–5].

The photochemical electron transfer properties of phthalocyanine complexes with cobalt and iron have been studied extensively [6–10]. I have reconstituted iron phthalocyanine complex with apomyoglobin and studied its ligand binding and autoxidation properties by visible spectroscopic methods.

Experimental

Analytical grade reagents and NANO pure water were used in all the experiments.

Reconstitution of (tspc)Fe(II)–apoMb

(tspc)Fe(III) was reduced to (tspc)Fe(II) with ascorbic acid in 1:1 stoichiometry and purified on Sephadex G-25 column prior to use. The solution containing 10 μM apoMb and 11 μM (tspc)Fe(II) were mixed in 250 ml of 0.1 m sodium phosphate buffer (pH 6). The mixture was stirred for 1 h under nitrogen gas at 4 °C. The solution was concentrated to 3 ml and passed through a Sephadex G-50 (2.5 × 45 cm) column, which was equilibrated with 0.1 M sodium phosphate buffer (pH 6) at 4 °C. The fractions containing metalloprotein were identified with UV and visible spectroscopy and dialysed against number of changes of distilled water and finally in 5 mM sodium phosphate buffer (pH 6) at 4 °C. The dialysed solution was adsorbed on CM-52, cation exchange column (1.5 × 10 cm), equilibrated with 5 mM sodium phosphate buffer (pH 6) at 4 °C. The colum was washed five times with the same buffer. The adsorbed metalloprotein was eluted with a potassium phosphate buffer whose concentration was increased gradually from 5 mM to 200 mM at pH 6 and temperature 4 °C The fractions containing metalloprotein were concentrated and used for physical studies.

Spectrophotometric measurements were made with Cary-219 and Shimadzu UV-250 recording spectrophotometer equipped with thermostatic assembly. The kinetic measurements were carried out with Shimadzu UV-250 spectrophotometer in a thermostatic cuvette control. The change in the absorbance was monitored at 630 nm for the photochemical, ascorbic acid reductions, peroxo and cyanide complex formation. The autoxidation was monitored at 672 nm. The samples were prepared just before use. The stock solution of (tspc)-Fe(II)–apoMb (100 μM) was diluted with 3 ml buffer of appropriate pH and 1-cm optical path length celi was used for all the measurements. The

*Abbreviations used are: (tspc)Fe(II)–apoMb, tetra sulphonated phthalocyanine iron(II)–apomyoglobin; e.u., entropy unit.

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TABLE I. First Order Rate Constant (k_{obs}) Measured for Autoxidation and Peroxo–Complex Formation of (tspc)Fe(II)–apoMb and Reduction of (tspc)Fe(III)–apoMb by Ascorbic Acid

Condition[a]	$10^5 \times k_{obs}$ (s^{-1})
Photochemical reduction of (tspc)Fe(III)–apoMb (6 μM) by 2 eV photon energy plus 10% of 100 W bulb of incandescent light measured at 630 nm	2.93 ± 0.05
Reduction of (tspc)Fe(III)–apoMb (6 μM) by ascorbic acid (4 mM) measured at 630 nm	9.62 ± 0.04
Peroxo-complex formation with H_2O_2 (4 mM) and (tspc)Fe(III)–apoMb (6 μM) measured at 630 nm	9.61 ± 0.02
Ligation kinetics of (tspc)Fe(III)–apoMb (6 μM) with KCN (1 mM) measured at 630 nm	11.33 ± 0.08
Autoxidation of (tspc)Fe(II)–apoMb (10 μM) measured at 672 nm[b]	4.57 ± 0.08

[a]In all experiments buffer contained 0.1 M sodium phosphate (pH 7.6) at 25 °C. [b]pH was 7.

concentration of the protein was calculated by the known absorptivity coefficient [11]. The first order rate constants (k_{obs}) were calculated by the linear plot of ln ($OD_t - OD_\alpha$) *versus* time (t). OD_α was recorded after the reaction run for 24 h. The plots were found to be linear over the 75% of the reaction.

Results and Discussion

A mechanism was proposed for the formation of (tspc)Fe(II)–apoMb complex, on the basis of fast coordination from the proximal histidine imidazole (no. 93) to the iron atom and slow rate determining step involves the charge transfer from the distal histidine imidazole (no. 64) to the central Fe(III) ion [12, 13].

