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Complexes of Iron, Cobalt and Copper Tetrasulfonated Phthalocyanines with Apomyoglobin≠

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The modified myoglobins containing the Fe(III), Co(II) and Cu(II) phthalocyanines in the place of heme were synthetized by recombination of those complexes with apomyoglobin. Such a modification was also carried out by replacement of the heme in myoglobin by metal tetrasulfonated phthalocyanine. This indicates that tetrasulfonated phthalocyanine and porphyrine combine with globin in a similar mode. The structure and properties of the complexes obtained with apomyoglobin were investigated by difference spectroscopy, electrophoresis, molecular weight estimation and the CD method.

Absorption spectra show the bands characteristic for these complexes, at 650 nm, 690 nm and 660, 700 nm for Fe(III)L-apoMb, Co(II)L-apoMb and Cu(II)-apoMb, respectively.

Coordination of iron and cobalt tetrasulfonated phthalocyanine with apomyoglobin increases the helical content of the protein, by 18% and 22%, respectively, to make it more similar to the structure of the native myoglobin.

The copper complex shows an increase in disordered structure of about 10% compared with apomyoglobin. Coordination of copper tetrasulfonated phthalocyanine with apomyoglobin is accompanied by a change in the electronic structure of the Cu(II) ion. Deformation of the phthalocyanine complex caused by the copper ion is responsible for the conformational changes in the protein. In the Soret band and in the VIS the studied compounds produce the induced Cotton effect, which indicates a change in symmetry of the tetrasulfonated phthalocyanine complexes because of the coordination with protein. Molecular weight and electrophoretic results indicate that the synthetized compounds are monomers.

Complexes Fe(II)L-apoMb and Co(II)L-apoMb have the ability to perform reversible oxygen fixation and can serve as models of myoglobin. That ability is not preserved in the complex Cu(II)L-apoMb. The redox properties of the obtained complexes indicate that coordination with apomyoglobin is responsible for stabilization of the II-oxidation state of the metal in the iron and cobalt tetrasulfonated phthalocyanines and for stabilization of the π-electronic system of the phthalocyanine ring in the complex Cu(II)L-apoMb.

Introduction

The interactions between the heme group and the surrounding protein are critical for the functional design of heme proteins [1, 2]. These interactions have been investigated by comparing the reactivity of the physical properties of model systems with those of the proteins [3, 4]. The effect of metal substitution on myoglobin conformational stability and the effect of the protein on the heme center have been reported for the following metallosubstituted myoglobins: Co(III)Mb, Zn(II)Mb, Cr(III)Mb, Cu(II)Mb, Mn(III)Mb, Ru(III)Mb [5–8]. Also the effect of chemical modification of porphyrin side chains and the role of peripheral substituents of the porphyrin in metalloporphyrin–globin complexes have been studied [6, 9, 10].

According to these results, the major role in the restoration of the helical content of a protein is played by the porphyrin ring itself, not by the metal component. For functionally active myoglobin, a hydrophobic environment of the prosthetic groups is required.

The compounds which closely resemble metalloporphyrins are tetrasulfonated phthalocyanines of metals. Earlier we showed that substitution of heme in hemoglobin by metal tetrasulfonated phthalocyanines gives dimeric complexes whose properties in many respects resemble those of hemoglobin [11, 12].

Combination of iron tetrasulfonated phthalocyanine with apocatalase, however, gives denaturated species and the physiological function of catalase can not then be performed [13].

≠Abbreviations: Mb = myoglobin; apoMb = apomyoglobin; L = tetrasulfonate phthalocyanine ligand ($C_{32}H_{12}N_8SO_3Na_4$); Fe(III)L = iron(III) tetrasulfonated phthalocyanine; Co(II)L = cobalt(II) tetrasulfonated phthalocyanine; Cu(II)L = copper(II) tetrasulfonated phthalocyanine.

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In this paper are reported the results concerning the structure and properties of artificial myoglobins obtained by incorporation of iron, cobalt and copper tetrasulfonated phthalocyanines into the heme binding site of apomyoglobin.

Experimental

Materials and Methods

Horse heart muscle myoglobin (purchased from Koch-Light Lab. Ltd., salt-free, lyophilized) was used without further purification or in some cases after purification on a column of carboxymethylcellulose with 10 m*M* Tris-HCl buffer, pH 8 [14]. The pooled protein solution was dialyzed against an appropriate buffer solution. The concentration of metmyoglobin was measured spectrophotometrically using $\epsilon_{410} = 157$ [15].

The globin was prepared according to the methods of Rossi-Fanelli and Antonini [16]. The concentration of globin was determined spectrophotometrically: the absorption coefficient, A_{280}^{1cm} for 1 mg/ml solution of globin was taken as 0.8.

The preparation and purification of iron, cobalt and copper tetrasulfonated phthalocyanines have been described by Fallab *et al.* [17]. Stock solution was obtained by weighing appropriate amounts of the solid and dissolving the latter in 100 ml of an appropriate buffer.

Globin complexes of the iron, cobalt and copper tetrasulfonated phthalocyanines were prepared by incubation of the reactants in 0.1 *M* phosphate buffer, pH 7.6 for 48 h at 4 °C.

Apomyoglobin and metal tetrasulfonated phthalocyanines were mixed in the molar ratio 1:10. Protein complexes were precipitated with saturated ammonium sulfate and dissolved in 10 m*M* potassium phosphate buffer, pH 7.6. The mixture was passed through a carboxymethylcellulose column equilibrated with 10 m*M* potassium phosphate buffer, pH 7.6.

