Spectral properties of cadmium porphyrin apomyoglobin and apohemoglobin complexes

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(Received March 6, 1991)

Abstract

Electronic spectra of cadmium porphyrin in various non-coordinating solvents were studied and found to depend on the refractive index of the solvent. The equilibrium constants of cadmium porphyrin with the nitrogen atoms of three ligands to form five-coordinated species were compared. Complexes of cadmium deuteroporphyrin with apohemoglobin and apomyoglobin were then prepared and studied by electronic spectroscopy. Small red shifts in the absorption spectra with concomitant changes in the ratio of the Q_0 to Q_1 bands were observed for both complexes. The maximum absorption bands of the two species were similar which suggests that any red shift is due primarily to the change in the coordination state of the cadmium from four to five and not the difference in the hydrophobic environment between the two proteins.

Introduction

The physical and chemical properties of Cd porphyrin have been of general interest because the Cd ion is a large, filled d-subshell ion which behaves like a good transmetalation precursor and can therefore be easily displaced by other atoms [1]. This displacement enables it to be used in the analysis of low levels of manganese [2], copper, cobalt, nickel and zinc. Another area of interest is the utilization of Cd porphyrin as a probe for the study of the stress placed at the metal site upon the $R \rightleftharpoons T$ allosteric transformation in hemoglobin [3]. Various techniques have been used to elucidate these properties and by far the most common one is the spectrometric method.

It has been found that the electronic spectra of Cd porphyrin are dependent upon the nature of the axial ligands [4] and hence this metalloporphyrin is a natural reporter of its immediate environment in hemoproteins. Furthermore, the metal exists solely in the +2 oxidation state and only one axial ligand can be coordinated to the Cd metal to form five-coordinated complexes. This makes interpretation of the spectra relatively unambiguous. However certain factors like the environment and the interaction between the peripheral groups of the porphyrin with the protein can possibly affect these spectra as well.

This paper reports the preparation and spectral properties of Cd porphyrin under different hydrophobic environments, in particular apohemoglobin and apomyoglobin. The equilibrium constants of Cd porphyrin with different nitrogenous ligands are also measured in order to determine the stability of five-coordinated Cd porphyrin–ligand complexes. Results from other metal substituted myoglobin and hemoglobin allow a detailed comparison to be made of the environment effects and the role of cadmium in biological systems to be ascertained.

Experimental*

$(TPP)H_2$ was prepared by the condensation of pyrrole and benzaldehyde followed by purification using a procedure adapted from Barnett *et al.* [5]. CdTPP (Fig. 1(a)) was obtained by refluxing $(TPP)H_2$ with $Cd(NO_3)_2$ in dimethylformamide [6]. It was recrystallized from toluene and dried under vacuum at 100 °C for a few days until there was no change in weight. The purity was checked by means of extinction coefficients and atomic absorption [7].

DPDME was synthesized from protohaemin and the purity checked by means of the melting point [8]. CdDPDME was prepared from DPDME and $Cd(NO_3)_2$ in dimethylformamide and the reaction was completed when the solution showed no ab-

*Abbreviations: TPP = tetraphenylporphyrin, DP = deuteroporphyrin, Mb = apomyoglobin, Hb = apohemoglobin.

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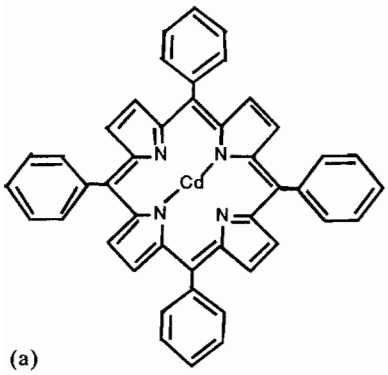
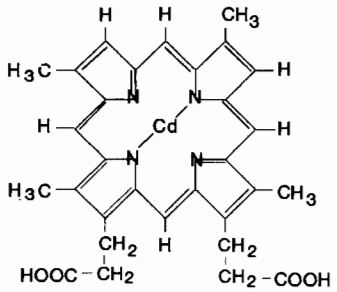


(a)



(b)

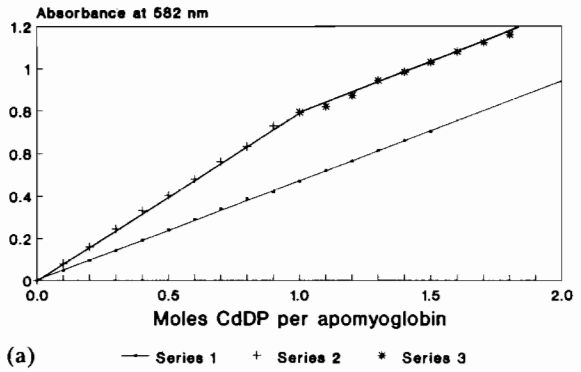
Fig. 1. Structure of (a) cadmium tetraphenylporphyrin (CdTPP); (b) cadmium deuteroporphyrin (CdDP).

sorption at 621 nm. Base hydrolysis by NaOH gave the desired CdDP (Fig. 1(b)); the purity was checked by visible spectroscopy as well as by thin layer chromatography whereby a single spot was obtained.

