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Effects of Organic Solvents on the Spectrum of Cytochrome *c*[*]

Lawrence S. Kaminsky and Allan J. Davison†

ABSTRACT: The effects of various alcohols, ethers, and amides on the absorption spectrum of cytochrome *c* have been investigated. A hemoundecapeptide, isolated from a peptic hydrolysate of cytochrome *c*, was similarly studied. (a) Increasing the concentration of organic solvent normally caused an initial increase in the intensity of the Soret peak, which subsequently decreased with further increases in solvent concentration. The maximum slope of a graph of absorbance change against mole per cent of organic solvent, S_M, was used as the criterion for comparing the effectiveness of the organic solvents. (b) The nature of the effects was similar in cytochrome *c* and the hemopeptide, but the magnitude of the change was usually two to three times greater in the hemopeptide. If the increase in Soret absorbance reflects denaturation, this result implies that the structure of the hemopeptide in aqueous solution is not completely random. (c) The addition of electrolytes significantly enhanced the S_M, presumably due to the encouragement of the disruption of ionic bonds in solutions of higher ionic strength. (d) The presence of urea similarly enhanced the S_M. (e) An increase in the length of the hydrocarbon chain of the alcohols and ethers increased S_M. Branching, however, produced a decrease. Hydrophobicity is thus important for conformational changes but accessibility of the solvent hydroxyl group or the interior of the polypeptide chain is also important. (f) The importance of hydrophobicity is also evident from the decrease in S_M which occurs on replacing a carbon by an oxygen or on inserting an oxygen into the chain. When a hydroxyl group replaces a hydrogen atom, the loss of effectiveness is even greater. (g) When chain length and oxygen content were simultaneously increased by adding a carbinol group, the above opposing effects balanced each other. (h) Hydrogen bond disrupting capacity is also important, as determined by comparison of formamide and dimethylformamide. On a volume per cent basis these are of roughly equal effectiveness in enhancing the Soret peak. Thus the increase in hydrophobicity only just balances the loss of hydrogen bonding capacity.

The above results are interpreted as qualitative evidence for contribution of hydrophobic, hydrogen, and ionic bonds to the stability of the native structure of both cytochrome *c* and the hemopeptide.

The effects of organic solvents on macromolecules have recently been studied with a view to estimating the relative importance of hydrophobic, hydrogen, and ionic bonds in stabilizing the preferred conformation. Among the molecules studied are DNA (Herskovits, 1962) and the proteins collagen (Herbage *et al.*, 1968), myoglobin, and α-chymotrypsinogen (Herskovits and Jaillet, 1969), and xanthine oxidase (Dastoli and Price, 1967).

In these studies the organic solvents denatured the protein. Despite the detailed studies of Gordon and Jencks (1963) on albumin, sufficient data are not yet available for derivation of a quantitative relationship between the properties of the solvent and the solvent susceptibility of the protein. Qualitative trends are, however, apparent in these reports. In the case of myoglobin, the more hydrophobic the solvent the greater is its denaturing power. In contrast, hydrogen-bonding capability is of primary importance in the case of albumin, where the hydrophobic character of the solvent contributes little to its effectiveness.

The properties of cytochrome *c* in a nonaqueous environment are of particular interest, since cytochrome *c* is thought to exert its physiological function within the relatively hydrophobic environment of the mitochondrial membrane (Ambe and Crane, 1959). The aerobic oxidation of cytochrome *c* by soluble cytochrome oxidase preparations is accelerated by the addition of lipids (Greenlees and Wainio, 1959) and cytochrome *c* has been shown to form complexes with phospholipids (Reich and Wainio, 1961).

A preliminary report on the three-dimensional structure

* From the Department of Physiology and Medical Biochemistry, University of Cape Town, Medical School, Cape Town, South Africa. *Received June 3, 1969.* Supported by grants from the South African C. S. I. R. and University of Cape Town Staff Research Fund.

† Present address: Department of Biochemistry, University of Oregon Medical School, Portland, Ore.

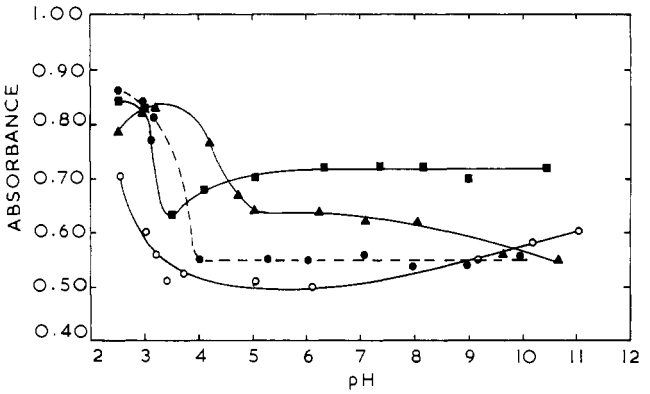

FIGURE 1: Effect of pH on the intensity of the Soret peak of an aqueous organic solvent of cytochrome *c*. (○) In 30% (v/v) methanol, (□) in 60% (v/v) methanol, (●) in 30% (v/v) dimethylformamide, and (■) in 60% (v/v) dimethylformamide. Cytochrome *c* concentration 5 μM. pH varied by addition of up to 0.005 ml of 15 *N* sodium hydroxide or 12 *N* hydrochloric acid.