The complex was absorbed onto the column, whereas the unbonded metal phthalocyanine passed through the column. The column was washed with 5 volumes of 10 m*M* potassium phosphate buffer, pH 7.6. The absorbed complex was slowly eluted with 50 m*M* potassium phosphate, pH 7.6. The concentration of the complexes was determined from the molar absorption coefficients. Their Focmol: Fe(III)-L-apoMb $\epsilon_{650} = 3.2 \times 10^4$, Co(II)L-apoMb $\epsilon_{680} = 2.6 \times 10^4$, Cu(II)L-apoMb $\epsilon_{670} = 4 \times 10^4$.

The reduced forms of the complexes were prepared by the addition of a few milligrams of sodium dithionite to their buffered solutions and removal of the reductant excess on a Sephadex G-50 column. Solid samples were obtained by lyophilization of the water solutions of the complexes.

Cyanide, azide and fluoride derivatives of model complexes were prepared by adding the sodium salt to the solution of the complexes dissolved in 0.1 *M* phosphate buffer, pH 7.6. The formation of derivatives was confirmed by their absorption spectra in the Soret and visible region.

Hydrogen peroxide compounds of the model complexes were prepared in a cuvette by adding hydrogen peroxide solution to 2 ml of solutions of the complexes. The molar concentration ratio of H_2O_2 to complexes was below 10. The metal tetrasulfonated phthalocyanines-apomyoglobin complexes were dissolved in 0.1 *M* borate buffer, pH 8.5. Hydrogen peroxide was diluted to the appropriate concentration with deionized glass-distilled water. All the chemicals used in the studies were of analytical purity.

Absorption Spectroscopy

Absorption spectra and difference spectra were recorded on a Cary 15 spectrophotometer with a cell compartment thermostatically controlled at 12 °C or on a Specord spectrophotometer. The measurements were made under equilibrium conditions.

Polyacrylamide Gel Electrophoresis

Electrophoresis was carried out according to the method described in [12]. The mobilities of the complexes were determined together with those of myoglobin and apomyoglobin.

Measurements of Catalytic Activity

These catalase-like activity of model complexes was measured at pH 8.5, by the method of Euler and Josephson [18]. The activity of the catalyst was characterized by the decomposition of H_2O_2 recorded against time.

Molecular Weight Estimation

Molecular weight measurements of model complexes were carried out by gel-filtration on a Sephadex G-75 column according to the method of Andrews [19]. The following proteins were used as reference substances: cytochrome c (12400), myoglobin(17800), pepsin(35000) and serum albumin(67000). The appropriate molecular weight of the model complexes was also estimated from the results of the electrophoretic studies.

Circular Dichroism Measurements

Circular dichroism (CD) spectra were recorded on a model ORD/UV-5 Japan Spectropolarimeter with CD attachment. The solutions of the metal phthalocyanines-apomyoglobin were prepared by dissolving the appropriate amount of lyophilized preparation in phosphate buffer, pH 7.6. CD results are expressed in molar ellipticity in units of degrees·cm^2 per decimole.

Results

The interaction of Fe(III)L, Co(II)L and Cu(II)L with myoglobin indicates that heme is partially displaced by phthalocyanine compounds. In all cases the Soret band at 418 nm, which is characteristic of free hemoprotein, disappears, and this is demonstrated in Fig. 1 by difference spectra of the Fe(III)-L–Mb, Co(II)L–Mb and Cu(II)L–Mb system against the same solutions unmixed. The complexes which are formed in the displacement reaction are characterized by the new band in the region of absorbance of phthalocyanine. Characteristic absorption of Fe(III)L (635 nm), Co(II)L (670 nm) and Cu(II)L (630 nm, 660 nm) decreased and the new bands of the complexes with myoglobin appeared at 650 nm, 680 nm and (670, 700 nm), respectively.

The displacement reaction of heme in myoglobin by phthalocyanine derivatives was investigated in the pH range 5.5–8. The intensity of decrease of the Soret band and increase of the new bands was strongest at pH 6.5.

Combination of Iron, Cobalt and Copper Tetrasulfonated Phthalocyanine with Apomyoglobin

The reaction between metal tetrasulfonated phthalocyanines and apomyoglobin was demonstrated by difference absorption spectroscopy experiments (Fig. 2). The difference spectrum of the Fe(III)L-apoMb system shows the positive peaks at 650 nm and negative ones at 635 nm, the difference spectrum of the Co(II)L-apoMb system exhibits a positive peak at 690 nm and two negative peaks at 625 nm and 670 nm. The difference spectrum of the Cu(II)L-apoMb system shows the decay of the peak at 630 nm and appearance of the new bands at 660 nm and 700 nm. These results indicate that apomyoglobin reacts with metal tetrasulfonated phthalocyanines to give the new complexes.

As shown in Fig. 3 the spectrophotometric titration curve of the combination of apomyoglobin with Cu(II)L gave the well defined inflexion point from which the molar stoichiometry of their binding was estimated as 1:1.

Combination of apomyoglobin with Fe(III)L and Co(II)L gave essentially identical results. The difference spectra recorded during the titration of Cu(II)L with apomyoglobin also showed well-defined isosbestic points at 640 nm, which indicates that the equilibrium involves two spectral species only. The new myoglobin complexes were isolated as green-blue solids by separation of the reaction mixture on carboxymethyl cellulose and by lyophilization. The solids were stable for a few weeks.

