Ruthenium Carbonyl Porphyrin as a Probe of Tertiary Structure of Myoglobin: Conformation of Bound Carbon Monoxide in Solution†

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Received 19 February 1979; accepted 23 April 1979

The ruthenium(II) carbonyl mesoporphyrin IX interacts with apomyoglobin in neutral pH forming a 1 : 1 protein complex. This protein complex shows a normal Soret, a split α band (separation 7.5 nm), and a broad β band in its absorption spectrum at neutral pH. The separation of α bands depends upon pH, becoming smaller as the pH is lowered from 8.0 to 4.5. The plot of this separation versus pH (8-4) is an S-shaped curve with a pKα value of 5.6. This pKα value suggests that the distal histidine is the titrable group. The splitting of α band and broadening of β band in the protein complex have been interpreted in terms of lowering of square planar symmetry of metal porphyrin as a result of constraint of distal histidine on linear RuCO grouping forcing it off the axis normal to the mean porphyrin plane. The denaturing studies on the protein complex by acid or urea show that there is no constraint of the distal histidine of RuCO grouping in the unfolded protein complex and thus the axis of linear RuCO grouping must be coincident with the axis normal to the mean porphyrin plane in the unfolded protein complex.

Materials and Methods

The preparation and purification of carbon monoxide complex of ruthenium(II) mesoporphyrin IX [Ru(II)(CO)MPIX] have been discussed elsewhere. The preparation and purification of carbon monoxide complex of ruthenium(II) myoglobin [Ru(II)(CO)Mb] was described earlier. The following instruments were used: Radiometer model 2S pH meter, Perkin-Elmer 124 and 323 spectrometers, Perkin-Elmer-Hitachi MPF-2A fluorescence spectrometer, and Perkin-Elmer 521 infrared spectrophotometer.

Results and Discussion

The Ru(II)(CO)MPIX forms a 1 : 1 complex with apomyoglobin. This complex shows three bands in the visible region at 554, 546.5, and 519 (broad) nm, and a Soret band at 398.5 nm as shown in Fig. 1. In this complex, the α band appears as split bands having absorption maxima at 554.5 and 546.5 nm and a separation between them of about 7.5 nm. On the other hand this complex in pyridine/NaOH shows normal α and β bands and a normal Soret band (Fig. 1). The separation between α bands is sensitive to changes in pH of the protein complex from 8 to 4. These changes are reversible. When the separation between maxima of two α bands is plotted as a function of pH in the range 8 to 4.6, an S-shaped curve is obtained. The pKα value of this heme-linked protonation group is 5.6 (50% of total change in separation between two α bands) and is very close to that of CO-myglobin (5.67) and is assigned to distal histidine.

Thus, the distal histidine is involved in regulating the changes in the separation of two α bands in the protein complex. The distal histidine and valine are present at the mouth of the ligand pocket in the protein complex and, therefore, their constraints force the linear RuCO grouping off the axis normal to the mean porphyrin plane. This results in lowering the symmetry of metalloporphyrin from D₅₃ to D₅₇ (ref. 7), which is responsible for two α bands and a broad β band. The distal histidine, present in the protein complex in the hydrophobic region, on protonation as a result of lowering of pH of the protein complex moves away from hydrophobic region to hydrophilic region, thereby releasing the constraint of the distal histidine on the RuCo grouping, which is responsible for the reduction of separation between two α bands on lowering the pH. The constraint of the distal histidine is completely released at pH 4 or below where the protein is unfolded. In the unfolded protein, a normal Soret and a normal α and β bands have been found, which is, in fact, expected in the absence of constraint of distal histidine. In order to check the above model the effect of

denaturing agents, e.g. urea, on the protein structure has been studied. Urea acts on a large part by a hydrophobic mechanism that favours exposure to the solvent of nonpolar groups in the interior of the protein molecule. The hydrogen atoms on the nitrogen atoms of urea with hydrogen bonding capability, also appear to contribute to some extent in denaturing action of this substance. The absorption spectra of Ru(II)(CO)Mb in the presence of 0, 4, and 8 M urea are shown in Fig. 2. In 4 M
urea, only a change in the visible region is observed, similar to the change observed in 2M NaCl (ref. 3). It implies that the folded form of the protein complex is different in 4 M urea than in absence of urea. In 8 M urea, the spectrum is changed markedly in both the Soret and the visible regions because of complete unfolding of protein. The extinction coefficient of the Soret band in 8 M urea is reduced and normal \( \alpha \) and \( \beta \) bands are observed with reduction of their extinction coefficients. In order to check that the changes in the absorption spectrum of Ru(II)(CO)Mb in 8 M urea is due to unfolding of protein, the fluorescence emission spectra of the protein in 0.4, and 8 M urea have been measured and are shown in Fig. 3. The intensities and positions of fluorescence emission maxima vary with exposure of tryptophan and tyrosine to an aqueous environment on increasing the urea concentration from 0 to 8M, thereby supporting the contention that in 8 M urea, the protein is completely unfolded. The denatured Ru(II)(CO)Mb in 8 M urea is found to revert to a folded conformation on passing it through a Sephadex G-25 column equilibrated with 0.1 M potassium phosphate of pH 7. This shows that the folding and unfolding process is reversible. The Co.Ru(II)-myoglobin and metmyoglobin\(^{12}\) behave very similar to urea denaturation and renaturation. In the unfolded Co.Ru(II)-myoglobin, there is no constraint of distal histidine on the linear RuCO grouping because of loss of tertiary and secondary structures in the protein complex. This gives rise to normal \( \alpha \) and \( \beta \) bands and a normal Soret band which are expected for \( D_{4h} \) symmetry of metalloporphyrin. In ligated Ru(II)(CO)MPPIX complex, the axis of linear RuCO grouping is coincident with the axis normal to the mean porphyrin plane\(^{13}\). The pyridine complex of Ru(II)(CO)MPPIX and the unfolded protein complex show very similar absorption spectra and this suggests the conformation of bound CO in both to be similar.

This work demonstrates that the tertiary structure of myoglobin controls the electronic structure of its prosthetic group by putting constraint on one of the axial ligands attached to the iron atom.

References