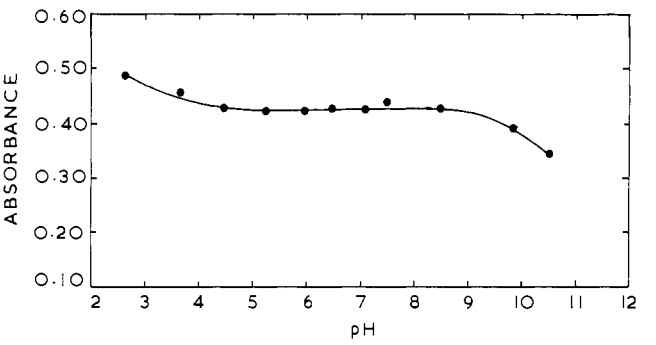

FIGURE 2: Effect of pH on the intensity of the Soret peak of a methanol–water (30% v/v) solution of 3.5 μM hemoundecapeptide.

of the cytochrome *c* molecule indicates that it contains a negligibly small proportion of α-helical structure of the peptide chain (Dickerson *et al.*, 1967). Further, the hemogroup of cytochrome *c* lies partially buried in a hydrophobic crevice (Theorell and Åkesson, 1941). It is inaccessible to ligands such as CO (Keilin, 1930) and is partially shielded from the effects of solvents (Stellwagen, 1967). The environment of the heme partly determines the maximum Soret absorbance of this group and thus any changes in the intensity of this band are indicative of disruption of this environment (Schechter and Epstein, 1968).

In the case of myoglobin, the decreases in the Soret peak intensity produced by organic solvents, were found to parallel other indices of protein conformational change (Herskovits, and Jaillet, 1969). In the current study, therefore, the effects of a range of concentrations of each of a variety of solvents on the Soret peak of cytochrome *c* were investigated. The spectral changes are interpreted as an indication of an unspecified modification of the environment of the heme group.

Similar studies on cytochrome *c* have been announced (Herskovits and Jaillet, 1968), but the data are not yet available for comparison.

Experimental Section

Materials. Horse heart cytochrome *c* (grade I) of 88% purity was obtained from Seravac Laboratories, Maidenhead, England. The hemoundecapeptide was prepared by peptic hydrolysis of cytochrome *c*, using the method of Harbury and Loach (1960). The amino acid composition of the isolated undecapeptide agreed with that reported by these workers.

The alcohols and amides were Analar grade, obtained from British Drug Houses and Hopkin and Williams, and were used without further purification. The ethers were British Drug Houses' Reagent grade. They were purified and the peroxides rigorously removed by the modified method of Dasler and Bauer (1946). The ethers were passed through a column of Hopkin and Williams aluminium oxide "Camag," pH 6, Brockmann activity 1, the system being maintained under an atmosphere of nitrogen throughout. Each ether was used immediately after purification. Water was redistilled and deionized.

Preparation of Solutions for Absorbance Readings. All final solutions were made up in the cuvet as follows: 0.2 M acetate buffer (pH 5.6, 0.3 ml), cytochrome *c* in aqueous solution[1] (concentration determined spectrophotometrically using extinction coefficients of Margoliash and Frohwirt (1959)) *or* hemopeptide in aqueous solution (0.1 ml), and water and organic solvents in varying ratios to give final volumes of 3 ml, with organic solvent contributions of 0–80% (v/v) in steps of 10% (v/v). The solutions were mixed in the cuvet with a microstirrer and the absorbances were read at wavelengths of 600–380 mμ using a Beckman DB spectrophotometer. The pH of each solution was determined in the cuvet using a Radiometer 27 pH meter. Corrections for solvent shrinkage, due to water–organic solvent interactions, were made to each absorbance reading. The shrinkage for each solvent–water mixture was determined with a micrometer syringe.

The effects of pH change on the Soret peak of aqueous organic solvent solutions of cytochrome *c* and the hemoundecapeptide were determined by adding very small quantities of concentrated hydrochloric acid or 15 N sodium hydroxide solution to solutions made up as previously described. The change in intensity of the Soret peak of the solution with altered pH, was then determined.

A study of the effect of removal of the organic solvent on the Soret peak of an aqueous organic solution of cytochrome *c* was performed. A solution of cytochrome *c* containing 60% (v/v) methanol was made up in a volumetric flask. The intensity of the Soret peak of this solution was compared with that of a solution of cytochrome *c* of the same concentration but containing only water as solvent. The methanol was then removed from the former solution under vacuum (approximately 2 mm of Hg) in a volumetric flask with an elongated neck, so as to prevent mechanical loss of solution during the removal. After complete removal of the methanol, the solution was brought back to its original volume with water and was scanned as before.