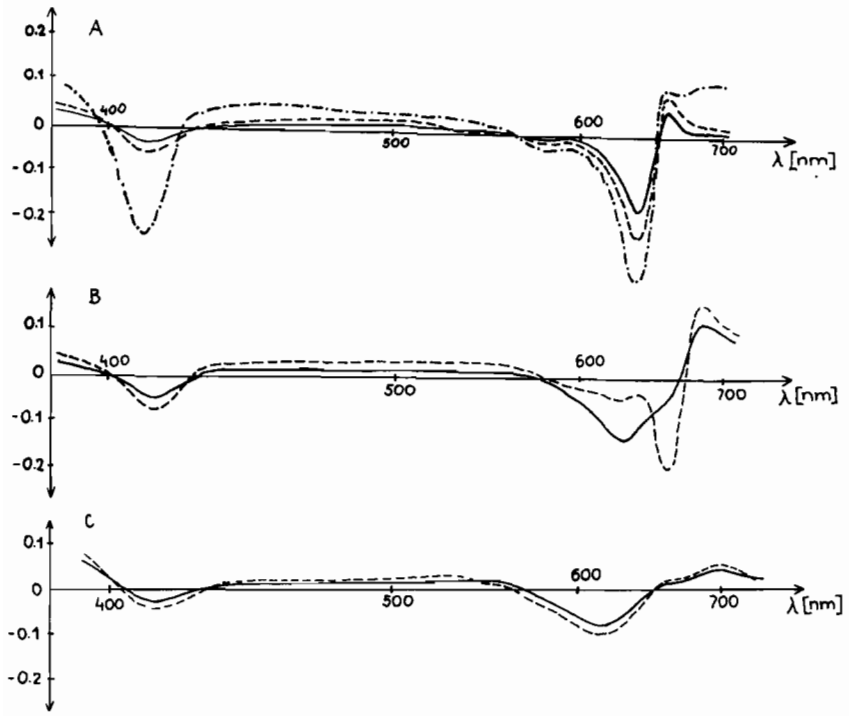


Fig. 1. Difference spectra of the mixture of Mb with Fe(III)L (A), Co(II)L (B) and Cu(II) (C) against unmixed solutions. (——) spectra were recorded after 30 min of the reaction run in 10 m*M* phosphate, pH 7.6. (– – –) spectra were recorded after 24 h of the reaction run in 10 m*M* phosphate, pH 7.6. (– · –) spectrum was recorded after 24 h of the reaction run in phosphate, pH 6. Concentrations: Mb = 5×10^{-8} *M*, Fe(III)L = Co(II)L = Cu(II)L = 3×10^{-7} *M*.

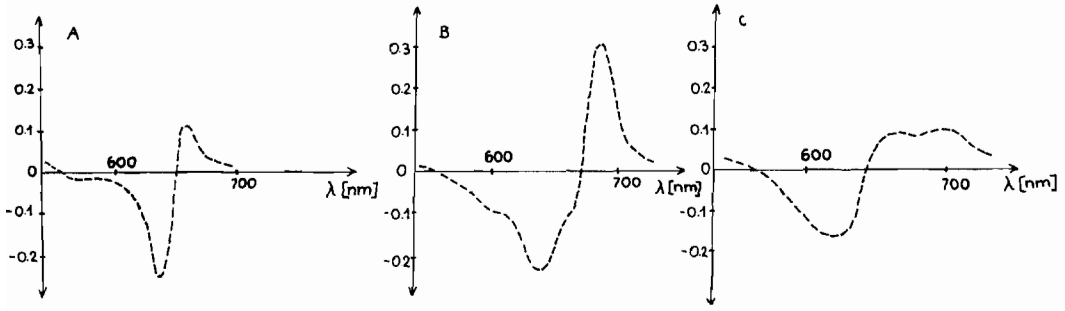


Fig. 2. Difference spectra of: Fe(III)L + apoMb (A), Co(II)L + apoMb (B) and Cu(II)L + apoMb (C) mixtures against the same solutions unmixed. Concentrations: apoMb = 6×10^{-8} *M*, Fe(III)L = Co(II)L = Cu(II)L = 9×10^{-8} *M*.

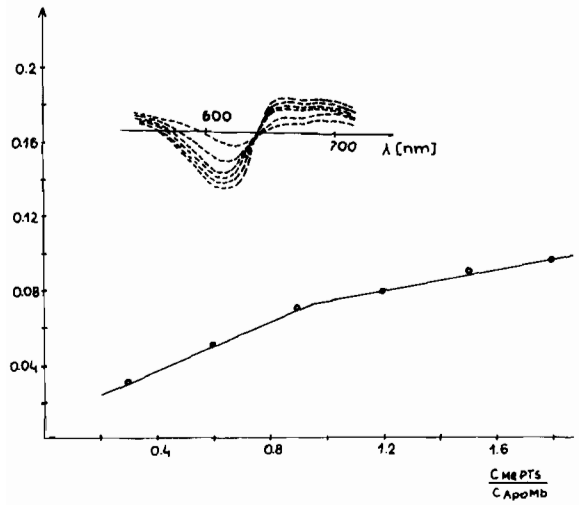


Fig. 3. Stoichiometric titration of Cu(II)L with apoMb. The changes in absorption were measured at 660 nm as a function of the molar ratio of Cu(II)L to apomyoglobin. Concentrations: apoMb = 6×10^{-7} *M*, Cu(II)L = 1.8×10^{-7} *M* – 10, 8×10^{-7} *M*.

