Effects of Solvent on the Absorption Maxima of Five-Coordinate Heme Complexes and Carbon Monoxide–Heme Complexes as Models for the Differential Spectral Properties of Hemoglobins and Myoglobins†

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ABSTRACT: Absorption spectra were recorded for 5- and 6-coordinate model ferrous heme complexes of hindered and unhindered ligands in aqueous, mixed aqueous, and detergent solutions. Heme complexes exhibited differences in absorption maxima up to 6 nm which were correlated with the polarity of the heme environment. Increasing polarity of the solvent resulted in a general blue shift of absorption maxima of both deoxy- and (carbon monoxy)heme complexes. The differences in absorption maxima of heme complexes with different heme environments are offered as a possible explanation for some of the differences in absorption maxima among hemoproteins such as hemoglobin, myoglobin, and leghemoglobin.

There has been an extensive effort to understand the relationship between the structure and properties of hemoproteins. The absorption spectrum is one property which has been widely used to characterize the coordination center of hemoproteins. The absorption spectra of oxygen-carrying hemoproteins have been shown to have a visible and Soret band in the 5-coordinate form and α, β, and Soret bands in the 6-coordinate oxygen and carbon monoxide complexes (Antonini & Brunori, 1971). Differences are observed, however, in the wavelength maxima of these proteins. Horse deoxymyoglobin has absorption maxima at 560 and 435 nm, while human deoxyhemoglobin has maxima at 555 and 430 nm (Antonini & Brunori, 1971), and soybean deoxyleghemoglobin has absorption maxima at 556 and 427 nm (Imamura et al., 1972). Horse carboxymyoglobin has maxima at 579, 540, and 424 nm, while human carboxyhemoglobin has maxima at 569, 540, and 419 nm (Antonini & Brunori, 1971), and soybean carboxyleghemoglobin has maxima of 563, 538, and 417 nm (Imamura et al., 1972). Similar differences are observed between oxymyoglobin and oxyhemoglobin.

Some model systems exhibit spectra which closely match the spectra of some, but not all, forms of these proteins. Traylor (Traylor et al., 1979) has synthesized a chelated protoheme model that has maxima at 558 and 430 nm for the deoxy form and at 569, 540, and 420 nm for the carbon monoxide complex. The latter values correspond closely to the absorption maxima of the carbon monoxide complex of human hemoglobin. A model heme complex using the sterically hindered ligand 1,2-dimethylimidazole and protoheme dimethyl ester has maxima at 557 and 431 nm in the deoxy form and at 564, 538, and 417 nm in the carbon monoxy form (Wang & Brinigar, 1979). The latter values correspond to the absorption maxima of the carbon monoxide complex of soybean leghemoglobin.

Differences are also observed between the spectra of hemoglobins in different states. Difference spectra of hemoglobin Kempsey ± inositol hexaphosphate (IHP)[1] and the difference spectra of NES-des-Arg-deoxyhemoglobin ± IHP reflect changes in wavelength due to R- to T-state transitions (Perutz et al., 1974). Wang & Brinigar (1979) have attempted to explain these differences in terms of the strength of the iron–nitrogen bond by varying the basicity or steric hindrance of the axial ligand.

A number of other factors may contribute to the maxima of a particular protein, including hydrogen bonding or deprotonation of the proximal imidazole (Peisach, 1975; Morrison & Schonbaum, 1976; Mincey & Traylor, 1979; Valentine et al., 1979; Swartz et al., 1979), interactions of the porphyrin with the side chains of the protein (La Mar et al., 1978), distortion of the distal ligand by the heme pocket (Caughey, 1970), and polarity or dipole orientation effects of the heme environment. The effect of solvent on the wavelength maxima of metalloporphyrins is well-known (Falk, 1964). Dipole orientation has been suggested to account for polarity effects in hemoproteins (Chang & Traylor, 1975). However, the importance of these solvent effects on the absorption spectrum has not been adequately studied.

The effect of solvent on the wavelength maxima of the absorption spectra of model heme complexes with sterically hindered ligands and the nonsterically hindered ligand 1-methylimidazole was investigated in this study. Wavelength maxima were measured for the deoxy and carbon monoxy forms of several model heme complexes in water, ethylene glycol/water, ethanol/water, benzene, and detergents. The extent and direction of the change in maxima of the heme spectra were correlated with the polarity of the solvent.

Experimental Procedures

Materials. Protohemin, equine (type III), sodium dithionite, and 1-methylimidazole (1-MeIm) were purchased from Sigma Chemical Co. Protohemin dimethyl ester was prepared by esterifying protohemin with methanol using sulfuric acid according to the procedure given by Falk (1964). The ligands 2-methylimidazole and 1,2-dimethylimidazole were purchased from Aldrich Chemical Co. *tert*-Butylamine and hexadecyltrimethylammonium bromide (CTAB) were obtained from Eastman Chemicals. Sodium dodecyl sulfate ($NaDodSO_4$) was obtained from Pierce Chemicals. Tween 20 was purchased from Fisher Scientific. Ultrahigh purity carbon monoxide was purchased from Matheson Gas Products. The

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[1] Abbreviations: 1-MeIm, 1-methylimidazole; 2-MeIm, 2-methylimidazole; 1,2-Me_2Im, 1,2-dimethylimidazole; IHP, inositol hexaphosphate; CTAB, cetyltrimethylammonium bromide; $NaDodSO_4$, sodium dodecyl sulfate; NES, *N*-ethylsuccinimide.

