Contribution of Aromatic Residue Interactions to the Stability of Myoglobin. III. Molecular Complexes of Aromatic Compounds with Hemin, Hematoporphyrin, and Myoglobin*

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ABSTRACT: Chlorpromazine interacts with hemin, hematoporphyrin, and myoglobin to form complexes showing new visible absorption bands. Comparison of these spectra indicates that chlorpromazine complexes with the heme in myoglobin. Moreover, it complexes much more strongly with hemin than does methyl viologen.

Iodobenzene and nitrobenzene form weak 1:1 complexes with hematoporphyrin in ethanol, the value of the association constant for iodobenzene being about four times greater than that for nitrobenzene. These findings support our previous proposal as to the mechanism whereby aromatic compounds enhance the rates of reaction of myoglobin.

Specific enhancement by aromatic compounds like benzene, iodobenzene, and chlorpromazine of the rate of the zinc–myoglobin reaction and the urea denaturation of this protein has been interpreted in terms of complexing of these compounds with the heme moiety of myoglobin (Cann, 1965, 1967). The complexes are evidently of the electron donor–acceptor type with an important hydrophobic contribution to stability. Independent spectroscopic evidence for such complexing was sought in support of this interpretation. Mauzerall (1965) has studied the molecular complexes of substituted pyridinium salts and various nitrogen heterocyclic compounds with uroporphyrin; Keilin (1943), complexes of caffeine with heme and porphyrins; and Tu and Chou (1965), FMN[1] with hematin. But, the literature reveals no reports on molecular complexing between hemin or porphyrin and aromatic compounds of the type found effective in our systems. Accordingly, an experimental search was made for such complexes using the results on urea denaturation (Cann, 1967) as a guide. The findings lend credence to our previous proposal as to the mechanism of action of these compounds on myoglobin.

Methods

The hemin was Nutritional Biochemical's recrystallized protohemin chloride supplied with assay. This material in pyridine showed the same spectrum, and within experimental error, the same molar extinction coefficient as reported by Drabkin (1959). The hematoporphyrin (free base) and sperm whale ferrimyoglobin were obtained from Mann Research Laboratories. BPA was Armour's crystallized bovine plasma albumin, lot no. A69908. Mann's methyl viologen was recrystallized from ethanol. Matheson Coleman and Bell's iodobenzene and Eastman's nitrobenzene were further purified by passage through a column of activated aluminum oxide, Woelm basic activity grade 1. The *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochloride was Eastman's and the dimethylaniline (free of mono), Matheson Coleman and Bell's. The chlorpromazine hydrochloride was kindly supplied by Smith Kline and French laboratories.

For experiments on aqueous solutions (Figures 1–4), hemin or hematoporphyrin dissolved in 0.1 M NaOH was diluted into either 0.05 M sodium acetate buffer or a similarly buffered solution of BPA. These were diluted in turn with a buffered solution of either tetraethylammonium chloride, sodium chloride, methyl viologen, or chlorpromazine, final pH 5.9–6.0. (This choice of pH was dictated by the insolubility of chlorpromazine at alkaline pH. It is also the pH at which urea denaturation was studied.) The experiments on myoglobin were also made in 0.05 M sodium acetate buffer at pH 6.0 (Figure 5a,b) or 6.4 (Figure 5c). The stock solution of myoglobin used for the experiment shown in Figure 5b was prepared by exhaustive dialysis of the protein against cold triple-distilled water followed by centrifugal clarification and redialysis against cold buffer.

The difference spectra were obtained with a Cary Model 14 spectrophotometer equipped with a 0–0.1 absorbance slide wire and the other spectra, with a Beckman Model DK-2 spectrophotometer thermostated at 28 ± 0.01°. Average noise level of the Cary instrument was ±0.0005 unit of absorbance, *A;* maximum

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[1] Abbreviations used: FMN, riboflavin mononucleotide; BPA, bovine plasma albumin.