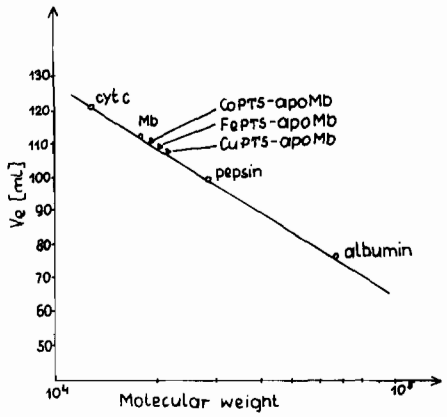


Fig. 4. Molecular weights of the Fe(III)L-apoMb, Co(II)L-apoMb and Cu(II)L-apoMb estimated by gel chromatography on Sephadex G-75. Plot of elution volume Ve *vs.* log(molecular weights) of known proteins and model phthalocyanine complexes. Column size = 2 × 50 cm, equilibrated with 20 m*M* potassium phosphate pH 7.6.

Electrophoresis and Molecular Weight Estimation

Figure 4 shows the results of the molecular weight estimation of Fe(III)L-apoMb, Co(II)L-apoMb and Cu(II)L-apoMb, using the Sephadex G-75 gel-filtration technique. The plot of elution Ve against log(molecular weight) revealed that the molecular weight of the iron complex was approximately 19000, of the cobalt complex 18500 and of the copper complex 19600. These values are comparable with that of myoglobin which indicates that the model complexes are monomers.

The new complexes were all electrophoretically homogeneous and they had similar electrophoretic mobilities. These compounds migrated slower than native myoglobin and had mobilities of 0.92–0.96 relative to that of myoglobin.

Circular Dichroism Measurements

These were carried out in the range 200–250 nm, 300–400 nm and 600–700 nm. Protein samples were in 0.01 *M* phosphate buffer, pH 7.6. The circular dichroism method was used to measure the α-helical content of apomyoglobin and the recombined product. The negative circular dichroism band near 220 nm is associated with the helical structure of proteins [20]. All phthalocyanine complexes, like apomyoglobin, showed the negative ellipticity band at 220–222 nm and 208 nm. The intensities of these bands however are different (Fig. 5A). The ellipticities of Fe(III)L-apoMb, Co(II)L-apoMb, Cu(II)L-apoMb and of the native apomyoglobin are at 221 nm: -3.4×10^4, -3.6×10^4, -2.45×10^4 and -2.8×10^4 deg. cm^2 dmol^{-1} respectively, and at 208 nm: -3×10^4, -3.3×10^4, -2.3×10^4 and -2×10^4 deg. cm^2 dmol^{-1}, respectively.

The results indicate that barring the remote possibility of a transition virtually coincident with the n – π peptide transition, the difference between metal tetrasulfonated phthalocyanine–apomyoglobin complexes and apomyoglobin represents a difference in helix content. Recombination of the apomyoglobin with iron and cobalt tetrasulfonated