0006-2960/82/0421-0880$01.25/0

Table I: Absorption Maxima (nm) of Heme Complexes in Different Solvents[a]

solvent	1-MeIm	2-MeIm	1,2-Me_2Im	*tert*-butylamine
H_2O[b]	556, 526, 422	553, 428	553, 428	555, 423
ethylene glycol/H_2O	557, 527, 423	554, 430	555, 430	556, 424
ethanol/H_2O	557, 527, 423	557, 431	557, 431	557, 424
2% CTAB	560, 530, 426	558, 433	557, 433	556, 426
2% Tween 20	560, 530, 426	557, 433	558, 433	556, 426
2% $NaDodSO_4$	560, 530, 426	558, 434	558, 434	556, 426
benzene	560, 530, 426	559, 430	559, 431	556, 426

[a] Heme concentrations were 6×10^{-6} M for Soret absorptions and 2×10^{-5} M for visible absorptions; all absorptions were taken at 23 ± 1 °C. [b] Values correspond to lowest concentration of heme measured.

Table II: Absorption Maxima (nm) of CO-Heme Complexes in Different Solvents[a]

solvent	1-MeIm	2-MeIm	1,2-Me_2Im	*tert*-butylamine
H_2O[b]	564, 534, 418	563, 536, 415	563, 536, 415	566, 536, 415
ethylene glycol/H_2O	565, 536, 418	564, 537, 418	563, 536, 417	566, 536, 415
ethanol/H_2O	566, 536, 418	564, 538, 418	563, 537, 417	566, 536, 415
2% CTAB	568, 539, 421	563, 541, 420	565, 540, 420	566, 536, 417
2% Tween 20	567, 539, 421	563, 541, 420	564, 539, 420	566, 536, 417
2% $NaDodSO_4$	568, 539, 421	563, 542, 420	564, 540, 420	566, 536, 417
benzene	561, 538, 422	564, 541, 420	564, 542, 420	566, 535, 417

[a] Heme concentrations were 6×10^{-6} M for Soret absorptions and 2×10^{-5} M for visible absorptions; all absorptions were taken at 23 ± 1 °C. [b] Values correspond to lowest concentration of heme measured.

ligands 1,2-dimethylimidazole and *tert*-butylamine were fractionally distilled before use.

Methods. The protohemin dimethyl ester was incorporated into the micelle by simultaneously dissolving both the protohemin dimethyl ester and the detergent in methanol. The methanol was evaporated and water added to the residue to form the detergent-solubilized heme solution. These solutions were placed in a modified Thunberg cuvette as previously described (Romberg & Kassner, 1979). The solutions were made anaerobic by two cycles of freezing, evacuating, and thawing followed by two cycles of freezing, evacuating, and equilibrating with nitrogen or argon.

Reduction was carried out with a minimum amount of a sodium dithionite solution or with a small amount of solid sodium dithionite in the side arm of the Thunberg cuvette. Both methods gave identical absorption maxima. It should be noted that excess sodium dithionite added to a solution in the presence of oxygen significantly degraded the heme and resulted in a weak absorption band at about 625 nm.

Heme complexes in benzene were prepared in a closed cuvette made of a rectangular glass with an upper 14/20 outer joint of ground glass and fitted with a Kontes 14/20 hypodermic injection inlet adaptor. The hemin dimethyl ester was reduced by adding 50 μL of sodium dithionite solution and then shaking the cuvette until reduction was complete. The cuvette was centrifuged to separate the two phases, yielding a clear benzene solution (Kassner, 1972).

Absorption spectra were recorded on a Cary 14R spectrophotometer with a scan rate of 1 Å/s. Each measurement was performed on at least three solutions to ensure reproducibility. In aqueous and mixed aqueous solutions, sterically hindered imidazole concentrations of 0.20 M were found to give a single visible absorption band characteristic of the 5-coordinate complexes (Wagner & Kassner, 1975; Collman & Reed, 1973; Brault & Rougee, 1974). No change was observed in the wavelength maxima upon increasing or decreasing the ligand concentration by 3-fold. A concentration of 1.0 M *tert*-butylamine was found to give one absorption band at 556 nm characteristic of the 5-coordinate complex. Ligand concentrations in benzene ranged from 0.20 to 0.002 M for 1,2-dimethylimidazole and 1-MeIm, from 0.02 to 0.002 M for 2-methylimidazole, and from 1.0 to 0.10 M for *tert*-butylamine. These ranges of concentrations of ligands were also found to give a single visible peak in benzene. The higher affinity of heme for 2-methylimidazole in benzene has been described in detail (Brault & Rougee, 1974).

Heme concentrations were determined by the pyridine hemochrome method as described by Falk (1964). Difference spectra were obtained by carefully preparing two solutions with identical heme concentrations.

Carbon monoxide complexes were prepared by passing CO over the solution until the CO–heme complex was completely formed as evidenced by no further change in the absorption spectra. All experiments were carried out at 23 ± 1 °C.

The possible formation of dimers was investigated for an aqueous solvent, and all other solvents used, by adding successive known aliquots of heme solution and then comparing the extinction coefficients and wavelength maxima. No change in these parameters with a 10-fold increase of heme concentration was taken as evidence that the heme was monomeric.