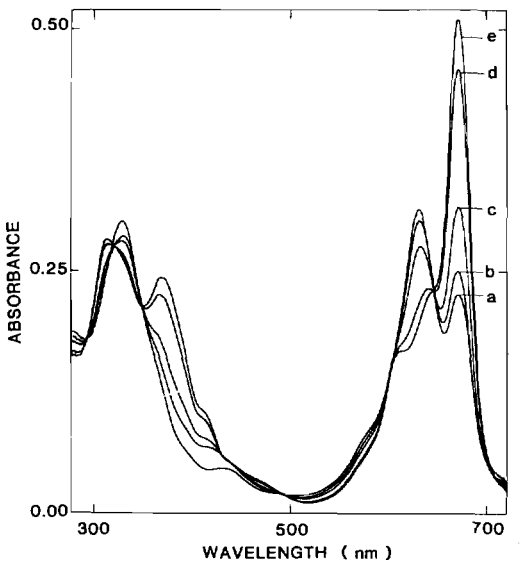
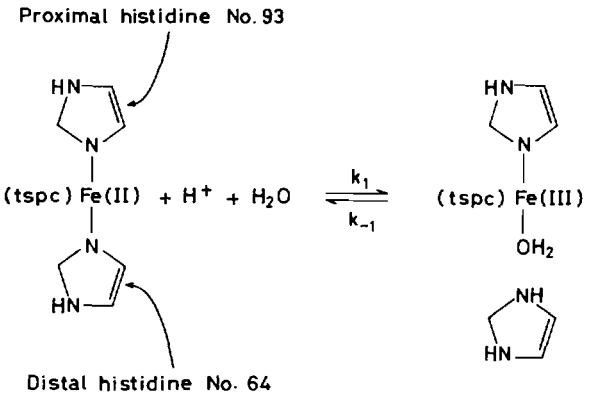


Fig. 1. Typical spectrum for ligand binding to (tspc)Fe(III)–apoMb (16 μM) with KCN (80 μM) at 25 °C and 0.1 M sodium phosphate (pH 7.6). (a) no KCN; (b) 30 min after the addition of KCN; (c) 90 min; (d) 180 min, and (e) 300 min.

The time course spectra of ligand binding with (tspc)Fe(II)–apoMb is shown in Fig. 1. The spectroscopic changes suggests that iron changes the spin states while coordinated with cyanide ion.

The rate of the photochemical reduction of (tspc)-Fe(III)–apoMb at an ambient condition of energy, which involve the slow rate determining step of charge transfer from ligand to the metal and was found to be $2.93 \pm 0.05 \times 10^{-5}$ s^{-1} at 25 °C and 0.1 M sodium phosphate (pH 7.6).

The autoxidation reaction of (tspc)Fe(III)–apoMb was studied through four half lives. The rate constants were calculated from the slopes of linear plots of ln ($OD_t - OD_\alpha$) *versus* time (t) and shown in Fig. 2. The rate constant was measured at various concentrations of hydrogen ion and at different temperatures. On the basis of dependence of the first order rate constant, k_{obs}, on [H^+] (Fig. 3), the following mechanism was proposed.



$$k_{obs} = k_1[H^+] + k_{-1} \quad (1)$$

and

$$k_d = k_{-1}/k_1 \quad (2)$$

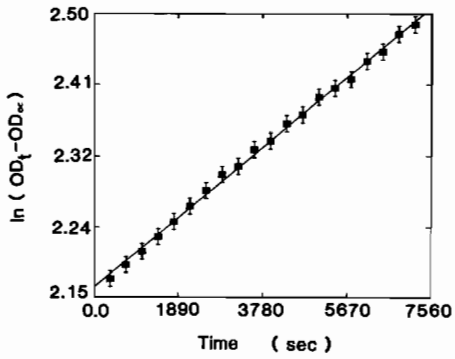


Fig. 2. Kinetic plot for autoxidation of (tspc)Fe(II)–apoMb (10 μM) at 25 °C and 0.1 M sodium phosphate (pH 7). k_{obs} = 4.57 ± 0.08 × 10^{-5} s^{-1}.