The relative change in Soret peak intensity $\Delta\epsilon/\epsilon_0$ (where $\Delta\epsilon$ represents the molar absorptivity difference, assuming Beer's law, between organic solvent–water solutions and aqueous

[1] Cytochrome *c* refers to horse heart ferricytochrome *c*; heme undecapeptide refers to ferriheme undecapeptide obtained by peptic hydrolysis of horse heart ferricytochrome *c*.

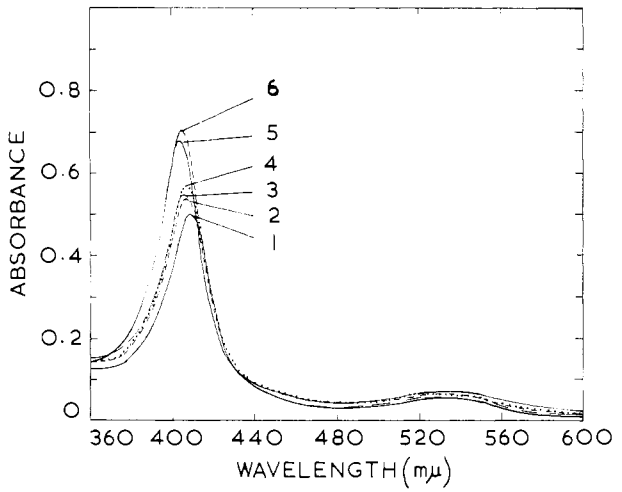

FIGURE 3: Absorption spectra of cytochrome *c*. (1) In aqueous solution, (2) in 10% (v/v) ethanol–water, (3) in 20% (v/v) ethanol–water, (4) in 30% (v/v) ethanol–water, (5) in 40% (v/v) ethanol–water, and (6) in 50% (v/v) ethanol–water. Cytochrome *c* concentration 5 μM. Final concentration of acetate buffer in cuvet, 0.02 M, pH 5.6.

solutions, and ϵ_0 is the molar absorptivity in aqueous solution) was plotted as a function of mole per cent solvent (the mole fraction of organic solvent expressed as a percentage). The maximum slope of this plot is designated S_M.

Results

Changes of pH in the range 5–7.5 produced no corresponding change in the Soret absorbance of aqueous organic solvent solutions of cytochrome *c*. At pH values out of this range, rapid increases in absorbance with decreasing pH were noted. These results are shown in Figure 1 with dimethylformamide and methanol as examples of organic solvents. In the case of the hemoundecapeptide, changes of pH produced less marked changes in Soret absorbance and a larger range of plateau, shown in Figure 2, with methanol as organic solvent. Since addition of organic solvents to buffered solutions of cytochrome *c* and hemopeptide produced increases in pH, all studies of the effects of organic solvents were initiated at pH 5.6, and the final pH was never greater than 7.5.

Most of the solvents tested produced a decrease of up to 5 mμ in the wavelength of maximum Soret absorbance of cytochrome *c*. Figure 3 shows the effect of a typical solvent (ethanol) on the absorption spectrum of cytochrome *c*.

The effect of increasing the amount of organic solvent on the spectrum of aqueous solutions of cytochrome *c* or hemoundecapeptide is initially an increase in Soret absorbance, which progresses until a solvent concentration is reached which produces a maximum effect, after which increases in organic solvent concentration produce a decrease in Soret absorbance.

In every case the effects of a particular solvent were more pronounced on the hemopeptide than on cytochrome *c*. Compared with cytochrome *c* the hemopeptide yielded greater maximum changes in Soret absorbance at lower levels of organic solvent concentration.