Results

Absorption maxima were measured for heme complexes of 2-methylimidazole, 1,2-dimethylimidazole, *tert*-butylamine, and 1-methylimidazole. Table I lists the absorption maxima of these complexes in the solvents water, ethylene glycol/H_2O (50:50), ethanol/H_2O (50:50), 2% CTAB, 2% $NaDodSO_4$, and 2% Tween 20. These values may be compared to the wavelength maxima of sperm whale myoglobin (556 and 434 nm), human hemoglobin (555 and 430 nm) (Antonini & Brunori, 1971), leghemoglobin (556 and 427 nm) (Imamura et al., 1972), and mono-3-(1-imidazoyl)propylamide–protoheme monomethyl ester in CTAB (558 and 430 nm) (Traylor et al., 1979). The absorption maxima for the corresponding CO–heme complexes are listed in Table II. Figure 1 shows the Soret and visible absorption spectra of the 1,2-dimethylimidazole–heme complex in the solvents 2% CTAB and ethylene glycol/H_2O (50:50). Figure 2 shows the CO–heme complex spectra for this model in both of these solvents.

A red shift is observed as the heme environment is varied from the polar ethylene glycol/H_2O (50:50) to the detergent and benzene solutions as seen in Tables I and II. The absorption spectra of the CO–heme complex in detergent micelles strongly resembles the absorption spectra of CO–heme com-

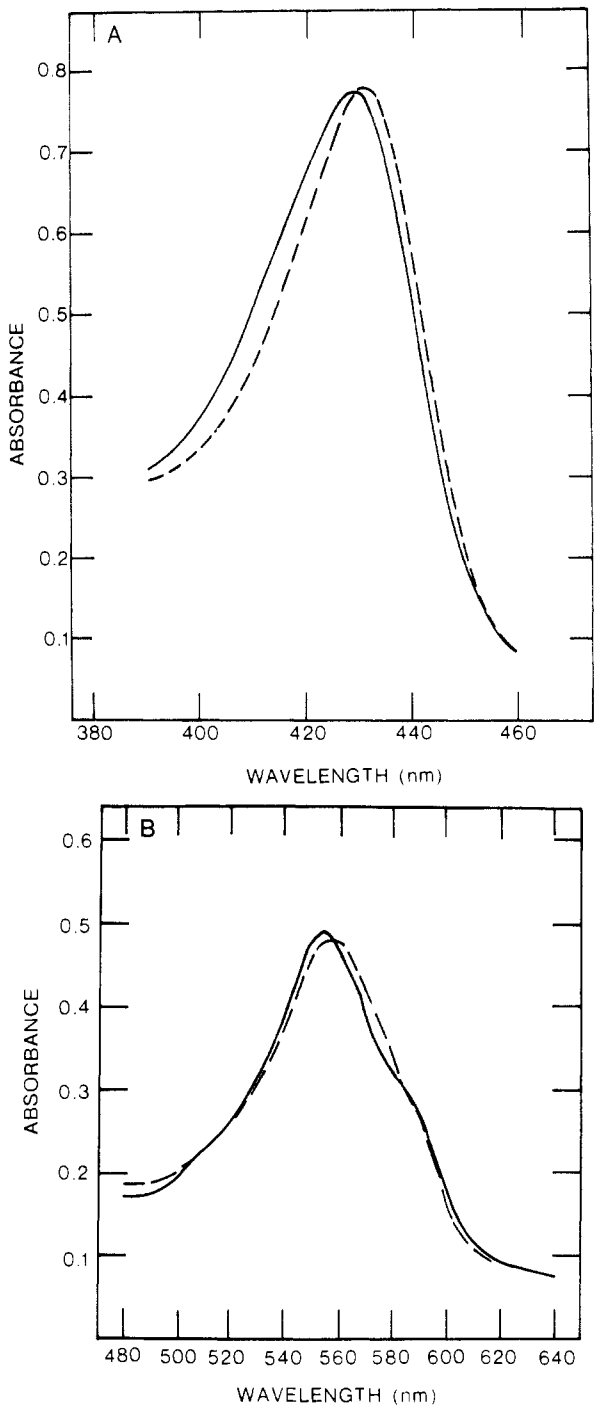

FIGURE 1: Soret (A) and visible (B) spectra of 1,2-dimethylimidazole protoheme in ethylene glycol/H_2O (50:50) (—) and 1,2-dimethylimidazole protoheme dimethyl ester in 2% CTAB (---).

plexes in benzene. The spectra of the deoxy complexes in detergent and benzene are similar in the visible region, but the Soret maxima of the sterically hindered imidazole complexes in benzene are 3 nm less than the observed Soret maxima for the same complex in the detergent systems. The absorption maxima of the heme complexes in ethylene glycol/H_2O (50:50) are similar or somewhat blue shifted relative to those in ethanol/H_2O. There is little variation between the cationic, anionic, and neutral detergents.

An oxyheme complex could not be observed at 23 °C; addition of O_2 led to oxidation as previously reported (Wang et al., 1958; Collman & Reed, 1973).