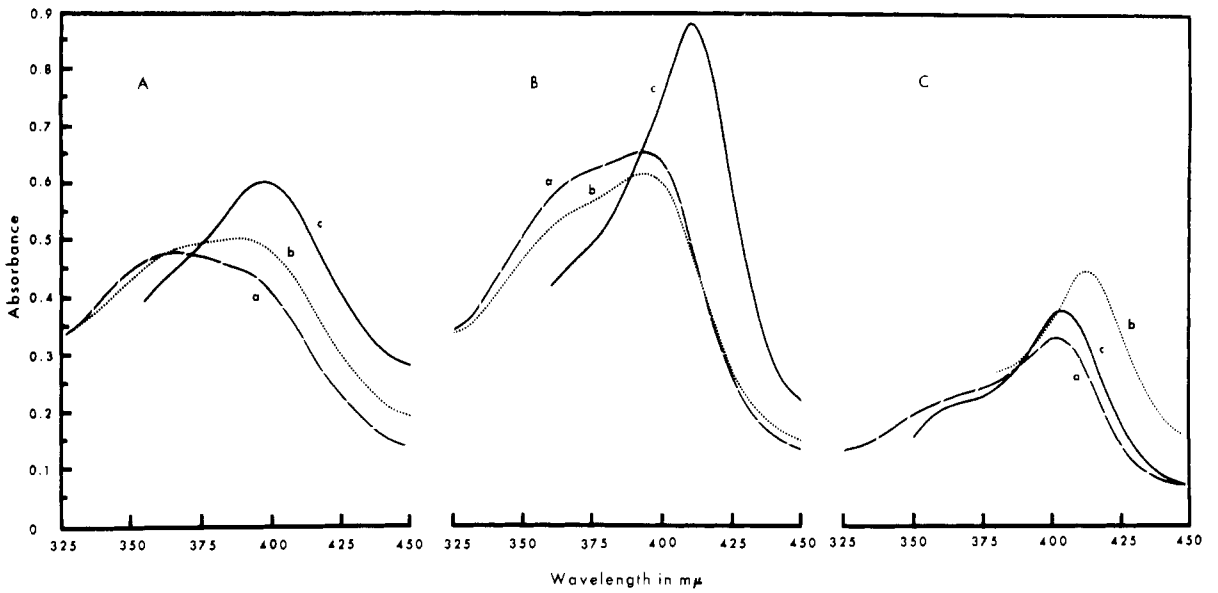

FIGURE 1: Reversible complexing of chlorpromazine and methyl viologen with hemin. Effect of these compounds on the Soret band. (A) Mixture of 1.3×10^{-5} M hemin with: (a) 1 or 3×10^{-2} M tetraethylammonium chloride, (b) 10^{-2} M methyl viologen, and (c) 10^{-2} M chlorpromazine. Chlorpromazine (10^{-3} M), while having a smaller effect on the Soret band than the higher concentration, was still considerably more effective than 3×10^{-2} M methyl viologen. (B) Mixture of 1.3×10^{-5} M hemin with 3.0×10^{-5} M BPA and: (a) 1 or 2×10^{-2} M tetraethylammonium chloride, (b) 10^{-2} M methyl viologen, and (c) 10^{-2} M chlorpromazine. (C) Hemin (5.2×10^{-7} M) with 3×10^{-5} M BPA and: (a) 4×10^{-4} M tetraethylammonium chloride and (b) 10^{-2} M chlorpromazine. Spectrum c of Figure C is that of mixture c of Figure B after dilution with buffered BPA solution to give the final composition, 5.2×10^{-7} M hemin–3.0×10^{-5} M BPA–4×10^{-4} M chlorpromazine. Light path: 1 cm in A and B, 10 cm in C. Dipole strengths[4] of bands in B: uncomplexed hemin, 4.8 A^2; hemin–chlorpromazine complex, 4.4 A^2.

noise level, ±0.001; resolution, 0.04 mμ at 580 mμ; wavelength accuracy, ±0.01 mμ at 579 mμ. Reproducibility of difference spectra was about ±0.7 mμ for wavelength of maximum ΔA and ±10% for ΔA; *e.g.*, Figure 3, chlorpromazine–hemin, maximum ΔA, ±0.0059; and Figure 5b, within average noise level. Repeatability upon repetitive scanning was within average noise level and ±0.7 mμ. The small peak between the 572.5- and 608.7-mμ bands of Figure 5b was always evident but statements as to its significance must await more sensitive instrumentation.

Fluorescence measurements were made with an Aminco-Bowman[2] spectrophotofluorometer equipped with a high-pressure Xenon source. A red-sensitive R 136 photomultiplier tube was used for the measurements presented in Figure 4a,c, and a 1P28 tube for those in Figure 4b.

Analysis of the data on complexing of iodobenzene (Figure 6) or nitrobenzene with hematoporphyrin indicated a 1:1 complex. The plots in Figure 7 are in accordance with the corresponding mass action relationship for large excess of aromatic compound

$$\frac{C_H l}{(A - A_0)} = \frac{1}{K(\epsilon_C - \epsilon_H)C_{\phi x}} + \frac{1}{(\epsilon_C - \epsilon_H)}$$

where C_H denotes molar concentration of hematoporphyrin; $A - A_0$, increase in absorbance at 410 mμ for light path, l, caused by the concentration, $C_{\phi x}$, of aromatic compound; $\epsilon_C - \epsilon_H$, difference between the molar extinction coefficients of complexed and uncomplexed hematoporphyrin; and K, association constant.

Results

Complexing of hemin with chlorpromazine and methyl viologen was investigated both in free solution and with hemin bound to serum albumin. Preliminary experiments were made in aqueous solution at pH 6 even though it is well known that hemin is aggregated in such solution. This approach was taken to ensure against the possibility that subsequent observations on hemin bound to albumin might be illusionary, simply reflecting, for example, possible alterations in protein

[2] The author wishes to thank Drs. Oscar K. Reiss and Wilhelm R. Frisell for kindly placing the Cary and Aminco-Bowman instruments at his disposal.