The effects of alcohols having different chain lengths and

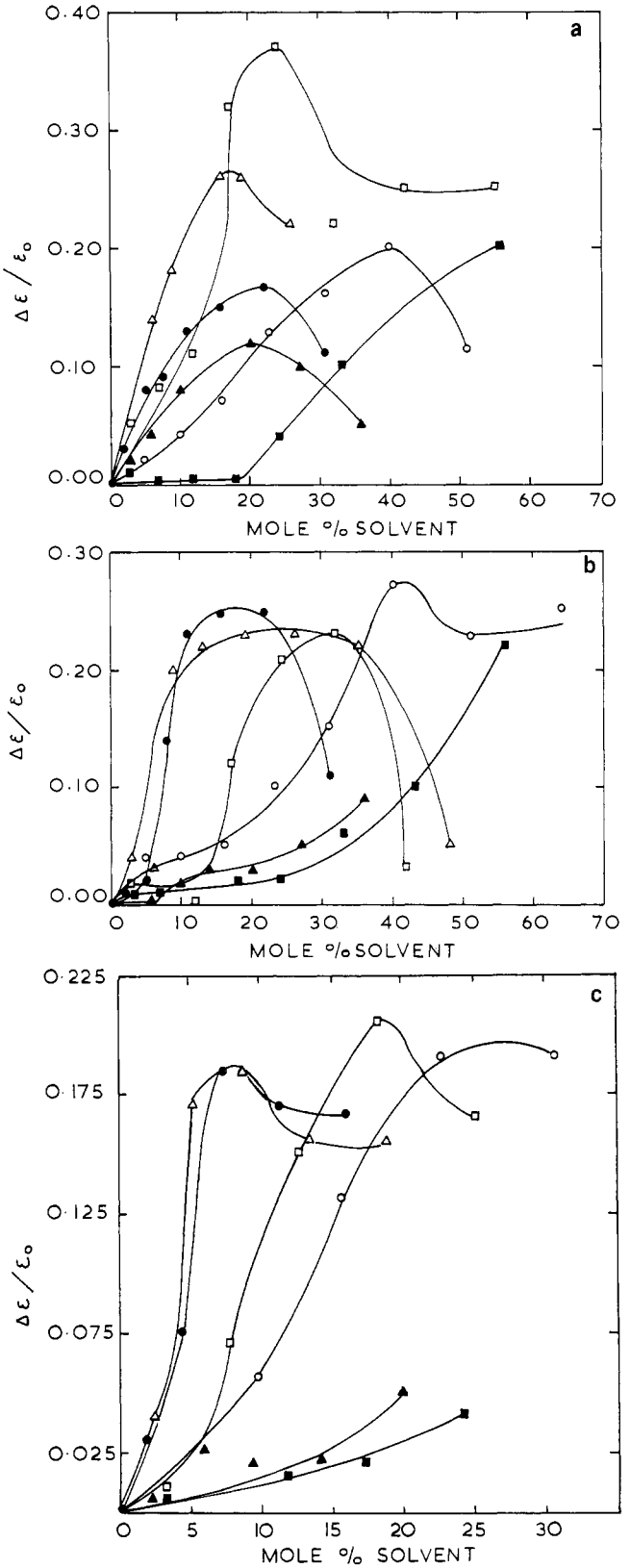

FIGURE 4: Soret studies. (a) Effect of alcohols on the Soret absorbance of cytochrome *c*. Relative change in molar absorptivity, $\Delta\epsilon/\epsilon_0$ is plotted as a function of alcohol concentration. (○) Methanol, (□) ethanol, (△) propanol, (●) *t*-butyl alcohol, (■) ethyleneglycol, and (▲) glycerol. Cytochrome *c* concentration 5 μM. Final concentration of acetate buffer in cuvet, 0.02 M, pH 5.6. (b) As in part a but in the presence of KCl, final concentration 10^{-1} M. (c) As in part a, but in presence of urea, final concentration 3 M.

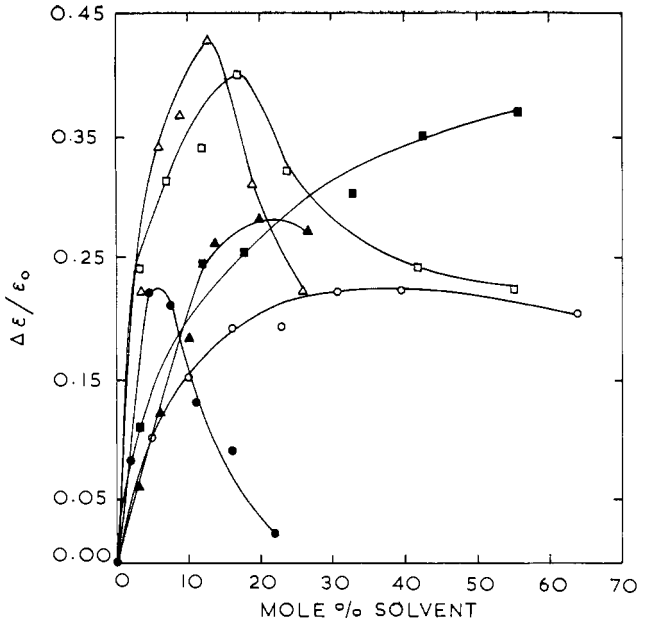

FIGURE 5: Effect of alcohols on the Soret absorbance of hemoundecapeptide. Relative change in molar absorptivity, $\Delta\epsilon/\epsilon_0$ is plotted as a function of alcohol concentration. (○) Methanol, (□) ethanol, (△) propanol, (●) *t*-butyl alcohol, (■) ethylene glycol, and (▲) glycerol. Hemoundecapeptide concentration 3.5 μM. Final concentration of pH 5.6 acetate buffer, 0.02 M.