Difference Spectra. The difference spectra of 1,2-dimethylimidazole protoheme in solutions of ethylene glycol/H_2O (50:50) and 2% CTAB for the Soret and visible regions

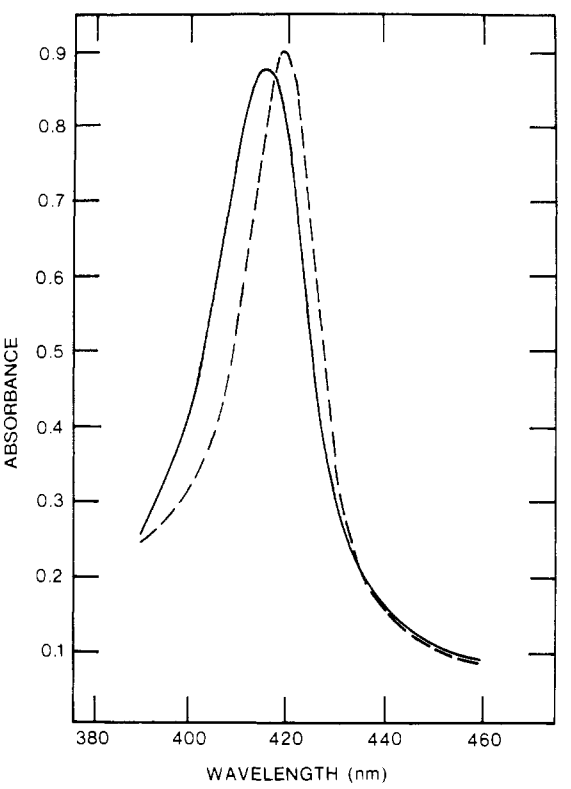

FIGURE 2: Soret spectra of (carbon monoxy)protoheme–1,2-dimethylimidazole in ethylene glycol/H_2O (50:50) (—) and (carbon monoxy)protoheme dimethyl ester–1,2-dimethylimidazole in 2% CTAB (---).

are shown in Figure 3. The difference spectra of the modified hemoglobin NES-des-Arg-deoxyhemoglobin ± IHP and hemoglobin Kempsey ± IHP (Perutz et al., 1974) are also shown for comparison. These hemoglobins exist in the R state in the deoxygenated form. Addition of IHP converts these hemoglobins to the T state. The difference spectra of these two hemoglobins ± IHP are similar in position to the difference spectra of 1,2-dimethylimidazole protoheme in ethylene glycol/H_2O (50:50) and 2% CTAB in both visible and Soret regions. Figure 4 shows the difference spectra of the CO–heme complex of 1,2-dimethylimidazole in ethylene glycol/H_2O and 2% CTAB together with that of human hemoglobin at low pH minus high pH (Soni & Kiesow, 1977) for comparison.

Absorption maxima were further blue shifted in 100% aqueous solution, although the resulting spectra show some evidence of dimer formation at higher concentrations. The measured extinction coefficients decrease as the concentration of heme is increased. The changes in the wavelength maxima for the carboxy complexes were greater than those for the deoxy complexes as the heme concentration increased. The absorption maxima for the 1,2-dimethylimidazole protoheme and its carboxy complex at 2×10^{-6} M heme were 553 and 428 nm and 563, 536, and 415 nm, respectively. At 5×10^{-5} M heme, the maxima for the 1,2-dimethylimidazole protoheme and its carboxy complex are 556 and 426 nm and 570, 543, and 411 nm, respectively.

In addition to the wavelength shifts, the α/β ratio of the carboxy complex changed from 0.97 to 1.10 as the heme concentration increased. The α/β ratio is defined as the quotient of absorbances of the long-wavelength visible peak (α) and the shorter visible wavelength peak (β). The addition of 20% ethylene glycol greatly diminished the wavelength changes, and 50% ethylene glycol totally eliminated these changes for the heme concentrations used in this experiment. There were no signs of dimer formation in any of the other