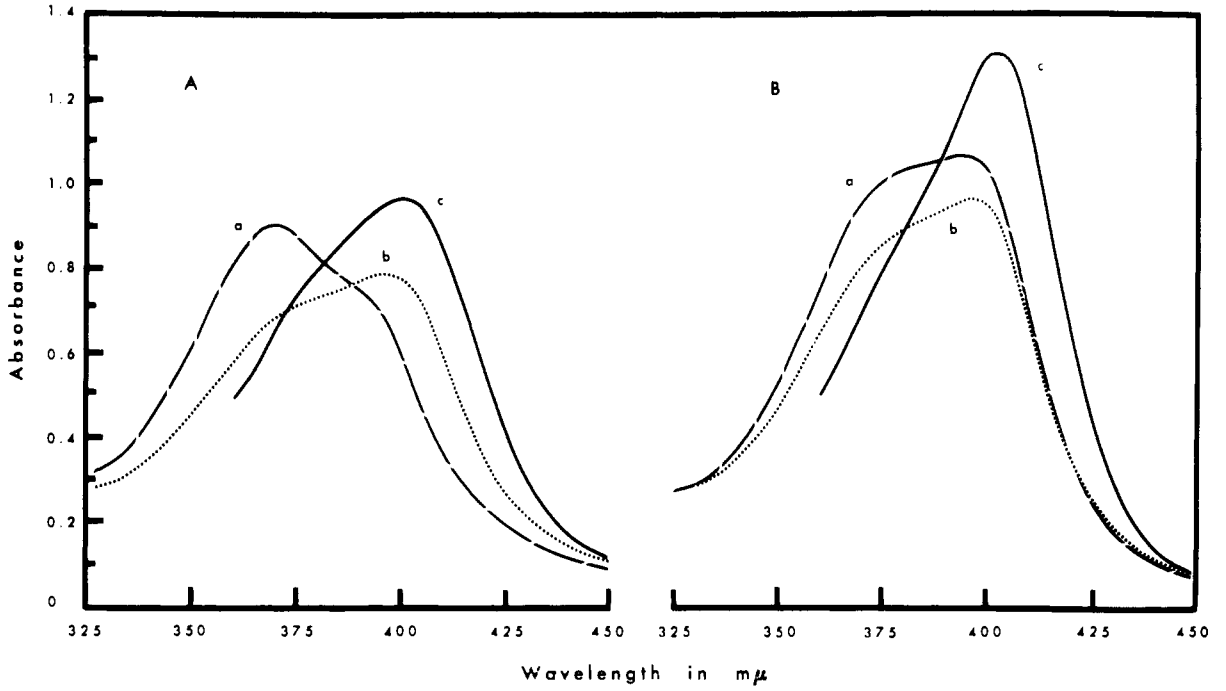

FIGURE 2: Complexing of chlorpromazine and methyl viologen with hematoporphyrin. Effect of these compounds on the Soret band. Mixture of 1.3×10^{-5} M hematoporphyrin with: (a) 1 or 2×10^{-2} M tetraethylammonium chloride, (b) 10^{-2} M methyl viologen, and (c) 10^{-2} M chlorpromazine. (A) Mixture contained no BPA. (B) 3×10^{-5} M BPA; 1-cm light path. Dipole strengths in B: uncomplexed hematoporphyrin, 5.3 A^2; methyl viologen–hematoporphyrin complex, 4.8; chlorpromazine–hematoporphyrin complex, 5.1.

structure by chlorpromazine or methyl viologen.

Addition of methyl viologen to hemin in free solution has little effect on the Soret band (Figure 1A), and the mixture is stable with time. When chlorpromazine is added, however, the color of the solution changes from brownish yellow to yellow, and the Soret band shifts to longer wavelength, sharpens, and increases in maximum intensity. Within 4 min, the hemin precipitates from solution. This observation is very important for our conclusion of chlorpromazine–hemin complexing, since it is hardly compatible with the notion that the spectral changes may merely reflect trivial deaggregation of the hemin. Nevertheless, in order to avoid these complications appeal was made to the fact that hemin binds to serum albumin (O'Hagan, 1961). When chlorpromazine is added to a hemin–albumin solution, no precipitation occurs but the Soret band[3] of the bound hemin still shifts into the red, sharpens, and intensifies (Figure 1B). This is so even for hemin concentration as low as 5×10^{-7} M and molar ratio of hemin to protein of only 2×10^{-2} (Figure 1C). In fact, the spectral changes are more striking in the presence of albumin, are readily reversed by dilution of the reaction mixture (Figure 1C), and occur with conservation of the integrated intensity of the band as indicated by approximate constancy of its dipole strength.[4] As with the free hemin, methyl viologen has only slight effect on the Soret band of protein-bound hemin. Quite similar results were obtained upon addition of chlorpromazine to hematoporphyrin (Figure 2), including precipitation of the porphyrin from its free solution but not from protein-containing solution.

In contrast to hemin, the Soret band of hematoporphyrin in free solution is quite sensitive to methyl viologen which causes a red shift accompanied by a decrease in absorbance (Figure 2A). But even here, methyl viologen does not cause precipitation. These observations agree qualitatively with those of Mauzerall (1965) for complexing of methyl viologen with unaggre-

[3] Bimodality of the Soret band is evidently due to different vibrational states (0–0 and 0–1) of the same electronic state (Gouterman, 1959).

[4] The dipole strength, which is a measure of oscillator strength and thus the integrated intensity of the absorption band, is given by

$$g^2 = \frac{1}{2500}\epsilon\frac{\Delta\lambda}{\lambda}$$

where g^2 is dipole strength is square angstroms; λ, wavelength of band maximum; $\Delta\lambda$, full band width at half-amplitude; and ϵ, maximum molar extinction coefficient (Gouterman, 1959, 1961).