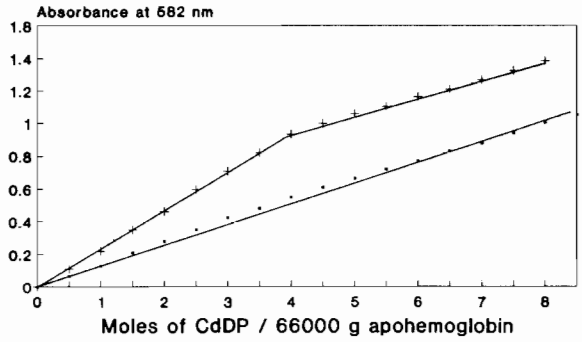
Sperm whale met-myoglobin was purchased from Sigma Chemical Company. Apomyoglobin was prepared by removing the heme group of met-myoglobin using 2-butanone [9]. Apohemoglobin was prepared from chicken blood and used within 5 days of preparation [10].

Standard solutions of CdDP in 0.05 M pyridine–water were titrated against standard apomyoglobin and apohemoglobin at 582 nm in order to determine the stoichiometry of the combination of these species (Fig. 2(a) and (b)). The concentrations of apoprotein used were between 4 and 10 μM.

Reconstitution of CdDP and apoprotein was carried out by the addition of a two-fold excess of standard CdDP in 0.05 M pyridine–water to standard apoprotein with gentle stirring. The concentrations of apo-Mb and apo-Hb were determined by the extinction coefficients of 15.8 mM [11] and 16.2 [12] mM cm^{-1}. Within half an hour, the mixture was placed on a long Sephadex G-25 column which was equilibrated with 10 mM phosphate solution to remove the pyridine and excess CdDP. The reconstituted complex was then passed through a carboxymethylcellulose column of pH 7 to remove any CdDP loosely bound to the surface of the protein. The



(a)



(b)

Fig. 2. Spectrophotometric titration of (a) apomyoglobin, (b) apohemoglobin, with CdDP in 0.1 M phosphate of pH = 7.0.

CdDP–protein complex was then eluted as a clean band with 10 mM phosphate solution and dialysed against 10 mM phosphate. Evidence for only one reconstituted product was obtained by the flow method [13]. All precipitate formed was removed either by filtering or centrifuging.

Analytical grade pyridine, piperidine, 1-methylimidazole, toluene and other neat solvents were used for the experiments and freshly distilled. Toluene and dimethylformamide were dried by using activated molecular sieves (4 Å) under a dry nitrogen atmosphere.

UV–Vis spectra were measured at controlled room temperature of 22.0 ± 0.1 °C using a Shimadzu spectrometer and 1.0 cm quartz cell. Reagents and apparatus were covered with aluminium foil and kept away from strong light. All protein preparatory work was carried out at room temperatures between 0 and 4 °C. Freshly prepared CdTPP in toluene was used for the equilibrium constant studies and used within 5 h in duplicate.

Results

Electronic spectra of CdTPP in neat solvents and with ligands

The visible absorption spectrum of CdTPP is strongly dependent upon the nature of the solvent

TABLE 1. Electronic absorption spectra of CdTPP in neat solvents

Solvent	Soret	Q_1	Q_0	Frequency of Q_0	$(n^2-1)/(2n^2+1)$
CH_2Cl_2	430.0	562.75	603.75	16653	0.2032
Cyclohexane	430.25	564.0	605.0	16528	0.2042
$CHCl_3$	432.75	569.0	609.5	16407	0.2105
Cyclohexene	433.0	569.5	610.0	16393	0.2107
CCl_4	428.0	562.0	601.5	16625	0.2159
Toluene	431.75	566.0	607.5	16461	0.2261
Benzene	433.25	567.5	608.75	16427	0.2276