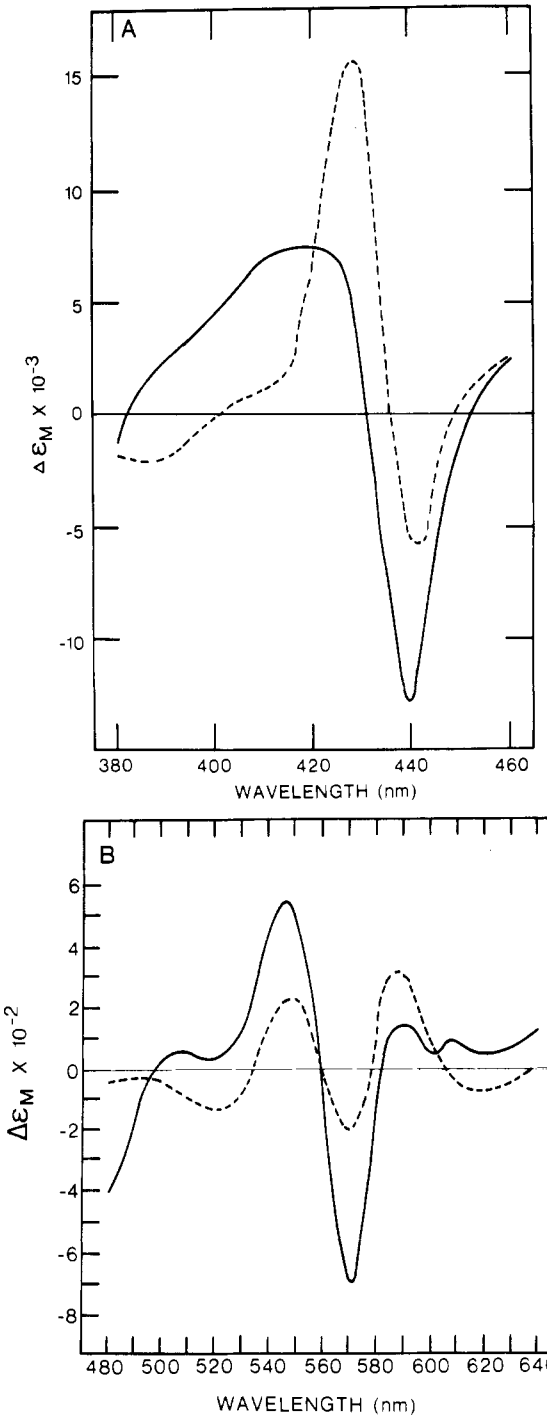

FIGURE 3: Soret (A) and visible (B) difference spectra of 1,2-dimethylimidazole protoheme in ethylene glycol/H_2O (50:50) minus 1,2-dimethylimidazole protoheme dimethyl ester in 2% CTAB (—) and reproductions of the difference spectra of NES-des-Arg-deoxyhemoglobin (A) and deoxyhemoglobin Kempsey (B) in the presence of IHP minus the spectrum in the absence of IHP (---) from Perutz et al. (1974).

solvents used up to 1×10^{-4} M.

Discussion

The spectra of heme complexes of sterically hindered ligands in different solvents have been measured as models for the effect of environment on the absorption spectra in hemoproteins such as leghemoglobin and myoglobin. Absorption changes up to 6 nm can be observed in the Soret and visible absorption bands of deoxyheme and carboxyheme complexes in changing from a polar aqueous environment to a less polar detergent environment. The results indicate that an increase

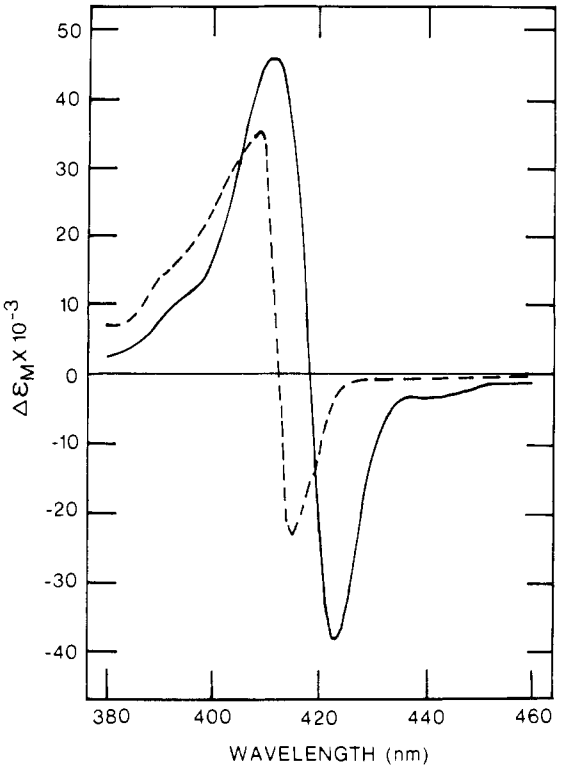

FIGURE 4: Soret difference spectra of (carbon monoxy)protoheme–dimethylimidazole in ethylene glycol/H_2O (50:50) minus (carbon monoxy)protoheme dimethyl ester–1,2-dimethylimidazole in 2% CTAB (—) and a reproduction of the difference spectrum of human hemoglobin at low pH minus high pH (---) taken from Soni & Kiesow (1977).

in the polarity of the solvent results in a general blue shift in the absorption maxima of the heme complexes investigated. It is notable, however, that the Soret maxima of the sterically hindered imidazole complexes and the visible maxima of the 1-methylimidazole–heme carboxy complex in benzene are blue shifted relative to the maxima in the detergent solution. The absorption maxima of heme complexes in detergents and benzene are in general similar, suggesting that detergent micelles provide a nonpolar heme environment. However, Traylor has observed that the oxygen affinity (Chang & Traylor, 1975) of a deoxy-chelated heme model and the absorption maxima (Traylor et al., 1979) of a carboxy-chelated heme model in a detergent (CTAB) are similar to values in dimethylformamide (DMF)/H_2O. These differences may be accounted for by differences in the location of the particular heme model in the micelle. The polarity of the heme environment in the interior may be expected to be more nonpolar while the environment near the micelle–water interface more polar. Fluorescence studies (Rehfeld, 1970; Schore & Turro, 1974; Gratzel & Thomas, 1976) indicate that the apparent polarity of the micelle depends on the particular probe. The chelated hemes may be more exposed to the solvent than the 2-methylimidazole–heme complexes used in the present study due to the polarity of the amide linkage and/or to less efficient packing of the detergent molecules around the locus of the linkage between the heme and the imidazole.