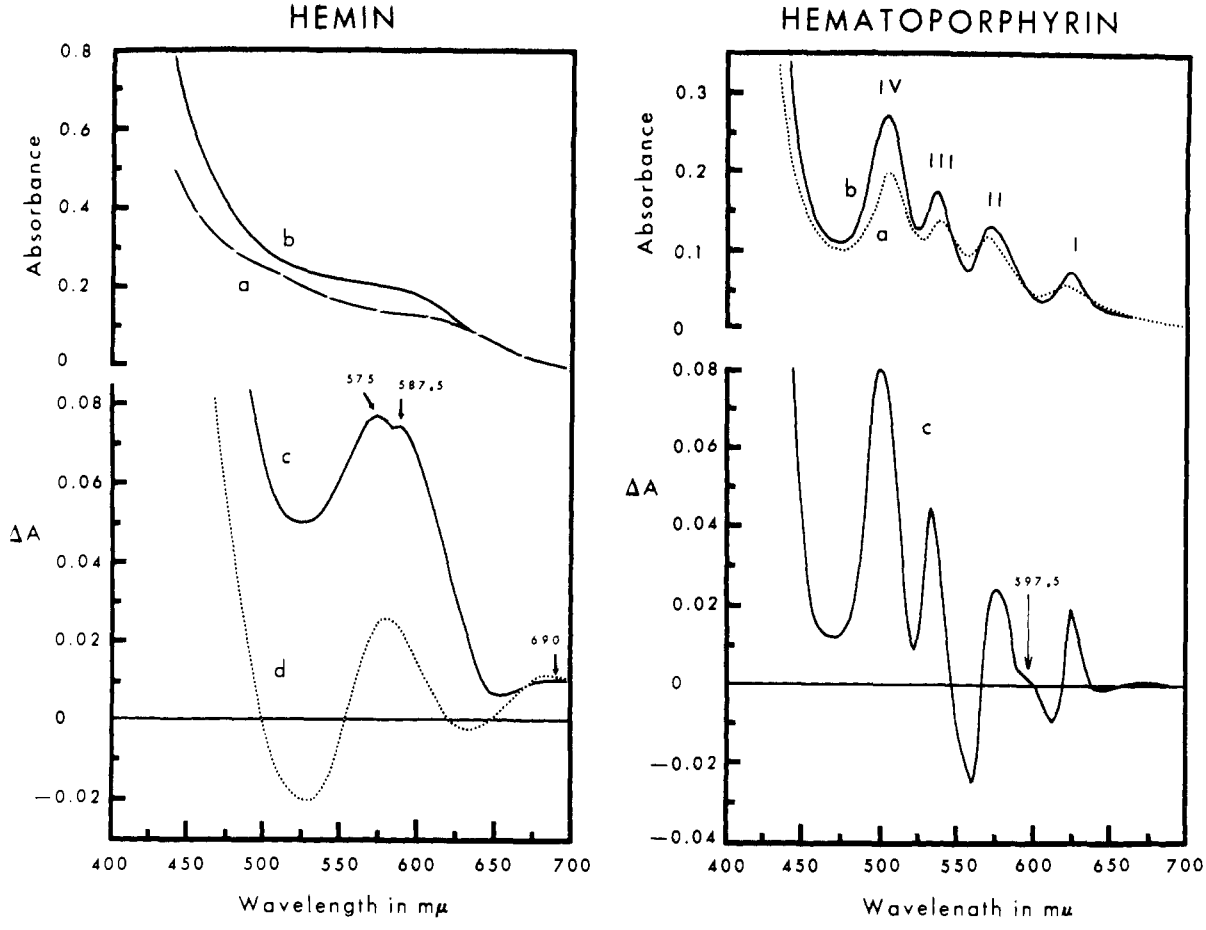

FIGURE 3: Complexing of chlorpromazine and methyl viologen with hemin, and chlorpromazine with hematoporphyrin. Visible absorption spectra and difference spectra of 2.2×10^{-5} M hemin and 2.9×10^{-5} M hematoporphyrin, all solutions containing 3.6×10^{-5} M BPA; light path, 1 cm. Hemin: (a) solution containing 10^{-2} M NaCl, (b) 10^{-2} M chlorpromazine, (c) difference spectrum of solution b *vs.* a, (d) difference spectrum of hemin solution containing 10^{-2} M methyl viologen *vs.* one containing 10^{-2} M NaCl. Hematoporphyrin: (a) solution containing 10^{-2} M NaCl, (b) 10^{-2} M chlorpromazine, and (c) difference spectrum of solution b *vs.* a; note new band at about 598 mμ. *A* denotes absorbance. Dipole strengths of hematoporphyrin bands: IV, 0.28 A^2 for uncomplexed and 0.27 for complexed hematoporphyrin; III, 0.11 and 0.14; II, 0.1 and 0.09; I, 0.047 and 0.045. The base line for the difference spectra was established using chlorpromazine–albumin or methyl viologen–albumin solutions; there was no evidence of visible chlorpromazine bands.

gated uroporphyrin. It is interesting that methyl viologen has a much smaller effect on the spectrum of protein-bound hematoporphyrin (Figure 2B).

Chlorpromazine not only alters the Soret band of hemin but also causes the appearance of new longer wavelength absorption bands. This is illustrated by the spectra and difference spectrum presented in Figure 3. The new bands are located at 575, 587.5, and 690 mμ. Here too, chlorpromazine has a considerably greater effect than does methyl viologen. In the case of hematoporphyrin (Figure 3), chlorpromazine sharpens the visible spectrum and causes a small blue shift of bands IV and III, virtually no shift of band II, and a small red shift of band I. These bands appear more intense in the presence of chlorpromazine, but, in fact, their integrated intensities are unchanged as indicated by constancy of their dipole strengths. More importantly, chlorpromazine causes the appearance of a new band at about 598 mμ. Furthermore, chlorpromazine at low concentration but not methyl viologen quenches the fluorescence of hematoporphyrin (Figure 4a,b). Quenching is not artifactual since at the wavelength of excitation in the Soret region the absorbance is actually greater in the presence than the absence of the quencher which itself is transparent. Accordingly, it is concluded that the degree of electronic coupling between chlorpromazine and hematoporphyrin is greater than between methyl viologen and hematoporphyrin. Nor is there necessarily a discrepancy between our observation on methyl viologen and that of Mauzerall (1965) who found that methyl viologen quenches the fluorescence of uroporphyrin in free solution.