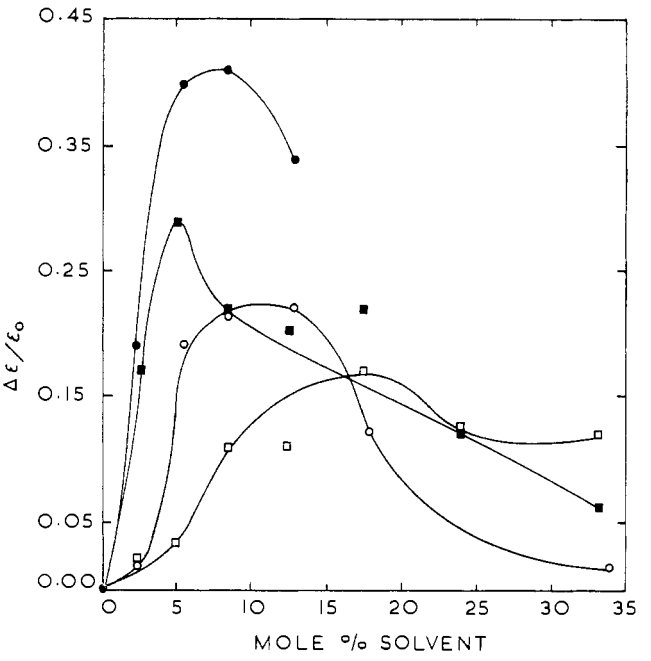

FIGURE 7: Effect of cyclic ethers on the Soret absorbances of cytochrome *c* and hemoundecapeptide. Relative change in molar absorbtivity, $\Delta\epsilon/\epsilon_0$ is plotted as a function of ether concentration. (○) Tetrahydrofuran on cytochrome *c*, (□) dioxane on cytochrome *c*, (●) tetrahydrofuran on hemoundecapeptide, and (■) dioxane on on hemoundecapeptide. Final concentration pH 5.6 acetate buffer 0.02 M; concentration of cytochrome *c* 5 μM, hemopeptide 3.5 μM.

different numbers of hydroxyl groups on the Soret absorbance of cytochrome *c* is shown in Figure 4a. The C* and S_M values for methanol (19.8%, 0.007), ethanol (13.5%, 0.020), 1-propanol (5.6%, 0.025), and 1-butanol (0.050) indicate the increasing effectiveness with increasing chain length. (The relative insolubility of 1-butanol prevents investigation at concentrations high enough to obtain the maximum effect.) *t*-Butyl alcohol is much less effective (5.5%, 0.016) in comparison with 1-butanol. An increase in the number of hydroxyl groups decreases the S_M; compare ethylene glycol (0.007) with ethanol (0.020) and propane-1,2-diol (0.004), and glycerol (0.006), with 1-propanol (0.025).

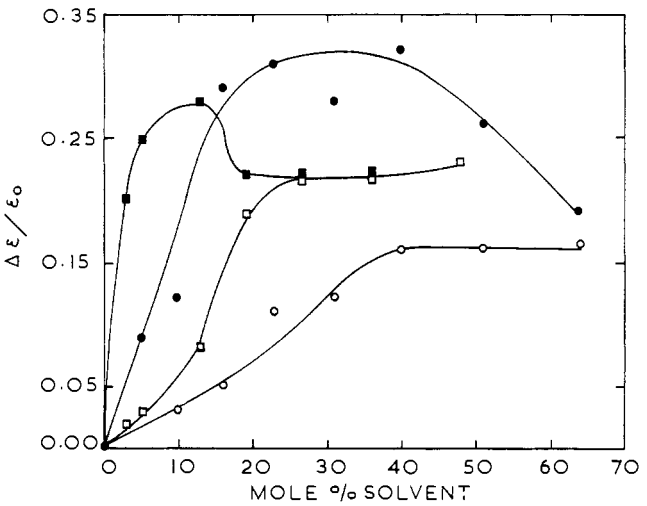

FIGURE 6: Effect of amides on the Soret absorbances of cytochrome *c* and hemoundecapeptide. Relative change in molar absorptivity, $\Delta\epsilon/\epsilon_0$ is plotted as a function of amide concentration. (○) Formamide on cytochrome *c*, (□) dimethylformamide on cytochrome *c*, (●) formamide on hemoundecapeptide, and (■) dimethylformamide on hemoundecapeptide. Cytochrome *c* concentration 5 μM; hemopeptide concentration 3.5 μM. Final concentration pH 5.6 acetate buffer, 0.02 M.

The effects of the same alcohols on the Soret absorption of the hemopeptide were more marked (Figure 5). The same trends of S_M were observed, methanol (0.013), ethanol (0.050), 1-propanol (0.063), *t*-butyl alcohol (0.026), ethylene glycol (0.023), and glycerol (0.018). The differences between S_M for cytochrome *c* and the hemopeptide for any particular organic solvent also followed the same trends.

The effects of electrolytes and urea on the action of alcohols on cytochrome *c* are shown in Figure 4b and c, respectively. The presence of 10^{-1} M potassium chloride increased the conformational modifying effects of the alcohols, methanol (0.010), ethanol (0.024), 1-propanol (0.043), and *t*-butyl alcohol (0.030), but had the opposite effect on the dihydroxy alcohols, ethylene glycol (0.004), and glycerol (0.002). Similar effects were noted in the presence of urea, methanol (0.008), ethanol (0.015), 1-propanol (0.042), *t*-butyl alcohol (0.037), ethylene glycol (0.003), and glycerol (0.005).

When the hydrogen-bonding capacity of formamide was decreased by the replacement of the amino hydrogen atoms by methyl groups, the conformational changes in cytochrome *c* were enhanced. Formamide (*C** 22.5%, S_M 0.004) dimethylformamide (*C** 15.0%, S_M 0.009) (Figure 6).