Different deoxymyoglobins and deoxyhemoglobins have absorption maxima that differ from one another by several nanometers (Antonini & Brunori, 1971). Wang & Brinigar (1979) have reported that differences in the absorption maxima of deoxyhemoproteins may be associated with differences in the strength of the axial histidine–iron bond based on differences in absorption maxima for model heme complexes of ligands with varying basicity or steric hindrance. Mincey &

Traylor (1979) have also reported differences in the absorption maxima of imidazole- and imidazolate–heme complexes as models for the effect of hydrogen bonding or deprotonation of the proximal imidazole in hemoproteins. The present results show that differences between the absorption maxima of these proteins may also be due in part to differences in the heme environment, more polar environments resulting in blue shifts relative to nonpolar environments.

Soybean leghemoglobin has a Soret absorption maximum at 427 nm (Imamura et al., 1972) which is blue shifted relative to the Soret absorption maximum of human hemoglobin at 430 nm and the Soret absorption maximum of sperm whale myoglobin at 434 nm (Antonini & Brunori, 1971). It has been proposed that the environment of the heme in leghemoglobin is more polar than the heme environment in myoglobin (Nicola & Leach, 1977a). This conclusion is supported by observations which indicate that the heme in leghemoglobin is much more accessible to the aqueous solvent than the heme in hemoglobins and myoglobins (Nicola et al., 1974, 1975; Vuk-Pavlovic et al., 1976; Nicola & Leach, 1977a,b; Lehtovaara, 1977; Ollis et al., 1981). At the lowest concentration of heme investigated in aqueous solution, the sterically hindered imidazole–heme complexes had absorption maxima at 553 and 428 nm, which are the lowest values reported to date, and close to the maxima observed for leghemoglobin (556 and 427 nm) (Imamura et al., 1972). In the less polar solvent of aqueous ethylene glycol, the sterically hindered imidazole–heme complexes had absorption maxima at 556 and 430 nm, which are similar to those of human hemoglobin (555 and 430 nm). The absorption maxima of the sterically hindered imidazole–heme complexes in detergent are very similar to those of sperm whale myoglobin (556 and 434 nm). This may suggest that the environment around the heme in deoxymyoglobin is similar to the environment of a detergent micelle. However, if the heme–imidazole bond in myoglobin is unhindered, then the environment around the heme in deoxymyoglobin may be more similar to that of an ethanol/H_2O mixture based on the blue shift associated with steric hindrance (Wang & Brinigar, 1979).

These observations are relevant to the oxygen binding properties of these proteins. It has been reported that an increase in solvent polarity increases the oxygen affinity of cobalt–porphyrin complexes (Stynes & Ibers, 1972). Likewise, an increase in the oxygen affinity of model heme complexes was observed when the polarity of the solvent was increased (Brinigar et al., 1974; Chang & Traylor, 1975). The oxygen affinity of leghemoglobins (Appleby, 1964; Imamura et al., 1972) is much greater than that of sperm whale myoglobin which is also consistent with a heme environment in leghemoglobin which is more polar than that in sperm whale myoglobin.

The effect of solvent may be compared to the proposed effects of the iron–nitrogen bond strength and hydrogen bonding or deprotonation of the proximal imidazole in interpreting the differences in the absorption maxima of leghemoglobin and myoglobin. Wang & Brinigar (1979) have reported that a decrease in the iron–nitrogen bond strength results in a blue shift in the absorption maxima of 5-coordinate ferrous heme complexes. If the blue shift in the absorption maxima of leghemoglobin relative to myoglobin is due to a weaker iron–nitrogen bond in leghemoglobin rather than a more polar heme environment, leghemoglobin would be expected to have a lower oxygen affinity than the myoglobin, based on model studies which show that strain in the iron–imidazole bond decreases the oxygen affinity (Geibel et al., 1978). Mincey & Traylor (1979) have reported that deprotonation of the imidazole results in a red shift in the absorption maxima of sterically hindered and nonhindered 5- and 6-coordinate ferrous heme complexes. If the blue shift in the absorption maxima of leghemoglobin relative to that of myoglobin is due to differences in hydrogen bonding, the proximal imidazole in leghemoglobin would be expected to be less strongly hydrogen bonded than the imidazole in myoglobin, which should lead to a lower oxygen affinity in leghemoglobin based on model studies which show that increasing proximal base electron donation increases oxygen affinity (Chang & Traylor, 1973). Thus, the solvent effect would appear to provide a better model for understanding the differences between the absorption maxima of leghemoglobin and myoglobin, although these other effects may be more important in other hemoproteins (Mincey & Traylor, 1979).

Differences between the absorption spectra of R- and T-state hemoglobins have been studied by using the abnormal hemoglobin Kempsey and the modified hemoglobins NES-des-Arg-deoxyhemoglobin and des-Arg-Tyr-deoxyhemoglobin (Perutz et al., 1974). These hemoglobins exist in the R state but can be converted to the T state by addition of IHP. The change from the R to the T state is accompanied by small ($\leq$2 nm) blue shifts in the absorption maxima and a decrease in oxygen affinity. These differences in absorption maxima and oxygen affinity have been attributed to increased tension in the iron–imidazole bond which results in the iron moving out of the plane of the heme toward the proximal imidazole and a lengthening of the iron–imidazole bond (Perutz et al., 1974). This proposal is supported by model studies which show that strain in the iron–imidazole bond decreased the oxygen affinity (Geibel et al., 1978). Difference spectra that resemble the R to T transition have also been observed by taking the spectrum of a heme complex with a more hindered ligand minus that of a less hindered ligand (Wang & Brinigar, 1979). Red shifts in absorption maxima have been observed for the deprotonation of imidazole in sterically hindered 5-coordinate ferrous heme complexes (Mincey & Traylor, 1979). Deprotonation or increased hydrogen bonding would be expected to increase the ligand basicity. The R- to T-state change is therefore also consistent with decreased deprotonation or decreased hydrogen bonding of the proximal imidazole based on the observation that oxygen affinity decreases as ligand basicity decreases (Chang & Traylor, 1973). It is noteworthy, however, that absorption spectral shifts resulting from the addition of IHP to methemoglobin fluoride have been interpreted to arise from a perturbance of the heme macrocycle conformation without movement of the iron out of the heme plane (Asher & Schuster, 1981).