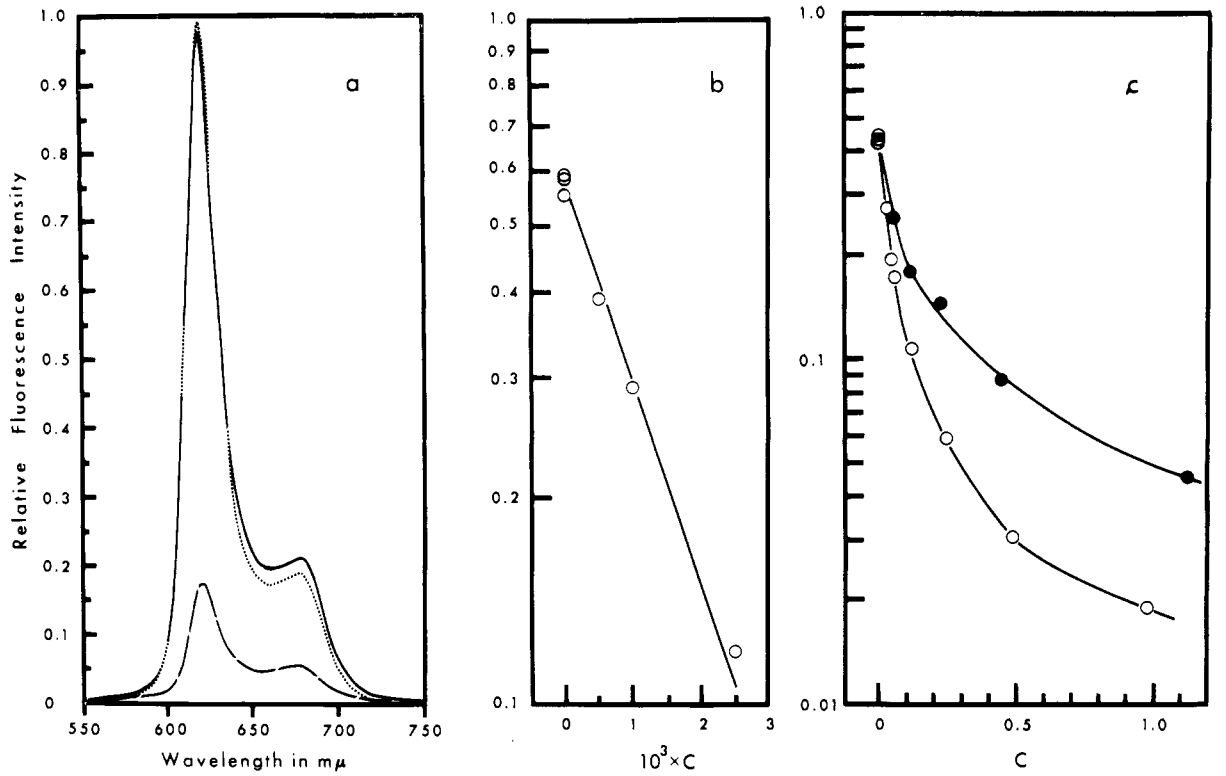

FIGURE 4: Quenching of fluorescence of hematoporphyrin by several aromatic compounds. (a) Emission spectra of 3.9×10^{-5} M hematoporphyrin bound to 3×10^{-5} M BPA in buffered solution containing 2.5 or 5×10^{-3} M tetraethylammonium chloride (———), 2.5×10^{-3} M methyl viologen (. . . . .), or 2.5×10^{-3} M chlorpromazine (— — —). (b) Corresponding quenching curve for chlorpromazine. (c) Quenching of fluorescence of 1.8×10^{-5} M hematoporphyrin in ethanol by iodobenzene (●) or nitrobenzene (○). Excitation wavelength (maximum): (a) 411, 411, and 408 mμ; (b) 404–407 mμ, and (c) 419 mμ. *C* denotes molar concentration.

Our experiments were made with hematoporphyrin bound to albumin.