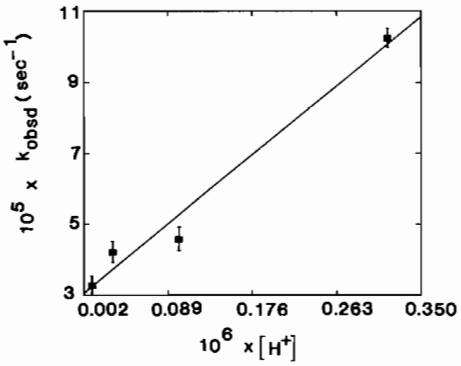


Fig. 3. Dependence of first order rate constant, k_{obs}, on [H^+] for autoxidation of (tspc)Fe(II)–apoMb at 25 °C and 0.1 M sodium phosphate.

The parameters calculated on the basis of equation 1 are k_1 = 2.24 ± 0.02 × 10^2 M^{-1} s^{-1} and k_{-1} = 3.01 ± 0.04 × 10^{-5} s^{-1}. The dissociation constant (k_d) for distal histinine of (tspc)Fe(II)–apoMb was found to be 74.4 nM.

The temperature dependence for the rate constants of autoxidation of (tspc)Fe(II)–apoMb is shown in Fig. 4. The thermodynamic parameters were calculated by the Eyring equation.

$$k_{obs}(T) = (RT/Nh) \exp(\Delta H^*/R(T) + \Delta S^*/R) \qquad (3)$$

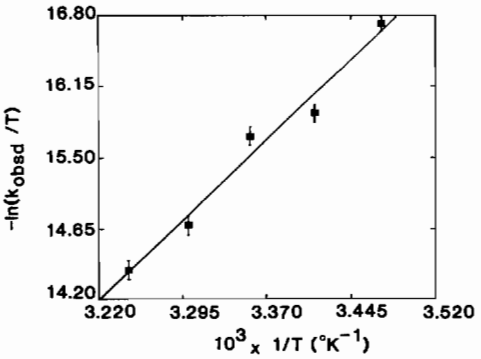


Fig. 4. Temperature dependence of first order rate constant for autoxidation of (tspc)Fe(II)–apoMb in 0.1 M sodium phosphate (pH 7).

The thermodynamic parameters were found to be ΔH^* = 19.1 ± 0.2 kcal/mol and ΔS^* = −13.7 ± 0.7 e.u. for the autoxidation of (tspc)Fe(II)–apoMb. The negative value of entropy suggests the high activation transition energy of unimolecular oxidation of (tspc)Fe(II)–apoMb.

References

1 H. Przywarska-Boniecka, L. Trynda and E. Antonini, *Eur. J. Biochem., 52,* 567 (1975).
2 H. Przywarska-Boniecka and L. Trynda, *Eur. J. Biochem., 87,* 569 (1978).
3 H. Przywarska-Boniecka and H. Swirska, *J. Inorg. Biochem., 13,* 283 (1980).
4 H. Przywarska-Boniecka and L. Trynda, *Inorg. Nucl. Chem. Lett., 7/8,* 247 (1981).
5 N. K. Kildahl, J. W. Kolis, J. Beckett, G. F. Holland and M. A. Patz, *J. Coord. Chem., 12,* 259 (1983).
6 R. E. Dickerson and R. Timkovich, in P. D. Bayer (ed.), 'The Enzymes', Vol. XI, Part A, 1975, p. 397.
7 C. Bartocci, F. Scandola, A. Ferri and V. Carassiti, *J. Am. Chem. Soc., 102,* 7069 (1980).
8 C. Bizet, P. Morliere, D. Brault, O. Delgada, M. Bazin and R. Santus, *Photochem. Photobiol., 34,* 35 (1981).
9 C. Bartocci, A. Maldotti, O. Traverso, C. A. Bignozzi and V. Carassitti, *Polyhedron, 2,* 97 (1983).
10 A. Maldotti, C. Bartocci, O. Traverso and V. Carassiti, *Inorg. Chim. Acta, 79,* 174 (1983).
11 L. Trynda, *Inorg. Chim. Acta, 79,* 229 (1983).
12 L. J. Kagen, 'Myoglobin., Columbia Univ. Press, New York/London, 1973, p. 11.
13 H. Przywarska-Boniecka, E. Panejko, L Trynda and J. Biernat, *Mat. Sci., IV/4,* 139 (1978).