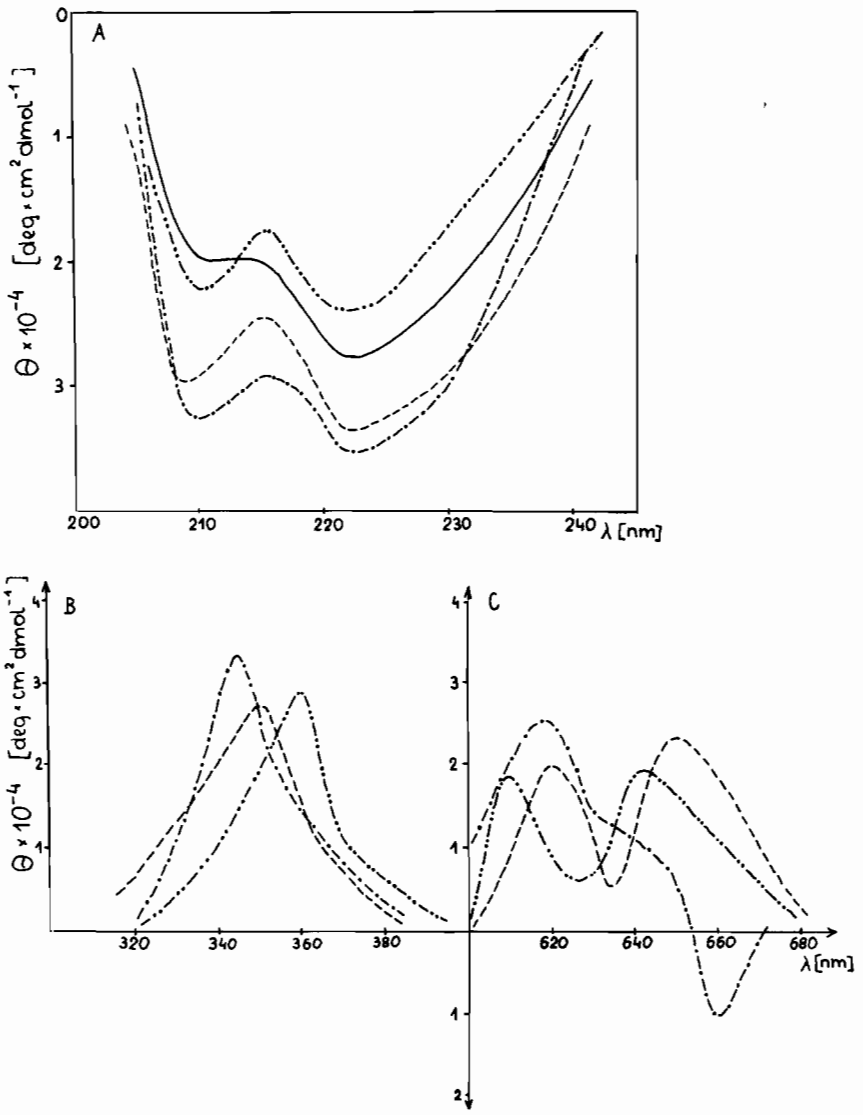


Fig. 5. Ultraviolet CD spectra of apoMb (——), Fe(III)L-apoMb (– – –) Co(II)L-apoMb (– · –) and Cu(II)L-apoMb (– · · –) in 10 m*M* phosphate buffer, pH 7.6 (A), in the Soret region (B) and in the visible region (C).

phthalocyanines increased the helical content of the apoprotein by about 18% and 22%, respectively.

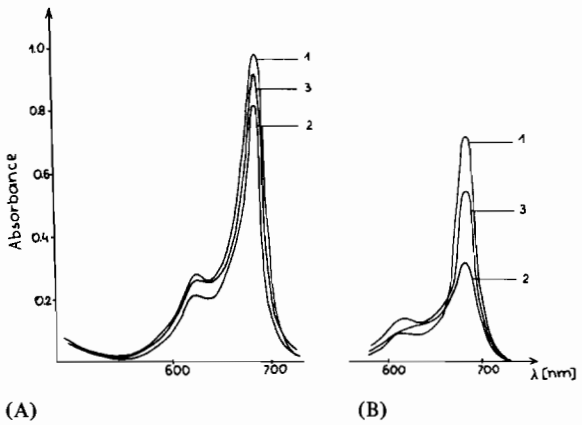
Recombination of the apomyoglobin with copper tetrasulfonated phthalocyanine decreased the α-helical content by about 10% compared with apomyoglobin.

In the Soret region (300–400 nm) the model complexes exhibit induced Cotton effects with the intensive positive CD band near 340 nm for the cobalt complex ($\theta = 3.4 \times 10^4$ deg. cm^2 dmol^{-1}), 350 nm for the ferric complex ($\theta = 2.8 \times 10^4$ deg. cm^2 dmol^{-1}) and 360 nm for the copper complex ($\theta = 3 \times 10^4$ deg. cm^2 dmol^{-1}) (Fig. 5B). The visible CD spectra of the complexes are characterized by one positive band at about 620 nm ($\theta = 2.6 \times 10^4$ deg. cm^2 dmol^{-1}) and another negative CD band at 660 nm ($\theta = 1 \times 10^4$ deg. cm^2 dmol^{-1}) for the cobaltic compound and two positive CD bands for the ferric complex ($\theta_{625\ nm} = 1.9 \times 10^4$ deg. cm^2 dmol^{-1}, $\theta_{650\ nm} = 2.4 \times 10^4$ deg. cm^2 dmol^{-1}) and copper complex ($\theta_{609\ nm} = 1.8 \times 10^4$ deg. cm^2 dmol^{-1}, $\theta_{642\ nm} = 1.9 \times 10^4$ deg. cm^2 dmol^{-1}) (Fig. 5C).

These spectra are associated with absorption bands of the examined complexes.

Reactions with Additional Ligands

The affinity of the model complexes for additional ligands was studied spectroscopically. The complexes of iron(II) and cobalt(II) tetrasulfonated phthalocyanines with apomyoglobin have the ability to perform reversible combination with oxygen as shown in Fig. 6, indicated by the change in absorbance between



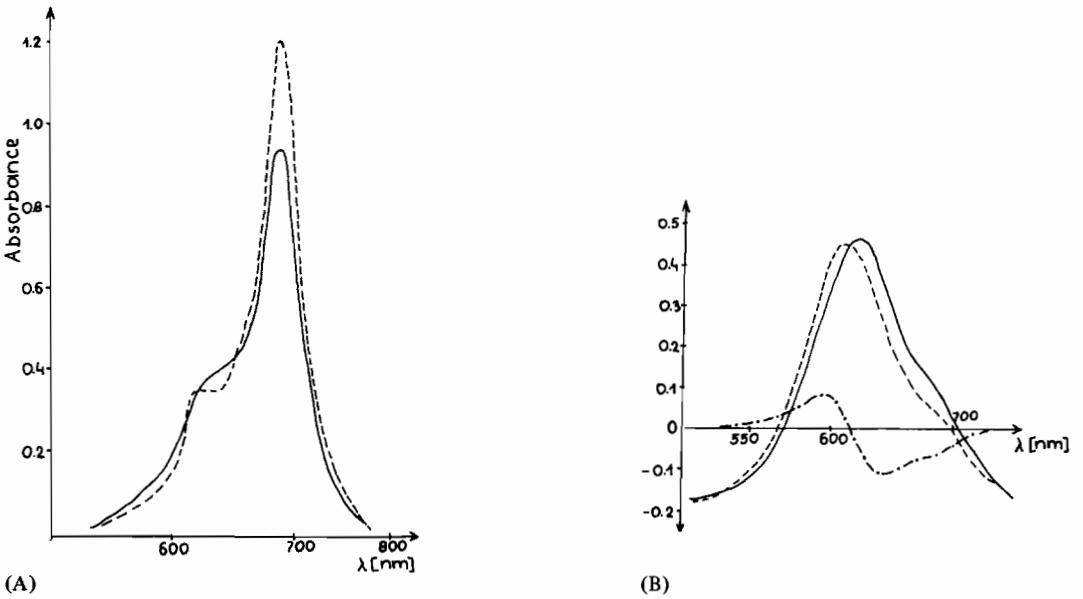
(A) (B)