The experiments described above demonstrate beyond reasonable doubt that chlorpromazine forms molecular complexes with hemin and hematoporphyrin. The question remains whether it also complexes with the heme moiety of myoglobin. The experiments presented in Figure 5 show that, in fact, this is so. When 4×10^{-3} M chlorpromazine is added to 5.5×10^{-4} M (0.93%) myoglobin, the difference spectrum relative to myoglobin solution containing NaCl (Figure 5b) is established immediately except for the 685-mμ band which grows with time for about 20 min as the shallow trough at 640 mμ deepens. It is concluded that the 685-mμ band and the 640-mμ trough represent a red shift. In contrast, the deep trough at about 510 mμ appears to arise from some spectral change other than a band shift. The 577.5- and 608.7-mμ bands and the 530–540-mμ band which appears as a prominent shoulder on the long-wavelength side of the 510-mμ trough are considered new ones. Increasing the concentration of chlorpromazine to 9×10^{-3} M (Figure 5c) causes the deep 510-mμ trough to deepen still further and the 685-mμ band to increase in intensity, both by the expected amount. On the other hand, the shallow trough at 640 mμ deepens more than expected and the new bands in the region of 670-610 mμ increase tremendously in intensity by about 10–20-fold. These also shift slightly into the blue. This disproportionality could conceivably reflect a change in protein conformation induced by increased binding of chlorpromazine. As reported previously (Cann, 1967) concentrations of chlorpromazine higher than those used in these experiments (1.4×10^{-2} M and higher) cause an appreciable decrease in Soret absorbance. In any event, these difference spectra share certain features with those in Figure 3 for chlorpromazine–hemin and chlorpromazine–hematoporphyrin, *viz.*, new bands in the 570–610-mμ region and, in the case of chlorpromazine–hemin, the 640–685-mμ red shift. An interesting feature of the difference spectrum of chlorpromazine–myoglobin not evidenced by chlorpromazine–hemin is the 530–540-mμ band. This is reminiscent of the 530-mμ band of the cationic free radical of chlorpromazine formed in concentrated sulfuric acid (Schieser and Tuck, 1962).

Iodobenzene and nitrobenzene were also found to complex weakly with hematoporphyrin in ethanol. As illustrated in Figure 6 for iodobenzene, increasing concentration of either aromatic causes the Soret

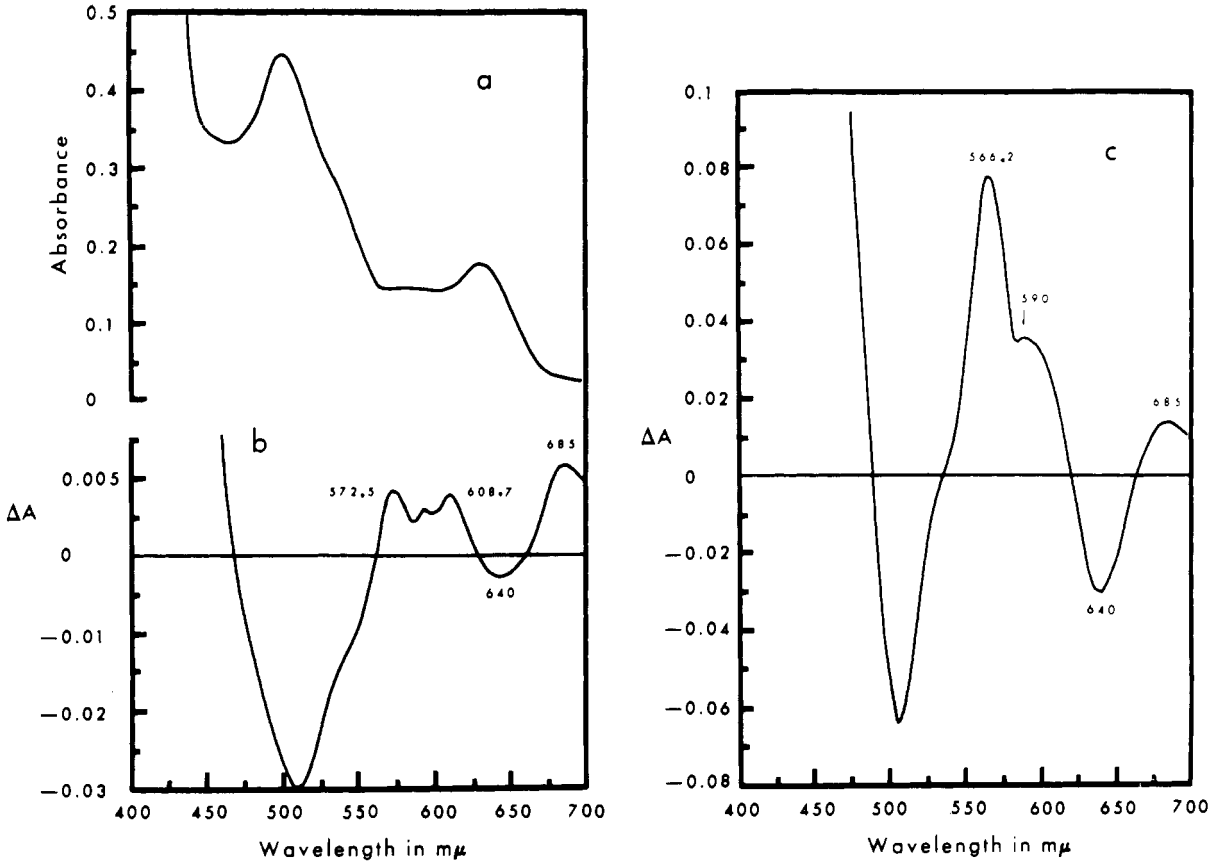

FIGURE 5: Complexing of chlorpromazine with myoglobin. (a) Spectrum of 5.5×10^{-4} M (0.93%) myoglobin in buffered solution containing 4×10^{-3} M NaCl, 1-mm light path. (b) Difference spectrum of myoglobin solution containing 4×10^{-3} M chlorpromazine *vs.* solution a, 1-cm light path. (c) Difference spectrum of myoglobin solution containing 9×10^{-3} M chlorpromazine *vs.* one containing the same concentration of NaCl, 1-cm light path. The base line for the difference spectra was established using chlorpromazine solutions which were transparent in this spectral region.