The present results show that difference spectra between heme complexes in different solvent environments resemble difference spectra between R and T states as indicated in Figure 3. Blue shifts comparable ($\leq$2 nm) to those observed for R to T shifts are also observed in changing from ethylene glycol/H_2O to ethanol/H_2O. The similarity of these shifts may suggest that the difference between the R and T states is due in part to differences in the environment of the heme associated with changes in polarity or dipole orientation. The T state is blue shifted and, therefore, would be expected to have the more polar heme environment. This conclusion appears to be inconsistent with the observation that an increase in solvent polarity increases oxygen affinity in model complexes (Stynes & Ibers, 1972; Brinigar et al., 1974; Chang & Traylor, 1975). Thus, differences between the iron–histidine bond strength (Wang & Brinigar, 1979), changes in proximal

histidine hydrogen bonding (Chevion et al., 1977), or a perturbation of the heme macrocycle conformation (Asher & Schuster, 1981) would appear to provide better models for differences between the R and T states of human hemoglobin. A tilt or change in the orientation of the heme to the imidazole has also been proposed for the conformationally induced change from the R to the T state (Gelin & Karplus, 1977).

The absorption maxima of the (carbon monoxy)heme complexes in different solvents may be compared to absorption maxima of the corresponding complexes of other model systems and hemoproteins. (Carbon monoxy)leghemoglobin has absorption maxima at 563, 538, and 417 nm (Imamura et al., 1972), human (carbon monoxy)hemoglobin has maxima at 569, 540, and 419 nm, and sperm whale (carbon monoxy)-myoglobin has maxima at 579, 542, and 423 nm (Antonini & Brunori, 1971). The absorption maxima at 568, 539, and 421 nm observed for the (carbon monoxy)protoheme–1-methylimidazole complex in 2% CTAB are very close to those of human hemoglobin. These values are similar to absorption maxima of 569, 540, and 420 nm for the unconstrained carbon monoxy complex of protoheme–mono-3-(1-imidazolyl)-propylamide monomethyl ester in DMF/H_2O (70:30) and 2% CTAB (Traylor et al., 1979). The absorption maxima at 563, 537, and 417 nm observed for the (carbon monoxy)protoheme–1,2-dimethylimidazole complex in ethanol/H_2O are most similar to absorption maxima of (carbon monoxy)leghemoglobin. These maxima are also in excellent agreement with those reported by Wang & Brinigar (1979) for the (carbon monoxy)protoheme dimethyl ester–1,2-dimethylimidazole complex in DMF. These investigators reported a blue shift in the maxima of the carbon monoxy complexes as the basicity of the fifth axial ligand is decreased or the steric interaction is increased. The data in Table II indicate a blue shift in the α and Soret absorption maxima between 1-methylimidazole– and 2-methylimidazole– or 1,2-dimethylimidazole–heme complexes in each solvent. Thus, the absorption maxima for (carbon monoxy)leghemoglobin may better be accounted for by a heme environment which is more polar than that of ethanol/H_2O (50:50) if the proximal histidine bond is unconstrained. It is noteworthy that the maxima of sperm whale (carbon monoxy)myoglobin are red shifted relative to those observed for unhindered as well as hindered imidazole complexes. While other factors must contribute to the electronic properties of the heme–ligand complex in myoglobin, these results suggest that the maxima in carboxymyoglobin are most consistent with an unhindered complex in a relatively nonpolar heme environment.

Differences between the carbon monoxy complexes of hemoglobin at high and low pH have been measured by following the transition between allosteric states of hemoglobin. The Soret maximum of hemoglobin exhibits a blue shift in changing from high pH to low pH (Soni & Kiesow, 1977). The blue shift has been assigned to a transition from the R to the T state which is stabilized at low pH (Perutz et al., 1974; Wang & Brinigar, 1979). By contrast, a red shift is observed in the transition from high to low pH for trout IV (carbon monoxy)hemoglobin (Giardina et al., 1975). The difference spectra observed for the (carbon monoxy)heme complexes in ethylene glycol/H_2O minus (carbon monoxy)heme complexes in detergent are similar to those reported for human hemoglobin at low minus high pH. Although the magnitude of the shifts observed for the model in the two solvents appears to be significantly greater than that for the carbon monoxy complexes of hemoglobin at different pHs, it is doubtful that the change between R and T states of (carbon monoxy)-hemoglobin corresponds to large changes in polarity. It has been suggested that the CO difference spectrum observed for hemoglobin is associated with changes in the ionization of the proximal histidine (Soni & Kiesow, 1977).