Fig. 6. Absorption spectra of Fe(II)L-apoMb (A) and Co(II)-L-apoMb (B) in 10 m*M* phosphate buffer, pH 7.6, in argon and oxygen atmosphere: 1, fresh solution; 2, after 60 min bubbling with argon; 3, after 30 min bubbling with oxygen. Concentration of Fe(II)L-apoMb = Co(II)L-apoMb = 10 m*M*.

670–680 nm in an argon atmosphere. The metal–oxygen bond is stronger in iron compounds than in the corresponding cobalt compounds. The copper compound is not able to combine reversibly with oxygen.

These compounds also form complexes with other additional ligands such as CN^-, F^-, and N_3. In the case of the Fe(II) and Co(II) compounds the addition of cyanide results in an increase in their absorption bands, Fig. 7A. The exposure of Cu(II)L-apoMb to cyanide causes a change in electronic spectrum shift absorption maxima towards shorter wavelengths, Fig. 7B. Oxygen and argon were not able to displace the cyanide ion. The reduction of the Fe(III)L-apoMb complex with dithionite involves a direct attack on the iron(III) atom by the reductant. The spectrum of the ferric compound with apomyoglobin reduced with dithionite is identical to that of the ferrous one (Fig. 8). In the case of unbonded Fe(III)L the mechanism of the reduction process suggests that dithionite attacks the complex at the phthalocyanine ligand edge. This suggests that the phthalocyanine compound is located deep inside the protein and that the reductant attacks only the iron atom instead of the phthalocyanine ring. The complex Fe(II)L-apoMb is stable and shows outoxidation. This indicates that coordination with protein stabilized the two-step oxidation of the iron. In the case of the Cu(II)L-apoMb compound the oxidation and reduction involve only the ligand orbitals. The spectra of the Cu(II)L-apoMb complex with dithionite show a two-electron reduction product, with maxima at 530 nm and 600–700 nm (Fig. 8). On exposure of the solution to oxygen the reduced species spectra were replaced by the oxidation product, which was reflected in a spectrum with only one peak in the 630 nm region. The reduction of unbonded Cu(II)L with dithionite involves a decomposition of complexes with time. The reduced species of Cu(II)L-apoMb did not show the same decomposition. These results show that coordination with protein stabilized the ligand π system.

The reduced species of Co(II)L-apoMb showed a spectrum similar to that of reduced unbonded



(A) (B)

Fig. 7. Absorption spectra of Co(II)L-apoMb (A) and Cu(II)L-apoMb (B) in phosphate buffer, pH 7.6, with KCN. Me(II)L-apoMb (——), Me(II)L-apoMb + CN^- (– – –), difference spectrum Cu(II)L-apoMb + CN^- against Cu(II)L-apoMb (– · –). Concentration of Co(II)L-apoMb = 10 m*M*, concentration of Cu(II)L-apoMb = 5 m*M*.

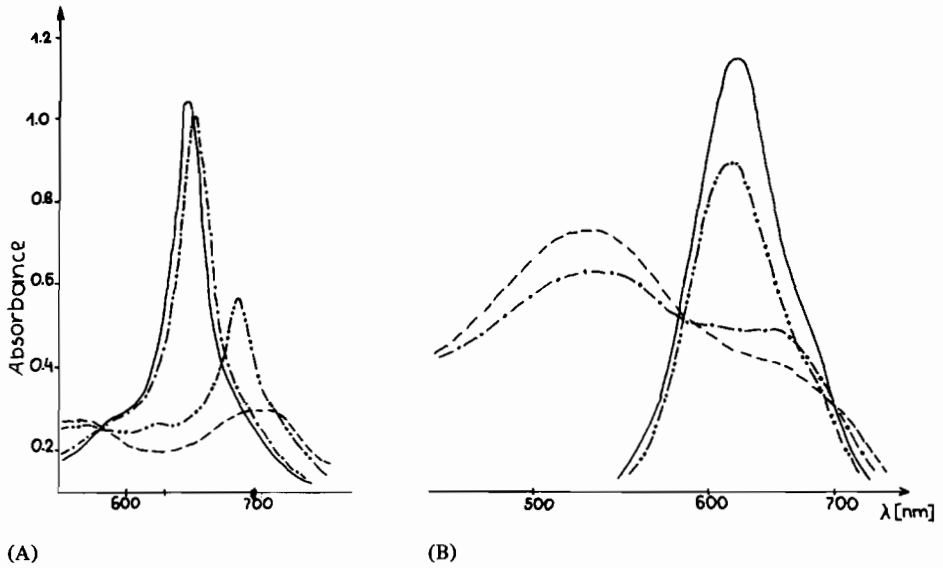


Fig. 8. Absorption spectra of Fe(III)L-apoMb and Cu(II)L-apoMb in phosphate buffer, pH 7.6 with dithionite. (A) Fe(III)L (——), Fe(III)L + $Na_2S_2O_4$ (– – –), Fe(III)L-apoMb (– · –), Fe(III)L-apoMb + $Na_2S_2O_4$ (– ·· –). Concentration of Fe(III)L-apoMb = 10 m*M*. (B) Cu(II)L-apoMb (——), Cu(II)L-apoMb + $Na_2S_2O_4$ in argon atmosphere (– – –), Cu(II)L-apoMb + $Na_2S_2O_4$ in oxygen atmosphere recorded after 10 min (– · –). Concentration of Cu(II)L-apoMb = 10 m*M* after 2 h (– ·· –).