band to shift progressively into the red with an isosbestic point. Analysis of these data indicates a 1:1 complex with association constant of 11×10^{-2} M^{-1} for iodobenzene and 3×10^{-2} M^{-1} for nitrobenzene (Figure 7). As shown in Figure 4c both compounds also quench the fluorescence of hematoporphyrin. But the fact that nitrobenzene quenches more effectively than iodobenzene need not necessarily indicate a greater degree of electronic coupling even though, in contrast to iodobenzene, its absorption band overlaps rather strongly with the Soret band. (But it is transparent at the excitation wavelength.) Both compounds quench at much lower concentrations than anticipated on the basis of complex formation alone, so that the quenching process is probably a dynamic one dependent among other things on diffusion coefficient. Accordingly, one might expect nitrobenzene with its lower molecular weight and presumably higher diffusion coefficient to be the more efficient quencher. In fact, the ratio of concentrations of the two compounds required for 50% quenching, 1.9, is only slightly larger than the ratio of their molecular weights, 1.66.

Finally, it is pertinent to inquire whether hematoporphyrin can act as an electron acceptor. The following experiments demonstrate that this is the case. When the strong donor *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (colorless in ethanol) is added to a solution of hematoporphyrin in ethanol (10^{-2} M donor as its dihydrochloride and 7.3×10^{-5} M hematoporphyrin), the color changes from rust to wine. The color change is due to the following spectral changes: (1) the Soret band is slightly intensified and shifted red from a maximum of 397 mμ to one at 408 mμ; and (2) the characteristic four-banded structure of the visible spectrum of hematoporphyrin (Figure 3) disappears, the spectrum now showing a major band ($\log \epsilon \sim 4$) at 554 mμ with a shoulder at 525 mμ and a minor band at 595 mμ. These features are similar to those of the spectrum of the radical cation (Würster's Blue) prepared in this instance by oxidation in ethanol of tetramethylphenylenediamine with either Fe^{3+} or H_2O_2: two bands of approximately the same intensity, one at 563 mμ with a prominent shoulder at 525 mμ and the other at 611 mμ. But, the reaction with hema-

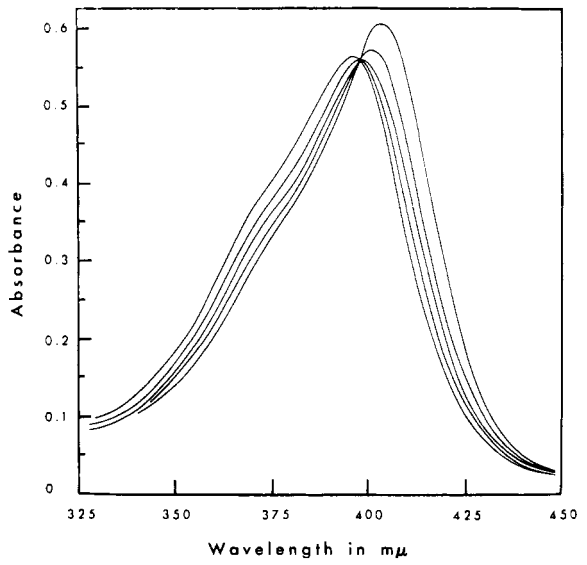

FIGURE 6: Complexing of iodobenzene with hematoporphyrin in ethanol as revealed by progressive shifting of the Soret band to longer wavelength with increasing concentration of aromatic compound; isosbestic point, 398 ± 0.5 mμ. Concentration of hematoporphyrin, 4.74 × 10^{-5} M; concentrations of iodobenzene, 0, 0.562, 1.12, 2.25, and 4.49 M; 1-mm light path. Dipole strength: no iodobenzene, 6.0 A^2; 4.49 M iodobenzene, 5.6 A^2.

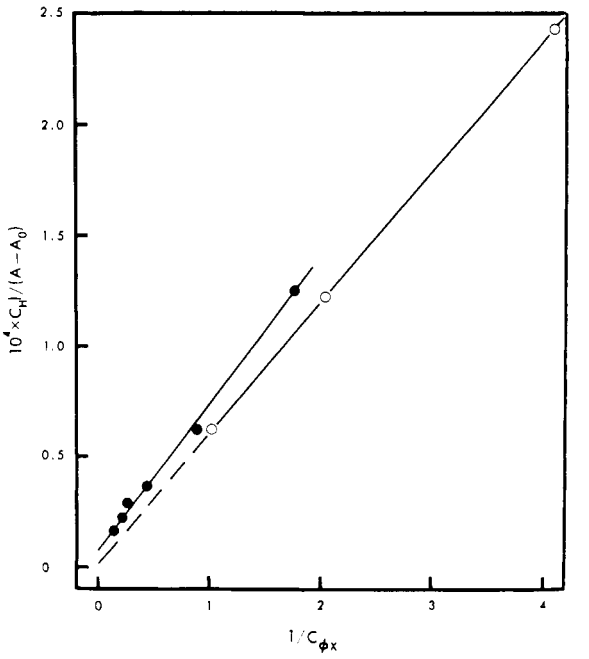

FIGURE 7: Molecular complexing between iodobenzene (●) or nitrobenzene (○) and hematoporphyrin in ethanol. For iodobenzene, $\epsilon_C = 2.1 \times 10^5$ M^{-1} cm^{-1} and $K = 11 \times 10^{-2}$ M^{-1}; nitrobenzene, 7×10^5 and 3×10^{-2}. $\epsilon_H = 7.1 \pm 0.4 \times 10^4$. The value of ϵ_C for nitrobenzene–hematoporphyrin is surprisingly large but not unreasonably so. Unfortunately, the absorbance of nitrobenzene precludes measurements at higher concentrations of this compound, thus necessitating the long extrapolation shown in the figure. It is apparent, however, that nitrobenzene complexes with hematoporphyrin less strongly than does iodobenzene.