The effects of various ethers on the Soret absorbance of cytochrome *c* are shown in Figures 7–9. The effects of the cyclic ethers, tetrahydrofuran (0.074) and dioxane (0.064),

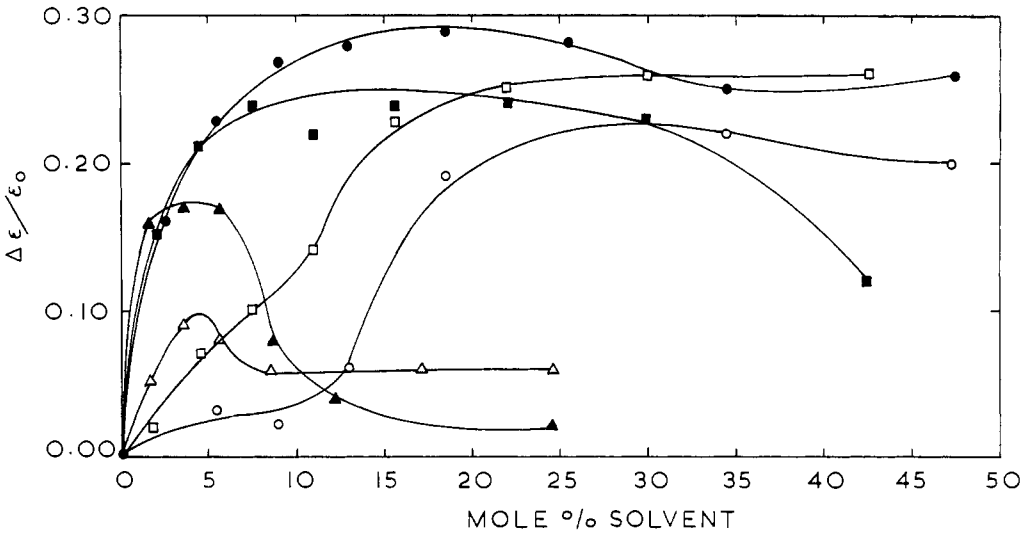

FIGURE 8: Effect of alkoxyethanols on the Soret absorbances of cytochrome *c* and hemoundecapeptide. Relative change in molar absorptivity, $\Delta\epsilon/\epsilon_0$ is plotted as a function of alkoxyethanol concentration. (○) Methoxyethanol on cytochrome *c*, (□) ethoxyethanol on cytochrome *c*, (△) *n*-butoxyethanol on cytochrome *c*, (●) methoxyethanol on hemoundecapeptide, (■) ethoxyethanol on hemoundecapeptide, and (▲) *n*-butoxyethanol on hemoundecapeptide. Final concentration pH 5.6 acetate buffer 0.02 M. Concentration of cytochrome *c* 5 μM; concentration of hemopeptide 3.5 μM.

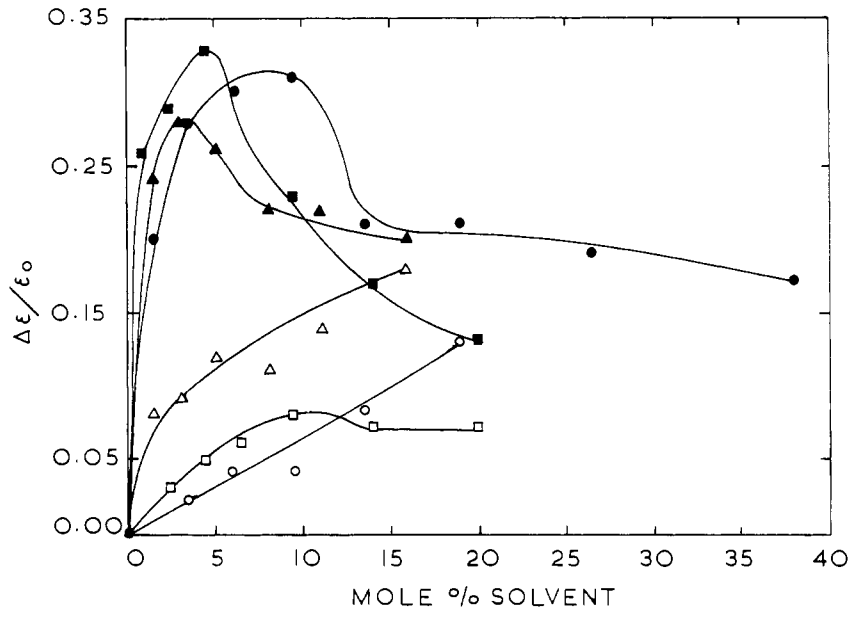

FIGURE 9: Effect of diethyleneglycols on the Soret absorbance of cytochrome *c* and hemoundecapeptide. Relative change in molar absorptivity, $\Delta\epsilon/\epsilon_0$ is plotted as a function of diethylene glycol concentration. (○) Methyldigol on cytochrome *c*, (□) *n*-butyldigol on cytochrome *c*, (△) dimethyldigol on cytochrome *c*, (●) methyldigol on hemoundecapeptide, (■) *n*-butyldigol on hemoundecapeptide, and (▲) dimethyldigol on hemoundecapeptide. Final concentration of pH 5.6 acetate buffer 0.02 M. Concentration of cytochrome *c* 5 μM; concentration of hemopeptide 3.5 μM.