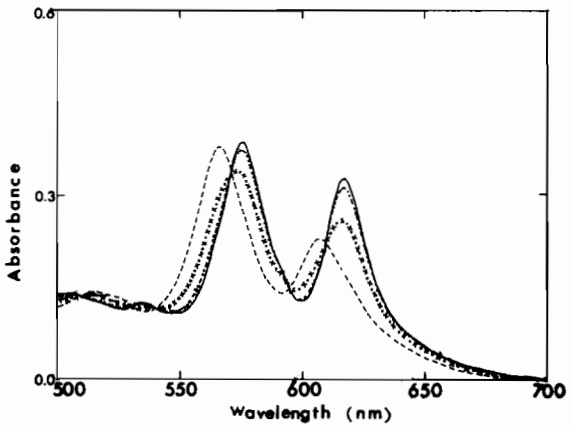


Fig. 3. Electronic absorption spectra of CdTPP under various 1-methylimidazole concentrations at 22.0 °C to determine the equilibrium constant.

TABLE 2. Equilibrium constants of ligands with CdTPP

Ligand	pK_a	Equilibrium constant
1-Methylimidazole	7.33	5.23 ± 0.03
Piperidine	11.1	4.18 ± 0.04
Pyridine	5.29	3.51 ± 0.05

it is dissolved in (Table 1). Addition of ligands to CdTPP in toluene give absorption spectra that are red-shifted relative to the CdTPP itself. Distinct isosbestic points for each ligand were observed and showed the presence of an equilibrium reaction between the CdTPP and that ligand (Fig. 3). This equilibrium constant was then determined according to the methods by Miller and Dorough [14] (Table 2).

Electronic spectra of CdDP–Mb and CdDP–Hb species

The sharp end-points obtained for the two titration graphs showed that well-defined CdDP–Mb and CdDP–Hb complexes in the ratio 1:1 and 1:4 were

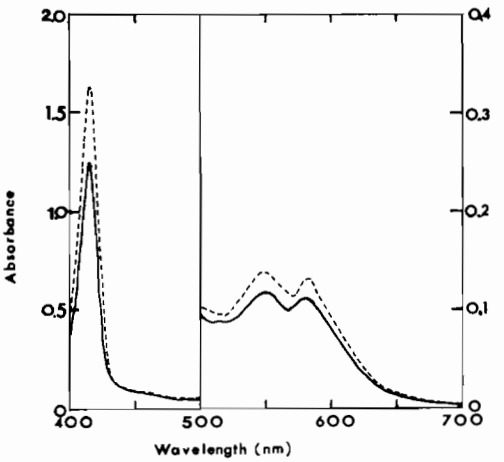


Fig. 4. Electronic absorption spectra of CdDP–Hb (——) and CdDP–Mb (– – –) in 0.02 M phosphate buffer of pH = 7.0.

formed. The absorption maxima wavelengths of both these complexes are similar (Fig. 4). There are red shifts in both complexes with respect to CdDP in $CHCl_3$–dimethylformamide solution. A new band at 280 nm which characterizes the presence of protein is observed. The ratio of the Q_0 to Q_1 bands is changed to a value of 0.94 for CdDP–Mb and 0.95 for CdDP–Hb (Table 3).

Discussion

The nature of the solvent affects the position of the bands in the absorption spectra by means of the solvation effect. This is seen when the position of the Q_0 band is plotted with $(n^2-1)/(2n^2+1)$ using the Bayliss method [15]. A linear plot which validates this effect is obtained (Fig. 5). This result is consistent with those obtained from ZnTPP by Nappa and Valentine [16]. The solvation effect affects the magnitude of the equilibrium constant between the metalloporphyrin and ligand significantly [17]. Generally, metalloporphyrins in a highly non-polar solvent have

TABLE 3. Electronic spectra of CdDP–protein complexes

Compound	Soret		Q_1		Q_0	
	λ_{max} (nm)	A[a]	λ_{max} (nm)	A	λ_{max} (nm)	A
CdDP–Mb	418	1.000	548.5	0.085	582	0.080
CdDP–Hb	418	1.000	548.5	0.105	582	0.101

[a]A = absorbance data normalized to 1.000 relative to the most intense transition.

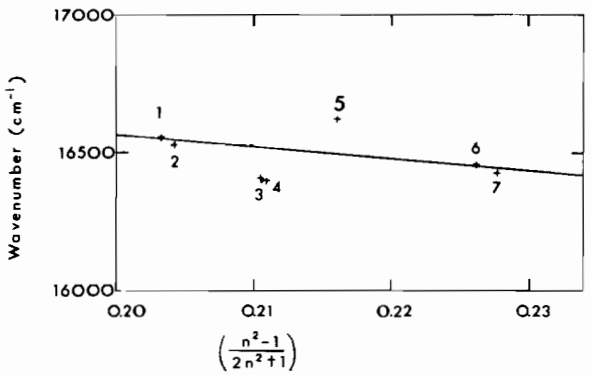


Fig. 5. Plot of the frequency of Q_0 band of CdTPP in neat solvents against $(n^2+1)/(2n^2+1)$ where n is the refractive index of the neat solvent.

a higher equilibrium constant than in a more polar solvent. In order to mimic the biological environment of apoprotein and to obtain an appropriate value for the equilibrium constant between Cd porphyrin and the apoprotein, toluene was used as the solvent.