Co(II)L. The reduction of the Co(II)L complex is assigned to the Co(II)–Co(I) process [21]. This process is reversible.

Interaction with Cytochrome C

Spectrophotometric studies of a mixture of the oxidized form of Fe(II)L-apoMb and cytochrome c revealed that Fe(II)L-apoMb is able to reduce ferricytochrome c. The changes in the spectra of a mixture of Fe(II)L-apoMb and ferricytochrome c in the Soret region and in the range 500–600 nm are shown in Fig. 9. The decrease at 406 nm and increase at 422 nm were attributed to the transformation from ferri- to ferro-cytochrome c. When the reaction was carried out under argon, the increase at 422 and 521 nm, 550 nm was much faster than in the presence of oxygen. This suggested that the reacting species was deoxy-Fe(II)L-apoMb rather than the oxy-compound. The changes in the phthalocyanine absorption region indicate that Fe(II)L-apoMb (680 nm) was oxidized to Fe(III)L-apoMb (650 nm).

Reaction of Model Complexes with Hydrogen Peroxide

The catalytic activities of ferric and cobalt tetrasulfonated phthalocyanines, hemin and catalase are compared and the mechanism of the reactions is known [22, 23].

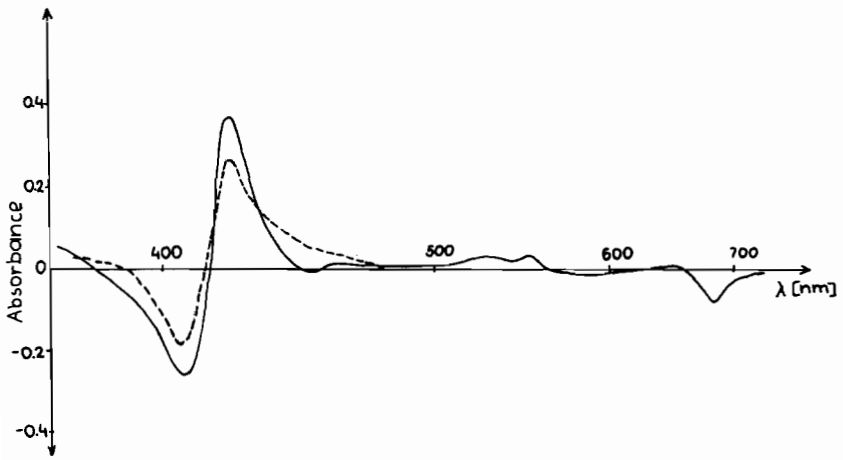


Fig. 9. Difference spectra of a mixture of Fe(II)L-apoMb and ferricytochrome c against unmixed solutions: in anaerobic conditions (——), in oxygen atmosphere (– – –). Concentration of Fe(II)L-apoMb = ferricytochrome c = 30 m*M*.

The catalase-like activity of Cu(II) complexes is strongly dependent on the saturation degree of the coordination sphere. In aqueous solution Cu(II) usually has a coordination number of 4 and forms inactive complexes [24, 25].

The Fe(III)L and Co(III)L catalyzed decomposition of H_2O_2 in phosphate buffer, pH 7.6, occurs with partial decomposition of the complexes. The catalyst in this system is a monomer complex and hence the catalase-like activity of tetrasulfonated phthalocyanines in aqueous solutions is small. The catalase-like activity of Cu(II)L is not observed. The model complexes with apomyoglobin catalyzed the decomposition of H_2O_2. The results of the catalase-like activity determination show the rate of decomposition of H_2O_2 expressed in terms of Kat. F was to be: 660 for Cu(II)L-apoMb, 850 for Co(II)-L-apoMb and 1000 for Fe(III)L-apoMb.

The model complex apomyoglobin containing Fe(III)L reacted with hydrogen peroxide to form 'peroxide compound'. This oxygenated derivative exhibited absorption spectra essentially similar to those of the Fe(II)L-apoMb, with the maxima at 680 nm, Fig. 10. These results could be compared with those for metmyoglobin derivatives [26, 27].

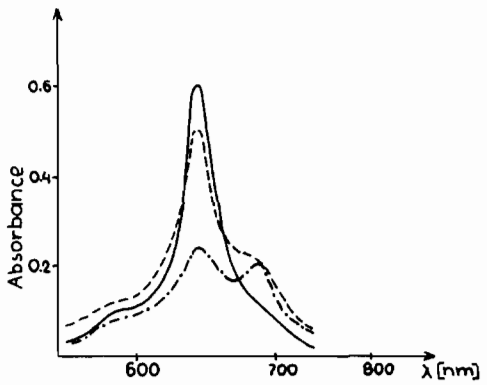


Fig. 10. Absorption spectra of Fe(III)L-apoMb in borate buffer, pH 8.5 with hydrogen peroxide. Fe(III)L-apoMb (——), Fe(III)L-apoMb + H_2O_2 immediately after mixing the reagents (– – –), after 2 h of the reaction run (– · –).