toporphyrin is molecular complexing rather than simple oxidation of the base to Würster's Blue, since the spectral changes are readily reversed by dilution. Consider, for example, a mixture of 2.5 × 10^{-4} M donor with 6.4 × 10^{-5} M hematoporphyrin. In this case, the spectrum is of an intermediate type exhibiting features of both the porphyrin and the complex, with the latter predominating; *e.g.*, the intensity of the 554-mμ band of the complex is about 70% of that expected for complete reaction and that of the 500-mμ band of the porphyrin only 30% of its value in the absence of the donor. When the mixture was diluted 1:20 with ethanol, the bands of the complex disappeared and the characteristic four-banded spectrum of the porphyrin reappeared, indicating virtually complete reversal of reaction. It is concluded, therefore, that tetramethylphenylenediamine forms an electron donor–acceptor complex with hematoporphyrin, the latter acting as the acceptor molecule. A test of this conclusion is provided by the prediction that the weaker donor dimethylaniline should complex less strongly with hematoporphyrin to give a charge-transfer band at a shorter wavelength (Briegleb, 1961). This has been verified. Thus, 2.6 M dimethylaniline changes the color of 5.3 × 10^{-5} M hematoporphyrin in ethanol from rust to orange. The Soret band is slightly intensified and shifted slightly red; each of the four visible bands is sharpened and shifted slightly red; and a new, relatively weak (log $\epsilon \sim 4$ as compared to log $\epsilon \sim 5$ for the Soret band) but well-resolved band appears at 383.5 mμ.

Discussion

It has been proposed (Cann, 1965, 1967) that aromatic compounds enhance the rates of reaction of myoglobin by forming electron donor–acceptor complexes with the heme. Such complexing is visualized as relieving the π-bonding interactions between the heme and the two aromatic rings of the phenylalanine residues, CD1 and 15H. This, in turn, labilizes the macromolecular structure to attack by zinc ions and urea. The experiments described above provide strong supporting evidence for such a mechanism.

Thus, chlorpromazine which enhances the rate of urea denaturation also forms molecular complexes with hemin, hematoporphyrin, and myoglobin. Moreover, it complexes with the heme moiety of myoglobin. Complexing with the hemeprotein is, of course, very much weaker than with hemin bound to albumin, as judged from molar difference absorbances calculated from the data in Figures 3 and 5. This is to be expected

since the hemin in myoglobin is tucked away in a hydrophobic pocket where it is relatively inaccessible to the chlorpromazine molecule. The possibility that chlorpromazine simply serves as a ligand for the iron seems to be eliminated by the similarities between hemin and hematoporphyrin with respect to change in solubility and alteration of the Soret band upon complex formation. Chlorpromazine evidently complexes with the protoporphyrin ring system.

The new visible absorption bands exhibited by these complexes could be charge-transfer bands, an interpretation which is not necessarily at variance with the structure of the bands. Although the charge-transfer band of a single electron donor–acceptor complex is characteristically structureless, it is conceivable that here we are dealing with a mixture of geometrically isomeric complexes each with its own distinct charge-transfer band (Orgel and Mulliken, 1957) formed by interaction of chlorpromazine with different parts (*i.e.*, ring system or side-chain vinyl groups) of the hemin molecule. In any event, the chlorpromazine–myoglobin complex also shows a band only slightly shifted from that characteristic of the cationic free radical of chlorpromazine. These observations are consistent with (1) the fact that chlorpromazine is a good electron donor (Karreman *et al.*, 1959); (2) the theoretical prediction (Pullman and Perault, 1959; Pullman *et al.*, 1960) that porphyrins and ferriporphyrins should be good π-electron acceptors (and also good donors); and (3) our experimental demonstration that hematoporphyrin can, in fact, act as an electron acceptor.

Further support for our proposed mechanism of action of aromatic compounds on myoglobin is provided by the correspondence between relative strength of complexing and degree of synergism with urea. The finding that chlorpromazine complexes much more strongly with hemin than does methyl viologen correlates very nicely with their relative effectiveness in enhancing the rate of urea denaturation of myoglobin. Likewise, iodobenzene complexes more strongly with hematoporphyrin than does nitrobenzene. This too correlates with the greater effectiveness of iodobenzene in enhancing the rate of denaturation.

Finally, these experiments and those on urea denaturation (Cann, 1967) could conceivably be of pharmacological significance as model systems for study of certain kinds of drug action. Chlorpromazine is a powerful tranquilizing drug whose mode of action is poorly understood. It has been suggested, however, that its therapeutic activity may possibly involve charge-transfer complexes with appropriate acceptor sites (Karreman *et al.*, 1959). In any event, myoglobin is an ideal protein for studying protein–drug interactions since its three-dimensional structure has been largely established by X-ray crystallographic analysis, thereby providing the fundamental information required for detailed description of its reactions (Kendrew, 1961, 1962; Kendrew *et al.*, 1961; Stryer *et al.*, 1964). It is of interest that not only does myoglobin bind chlorpromazine but also the anesthetic, Xenon, which fits into a cavity where it makes contact with both the heme and the proximal imidazole group of the F8 histidine residue (Schoenborn *et al.*, 1965).

Acknowledgment

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