The spectrum of four-coordinated CdTPP in toluene is a representative example of a normal metalloporphyrin. The change in the intensity ratio of Q_0 to Q_1 upon complexation and a red shift confirm the formation of a five-coordinated CdTPP complex. The order of the equilibrium constant is 1-methylimidazole > piperidine > pyridine. Kirksey and Hambright have shown that the equilibrium constants of CdTPP with certain ligands obey a linear relationship with the pK_a of these ligands [4]. However this order here does not fully correlate with that of the basicity of the ligands alone, where the pK_a values are piperidine > 1-methylimidazole > pyridine. X-ray studies of the CdTPP(piperidine) complex indicate that the nitrogen lone pair electrons are directed towards the Cd atom [18]. The reverse order for 1-methylimidazole is probably due to the smaller ring angle of the imidazole which gives a bigger exposure of these nitrogen lone pair electrons of the imidazole to CdTPP, thereby leading to the higher equilibrium constant.

The large equilibrium constant with 1-methylimidazole suggests that when Cd porphyrin is added to apomyoglobin and apohemoglobin, it will be coordinated quickly into the nitrogen atoms of the hydrophobic protein environment. The red shift and the significant change in the intensity band ratio of Q_0 to Q_1 once again confirm that the fifth coordination sites of both CdDP–Mb and CdDP–Hb complexes are occupied by the nitrogen atoms of the imidazole groups in the F-8 histidine residues.

Several factors can enhance this red shift and of particular interest is whether the difference in the hydrophobic protein environment between apomyoglobin and apohemoglobin can exhibit this effect. The results from CdTPP in neat solvents exhibited significant differences in the positions of the electronic bands and hence there was a dependence upon the type of solvent environment. Romberg and Kassner have highlighted that for model heme complexes the red shift correlated with the polarity of the solvent [19] and any change in the polarity of the heme environment will therefore affect the red shift. In order to study this effect, other factors must be kept constant. These include interactions of the 2,4 disubstituents of the porphyrin ring with the protein environment [8] and the deprotonation of the coordinated imidazole side chain of the F-8 histidine [20].

X-ray studies show that in myoglobin, the heme pocket is more rigid than in hemoglobin [21]. Consequently, the peripheral substituents of the porphyrin ring are more likely to interact with the side chains of the amino acid groups in myoglobin than in hemoglobin. Sono and Asakura obtained direct evidence for this interaction by recombining two isomers, spirographis (2-formyl-4-vinyl-deuterohemin) and isospirographis (2-vinyl-4-formyl-deuterohemin) into apomyoglobin [22]. The oxygenated species of these two compounds showed significant differences in their absorption spectra, with the spirographis–Mb being more red-shifted than the isospirographis–Mb. This shows that there is more interaction between the protein and the peripheral groups at the 2-substituted position than the 4-substituted position. In natural hemoproteins, the 2,4 disubstituents are vinyl groups and, being electron-

withdrawing in behaviour, they can transfer charge to the side chains of the amino acid groups by direct charge transfer [23]. This affects the electronic cloud of the porphyrin ring and leads to a change in the red shift. To overcome this effect, Cd deuteroporphyrin, where the vinyl groups are substituted by hydrogen atoms, was used. This ensures that there is no electronic interaction and any steric effect is also eliminated [24].

Mincey and Traylor have shown that different imidazolate axial ligands red-shifted the absorption bands by different extents [20]. This effect by the deprotonation of the coordinated imidazole in the F-8 histidine of the heme pocket was kept constant by placing both CdDP–Mb and CdDP–Hb complexes in the same buffer of pH = 7. The degree of protonation or deprotonation in both complexes was then the same. Furthermore, increasing the pH to 9.28 did not produce any significant changes to the spectra of either complex.

The similarities in the band positions of CdDP–Mb and CdDP–Hb complexes suggest that the effect of the change in hydrophobic environment from apomyoglobin to apohemoglobin does not affect the electronic spectra significantly. In conclusion, any red shift in the electronic spectra of these complexes compared to those in organic solvents, is caused by the change in the coordination state of the Cd ion.

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