on the hemopeptide, parallel those on cytochrome *c*, tetrahydrofuran (0.030) and dioxane (0.013), but are more pronounced (Figure 7). An increase in effectiveness against cytochrome *c* with an increase in alkyl chain length is seen in the alkoxyethanol series, in C^*, but not in the values of S_M. Methoxyethanol (14.5%, 0.021), ethoxyethanol (9.8%, 0.014) and *n*-butoxyethanol (1.1%, 0.029), and in the case of hemopeptide, methoxyethanol (2.0%, 0.054), ethoxyethanol (1.4%, 0.060), and *n*-butoxyethanol (0.3%, 0.050) (Figure 8). The digols (diethylene glycols) were less effective in their effect on cytochrome *c;* methyl digol (0.007), *n*-butyldigol (0.011), and dimethyldigol (0.030), but more effective on the hemopeptide; methyl digol (0.109), *n*-butyldigol (0.278), and dimethyldigol (0.192) (Figure 9).

In the case of the one solvent tested (methanol) removal of the organic solvent resulted in a change of absorbance to its original value in aqueous solution.

Discussion

Criteria which have been used previously to compare effectiveness of the solvents in modifying the structure of proteins include: (a) $\Delta\epsilon/\epsilon_0$ and (b) C^*, the mole per cent of denaturent required to effect 50% of the maximum change (Tanford, 1964). In the case of the effects of organic solvents on cytochrome *c* these criteria apparently represent the resultant of at least two effects. The first effect is a modification in protein structure resulting in increasing Soret absorbance, while a second effect results in a decreased absorbance. This latter effect is probably not a refolding of the peptide chain, as observed with β-lactoglobulin and γ-globulin (Tanford, *et al.* 1960), since it occurred to no less extent in the presence of urea. Since both peak height and peak position reflect a net result of at least two opposing effects, a better criterion of the initial conformation modifying power of the solvents, in this case, is the maximum slope, S_M, of the graph of relative molar absorptivity against mole per cent of solvent.

The effect of all the solvents studied, on the Soret peak of cytochrome *c*, was identical in character with that produced by urea (Stellwagen, 1967) and (Myer, 1968) and probably reflects a conformational change analogous to denaturation. In contrast, denaturation of myoglobin by urea (Schechter and Epstein, 1968) and by organic solvents (Herskovits and Jaillet, 1969) resulted in a decrease in Soret absorbance. This difference is presumably a consequence of the differing environment of the heme in the myoglobin. Possibly the difference in effects is due to the difference in accessibility of the heme in the two molecules. Addition of an organic solvent to cytochrome *c* increases the accessibility of the heme, which is then exposed to a more aqueous environment. At high levels of solvent the environment of the exposed heme becomes less aqueous and once more hydrophobic. In contrast the heme group in myoglobin is initially accessible to oxygen and even initial additions of organic solvent may increase the hydrophobic environment of the heme.

Since it has been suggested that the hemohexadecapeptide, isolated from cytochrome *c*, represents the fully exposed heme group of cytochrome *c* (Stellwagen, 1967), a similar compound, the hemoundecapeptide, was used as a model for the effect of organic solvents on the Soret absorbance of the heme group itself. Aggregation of this peptide may affect the results although it has been shown that organic solvents mainly disperse aggregates of heme peptides (Urry and Pettegrew, 1967). The parallel effects of the various solvents on cytochrome *c* and the hemoundecapeptide, however, imply that the structure of the peptide is not completely random in aqueous solution. Although it is much more sensitive to the solvents, the stabilizing forces in the hemopeptide are qualitatively similar to those determining the native conformation of cytochrome *c*. The maximum molar absorptivity of the Soret band of cytochrome *c*, in any particular aqueous organic solvent mixture, should thus tend to that of the hemopeptide. The absorbance maximum of the peptide was always greater than that of cytochrome *c*, under the same conditions of organic solvent concentration, suggesting that no solvent produced complete unfolding of the protein molecule. The difference between S_M for cytochrome *c* and for the heme peptide, at any particular solvent concentration, presumably results from the more complete protein environment in the cytochrome *c*.