The present studies show then that the solvent effect must be taken into account when trying to explain electronic differences based on absorption maxima. All of the differences in wavelength maxima can not be understood in terms of tension of the iron–imidazole bond or hydrogen bonding of the proximal imidazole with the protein.

From the data given, it is apparent that a polar environment can blue shift the absorption maxima of both the deoxy- 5-coordinate and the 6-coordinate carboxyheme complexes relative to a nonpolar environment. Differences in wavelength maxima of hemoproteins may be attributed in part to a change in the polarity of the environment around the heme.

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Phosphorylation and Hydrolysis of 7-Deazaadenine Nucleotides by Rat Liver and Beef Heart Mitochondria†

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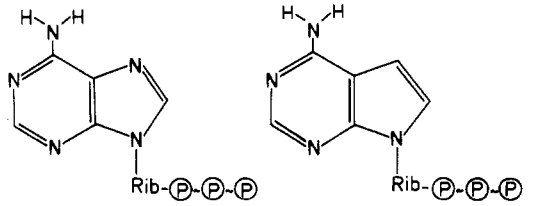
ABSTRACT: Tubercidin nucleotides [tubercidin 5′-monophosphate (TuMP), 5′-diphosphate (TuDP), and 5′-triphosphate (TuTP)] were tested as potential substrates for the mitochondrial phosphotransferases from rat liver and beef heart. TuDP is recognized by the mitochondrial translocase and phosphorylated by the respiratory chain enzymes in both mitochondria and submitochondrial particles from rat liver and beef heart; the low transport rate of the analogue into the matrix space of the intact organelles seems to be not a limiting step in the formation of TuTP. The phosphorylation of TuDP is significantly lower in beef heart mitochondria because of a higher specificity for ADP of the heart oxidative phosphorylation system. On the basis of the kinetic parameters of the partially purified liver mitochondrial adenylate kinase, one can conclude that the liver mitochondria are able to phosphorylate in vivo TuMP at a rate practically equal to the rate of AMP phosphorylation. The liver mitochondrial NDP kinase ensures a further fast phosphorylation of TuDP without the direct involvement of respiratory chain enzymes. In the case of heart mitochondria, two factors limit the rate of TuMP phosphorylation to TuTP: the lower acceptor activity of adenylate kinase with TuMP as compared with AMP and the different localization of heart NDP kinase situated on the inner face of the inner mitochondrial membrane. TuDP and TuTP preserve the ability of the natural nucleotides to interact with the "tight" nucleotide binding sites of isolated or membrane-bound F_1. The low hydrolytic rate of TuTP with F_1 may be related to the unusual flexibility of the glycosyl bond of tubercidin nucleotides in aqueous solution, with a high accessibility to syn conformation.

Tubercidin[1] is a highly cytotoxic nucleoside to microbial as well as to mammalian cells (Acs et al., 1964; Nishimura et al., 1966; Bloch et al., 1967). This compound is not a substrate for adenosine deaminase (Agarwal et al., 1975), and it is rapidly phosphorylated to TuTP which may substitute for ATP in a large variety of reactions (Henderson & Khoo, 1965; Smith et al., 1970; Suhadolnik, 1970; Bhuyan et al., 1971; Weiss & Pitot, 1974; Seela et al., 1981). However, despite the great diversity of its actions, the mechanism responsible for inhibition of growth and metabolism of cells is still obscure.

In the present study the role of various compartments of rat liver and beef heart mitochondria in phosphorylation and hydrolysis of 7-deazaadenine nucleotides is described. Our interest was aroused by the fact that participation of 7-deazaadenine nucleotides in mitochondrial reactions of phosphoryl group transfer may offer a better explanation for the cytotoxic effects of tubercidin on eucaryotic cells (Bisel et al., 1970; Grage et al., 1970; Ross & Jaffe, 1972; Bloch, 1975). On the other hand, the close structural resemblance of both adenine and 7-deazaadenine (Chart I) might provide more

Chart I: Structure of ATP (a) and TuTP (b)[a]



a. b.

[a] Rib-P-P-P = ribosyl 5′-triphosphate.

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[1] Abbreviations: tubercidin, 4-amino-7-β-D-ribofuranosyl-7*H*-pyrrolo[2,3-*d*]pyrimidine or 7-deazaadenosine; TuMP, TuDP, and TuTP, tubercidin 5′-mono-, 5′-di-, and 5′-triphosphate; 8-BrIDP, 8-bromoinosine 5′-diphosphate; o^{1}ADP and o^{1}ATP, adenosine N^1-oxide 5′-di- and 5′-triphosphate; Ap_5A, P^1,P^5-di(adenosine-5′) pentaphosphate; AMP-P-(NH)P, 5′-adenylyl β,γ-imidodiphosphate; NDP and NTP, nucleoside 5′-di- and 5′-triphosphate; NDP kinase, nucleosidediphosphate kinase (EC 2.7.4.6); G6P, glucose 6-phosphate; 6PG, 6-phosphogluconic acid; F6P, fructose 6-phosphate; FDP, fructose 1,6-bisphosphate; MA, MgATP submitochondrial particles; MAU, MgATP submitochondrial particles treated with urea; FCCP, carbonyl cyanide (trifluoromethoxy)-phenylhydrazone; EDTA, ethylenediaminetetraacetic acid; Tris, tris-(hydroxymethyl)aminomethane.

0006-2960/82/0421-0886$01.25/0