Conclusions

In myoglobin and in similar hemoproteins, the heme group is bound to the polypeptide chains by non-covalent and reversible dissociation into heme and apoproteins may be obtained [28]. The reversible nature of heme-globin association in myoglobin can be demonstrated by the displacement of heme in myoglobin by phthalocyanine derivatives. The difference spectra of myoglobin with Fe(III)L, Co(II)L and Cu(II)L show that heme is partially displaced by phthalocyanine compounds. In all cases the Soret band characteristic for the free hemo-protein disappears and new bands in the region of phthalocyanines absorbance are seen at the same time. This suggests that the metal phthalocyanine binding site in apomyoglobin is the same as that of heme. The reaction between myoglobin and phthalocyanine derivative is dependent on the pH of the medium. The fact that the optimum pH of this reaction was 6.5 suggested that displacement of heme by phthalocyanine depended on dissociation of the myoglobin into heme and globin. It is known from previous investigations [29] that tetrasulfonated phthalocyanines exist in aqueous solution as dimers. The stability of the dimers was found to decrease in the order: CuPTS > FePTS > CoPTS. It involves the differences in the displacement reaction of myoglobin with these phthalocyanine derivatives.

The Soret band at 418 nm characteristic for myoglobin decayed more quickly in the reaction with CoPTS than with CuPTS.

These results are compared with our previous investigations [11] which suggest that the monomer is a species displacing heme from hemoglobin.

The interaction between iron, cobalt and copper tetrasulfonated phthalocyanines and apomyoglobin leads to the formation of artificial myoglobin containing phthalocyanine derivatives in place of protoheme. Molecular weight and electrofocusing demonstrate that these compounds are single chains. Their approximate molecular weight (18500–19600) is close to that of myoglobin. Incorporation of the metal tetrasulfonated phthalocyanine into apomyoglobin changes the conformation of a protein.

Recent studies of the myoglobin and its derivative apoprotein have demonstrated the significant differences between these two proteins [30, 31]. The helical content of myoglobin declines from about 70% to 50% on removal of heme [32]. It has also been shown that myoglobins reconstituted with metalloporphyrins different from protoheme all have the same optical activity in the far ultraviolet [33].

The same effect is observed in the combination of metal tetrasulfonated phthalocyanines with apomyoglobin. Calculations of the helical contents from the present CD spectra show that addition of Fe(III)L and Co(II)L to apomyoglobin results in an increase in helix content of about 18% and 22%, respectively. This points to the stabilization of the secondary structure of the protein by phthalocyanine complexes, like metalloporphyrins. CD studies confirmed the presence of a small but definite conformational difference between Fe(III)L-apoMb and Co(II)L-apoMb. This difference is probably the result of differences in the dissociation process which determines the mechanism of the reaction, as suggested earlier [11], and involves conformational differences in these complexes. The complexes Fe(II)L-apoMb and Co(II)L-apoMb show reversible oxygen binding. These complexes are stable and

resistant to oxidation, unlike the free Fe(II)L and Co(II)L [34].

The removal of oxygen from the oxygenated phthalocyanine compound is more difficult than in the model complexes with apomyoglobin. The metal-oxygen bond is stronger in the iron compound than in the corresponding cobalt compound. The copper compound is not able to combine reversibly with oxygen. All these compounds have affinity with additional ligands such as CN^-, F^-, N_3.

The reduction of Fe(III)L-apoMb with dithionite and oxidation of Fe(II)L-apoMb with ferricytochrome c involves a direct attack on the iron atom. The reaction between unbonded Fe(III)L and dithionite and the oxidation of Fe(II)L with ferricytochrome c involves decomposition of the phthalocyanine ligand. That suggests that the phthalocyanine compound is located deep inside the protein and that a protein stabilized phthalocyanine ring is formed by providing a hydrophobic environment for this complex. The interpretation of the results of the studies on redox properties of the new modified myoglobins leads to the conclusion that the apomyoglobin in its combination with iron and cobalt tetrasulfonated phthalocyanines stabilized the oxidation state (+2) of the metal ions. Coordination of copper tetrasulfonated phthalocyanine with apomyoglobin stabilized the ligand π-system phthalocyanine. The model complex apomyoglobin containing Fe(III)L reacted with hydroperoxide to form 'peroxide compound'. It is suggested that the iron is in the oxidation state rather than in the ferric state, and that the phthalocyanine structure may be modified by the reaction with hydroperoxide.

The combination of apomyoglobin with copper tetrasulfonated phthalocyanine decreases the helical content of the protein and gives values for the α-helical structure lower by about 10% compared with those of apomyoglobin.

The binding of additional ligands and catalase-like activity of Cu(II)L-apoMb indicate that the copper complex, which is of the d^9 type, exhibits considerable Jahn-Teller distortion. Deformation of the Cu(II)L molecule should give rise to conformational changes in the apoprotein that could account for a particular tendency of Cu(II)L-apoMb to denaturation.

The lower content of the helical structure in the Cu(II)L-apoMb is in agreement with suggested conformational changes of protein due to the change of the Cu(II) ion electronic configuration.

The results presented here, although incomplete, show that iron and cobalt tetrasulfonated phthalocyanine-apomyoglobin complexes may serve as models of myoglobin. The physiological function of myoglobin as an oxygen cannot be performed when its heme is displaced by copper tetrasulfonated phthalocyanine.

Therefore the reaction between model complex Fe(II)L-apoMb and ferricytochrome c may serve as a model system for exploring the mechanism of electron transport between hemoprotein molecules. Structural investigations of these complexes are in progress.

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