The following conclusions on the effects of the organic solvents on the conformation of cytochrome *c* are apparent. (a) Increasing the length of the hydrocarbon chain increased the Soret enhancing effect of the solvent, *i.e.*, its conformation-modifying effect. Branching however, produced a decrease in this effect. The fact that *t*-butyl alcohol is less effective than ethanol, moreover, indicates that hydrophobicity is not the only factor, and that considerations of accessibility of the hydroxyl group, or of the interior of the polypeptide chain may determine the effectiveness in some cases. On the other hand, cyclization of a particular chain had negligible effect in the case of ethoxyethanol (compare dioxane). (b) The addition of electrolytes significantly enhanced the maximum slope, presumably due to the encouragement of the disruption of ionic bonds in solutions of higher ionic strength. (c) The presence of urea similarly enhanced the solvent effects, thus indicating incompleteness of the unfolding of the protein by the organic solvent. (d) The importance of hydrophobicity is also evident from the decrease in the Soret enhancing effect which occurs on replacing a carbon by an oxygen (compare *n*-butoxyethanol and methyldigol) or on inserting an oxygen into the chain (compare tetrahydrofuran and dioxane or propanol and methoxyethanol). When a hydroxyl group replaces a hydrogen atom, the loss of effectiveness is even greater (compare ethanol and ethylene glycol). (e) When chain length and oxygen content were simultaneously increased by adding a carbinol group, the above opposing effects balanced each other (compare ethylene glycol and glycerol or methanol and ethylene glycol) when peak enhancing effectiveness is considered. The effectiveness of methoxyethanol is likewise identical with that of ethanol, but methyldigol is less effective than ethoxyethanol. (f) That hydrogen bond disrupting capacity is also important, is seen in the comparison of formamide and dimethylformamide. On a volume per cent basis these are of roughly equal effectiveness in enhancing the Soret peak. Thus the increase in hydrophobicity only just balances the loss of hydrogen bonding capacity, as argued by Herskovits and Jaillet (1969). Thus the absence of any significant α-helical structure in cytochrome *c* (Dickerson *et al.*, 1967) does not negate the importance of hydrogen bonding in stabilizing its native conformation. In the case of the hemopeptide, however, hydrogen bonding is considerably less important.

The above results contain qualitatively interpreted evidence for contribution of hydrophobic, hydrogen, and ionic bonds to the stability of the native structure of cytochrome *c* and of the hemopeptide. Estimation of the magnitude of the contribution of each kind of bonding will have to await derivation of a satisfactory theoretical or empirical relationship between the solvent sensitivity of a protein and the physical properties of the attacking solvents. Similar studies on a variety of proteins are urgently required toward this end. The above data, like that of Herskovitz and Jaillet (1968, 1969), Schechter and Epstein (1968), and others should permit more informed selection of solvent series for further experiments, which will in turn be capable of a more exact quantitative interpretation.

Acknowledgments

The skilled assistance of Mr. R. Douglas is acknowledged with thanks.

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Concerning the Tertiary Structure of the Soluble Surface Proteins of Paramecium*

A. H. Reisner, Janet Rowe, and R. W. Sleigh

ABSTRACT: Six of the water-soluble globular proteins found on the surface of *Paramecium aurelia* (serotypic or immobilization antigens) are compared with respect to their amino acid compositions. They contain a remarkably high percentage of cystine (>10%) and hydroxylamino acids (24–29%), particularly threonine (12–16%).

The complement of hydrophobic amino acids is low, their average hydrophobicities, $H\phi_{av}$, ranging from 818 to 892 cal per mole of residue. The sedimentation coefficients of these molecules show marked concentration dependence and although hydrodynamic data indicate that the molecules are effective hydrodynamic prolate ellipsoids with axial ratios of about 11:1, the validity of this finding as regards their true shape is not supported by electron microscopy which presents a rough circular image about 175 Å in diameter. Analyses of the amino acid composition using Jennings' equation, predict that the oblate ellipsoidal dimensions should be about 200 × 15 Å while the prolate ellipsoid would measure about 1000 × 24 Å. We suggest that these molecules are in fact disk like. Optical rotary dispersion measurements indicate that little helical structure is present. A model for these molecules is presented in which it is suggested that they might resemble double bimolecular leaflets and may act as surface protectants.

Coating the surface of the ciliated protozoan, *Paramecium*, are a family of proteins termed the serotypic or immobilization antigens. Any one animal may have the genetic potential for expressing over a dozen members of the family but normally only one of the group is detectable at a given time. In short, a system of mutual exclusion operates (see Beale, 1954, Beale and Wilkinson, 1961, and Preer, 1968, for reviews).

Preer (1959) demonstrated the proteinaceous character of these molecules and estimated that one of them (51A from *Paramecium aurelia* syngen 4) has a molecular weight of about one-quarter million (±20%). Later studies by Jones (1965) and Steers (1965) have extended Preer's findings, so that amino acid analyses are available for antigens 90D, 178D, and 90G (syngen 1) and 51A. In addition Steers determined the molecular weight of 51A to be 310,000 g/mole while Jones estimated the syngen 1 molecules to have molecular weights of 250,000 g/mole.

Comparative analyses of these data together with our findings on 51A, 51B, and 51D show that the serotypic anti-

* From the C. S. I. R. O. Division of Animal Genetics, P. O. Box 90, Epping, N. S. W. 2121, Australia, and the C. S. I. R. O., Division of Food Preservation, Delhi Road, North Ryde, N. S. W. 2113, Australia. *Received June 30*, 1969.