EFFECTS OF ORGANIC SOLVENTS ON CYTOCHROME *c*

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1. Introduction

The effects of organic solvents on the conformation of DNA and of proteins have recently been studied with a view to estimating the relative importance of hydrophobic, hydrogen, and ionic bonds in stabilising the preferred conformation [1–3]. The effects of a non-aqueous environment on cytochrome *c* is of particular interest since its physiological function is in relation to lipid bound enzymes [4]. An investigation of the effect of a range of concentrations of each of a series of organic solvents on the Soret peak of ferricytochrome *c* and a haem containing undecapeptide [5], isolated from cytochrome *c*, was therefore undertaken. We investigated the influence of chain length, shielding of hydrogen bonding groups, addition of hydrogen bonding groups, and of the addition of urea or electrolytes on the effectiveness of the solvent in modifying the spectrum.

2. Materials and methods

In a cuvette, 0.1 ml of a 150 μM aqueous solution of horse heart cytochrome *c* (Grade 1, 88% purity, from Seravac Laboratories, Maidenhead, England) or 106 μM haempeptide was diluted with 0.3 ml of 0.2 M acetate buffer pH 5.6, water and organic solvent to the stated solvent concentration in 3 ml final volume. Since increases in pH, upon the addition of organic solvents, were unavoidable it was established that the spectrum was not altered over the pH range 5.6 to 7.5. In every experiment it was confirmed by measurement that the pH remained within these limits. The solution was scanned from 600 to 380 mμ using a Beckman DB spectrophotometer and 10″ recorder.

3. Results and discussion

In every case increase in the organic solvents concentration produced an increase in the intensity of the Soret peak similar to that produced by the action of urea [6] on cytochrome *c*, which probably reflects a conformational change analagous to denaturation. It has been shown that the magnitude of changes in Soret peak intensity parallels the degree of denaturation [6]. At higher concentrations of most solvents the peak decreased in intensity, in some cases almost to the initial value in aqueous solution (figs. 1a and b). For each solvent the relative molar absorbtivity ϵ-ϵ_0/ϵ_0 (where ϵ_0 is the molar absorbtivity in aqueous solution) was plotted as a function of solvent concentration. Tanford [7] and Herskovits [1] have used as their criterion of solvent effectiveness, the solvent concentration required to produce half the maximum response (C^*-Tanford). As a criterion of the denaturing power of the solvent the maximum slope (S_m) of the graph of relative molar absorbtivity against mole fraction of solvent or volume fraction of solvent was used in preference, since the height and position of the peak represent a balance between absorbance enhancing and depressing effects. Table 1 shows several criteria for the relative effectiveness of the various solvents examined, on cytochrome *c* and the haempeptide.

The following conclusions are apparent. (a) The nature of the effects was similar in cytochrome *c* and the haempeptide, but the magnitude of the change was usually two to three times greater in the haempeptide. Their relative sensitivities to any specific solvent were for the most part parallel, the exception being the substituted digols to which the haempeptide was (inexplicably) abnormally sensitive. If the increase

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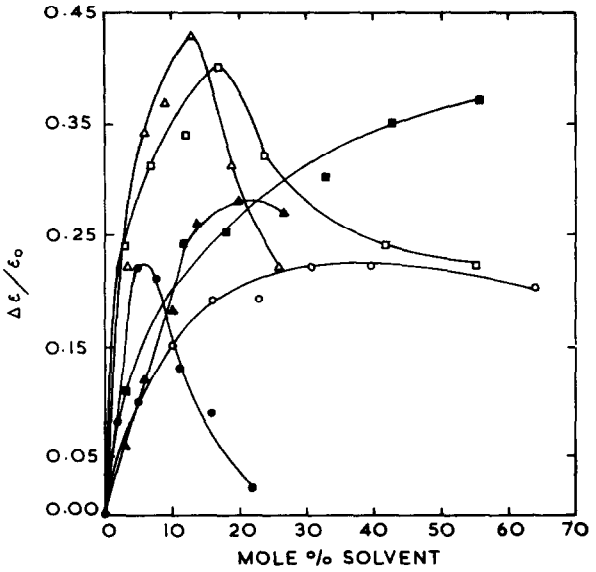

Fig. 1a. Effect of alcohols on the Soret absorbance of cytochrome *c*. ○ methanol; □ ethanol; △ 1-propanol; ● *t*-butanol; ■ ethylene glycol; ▲ glycerol.

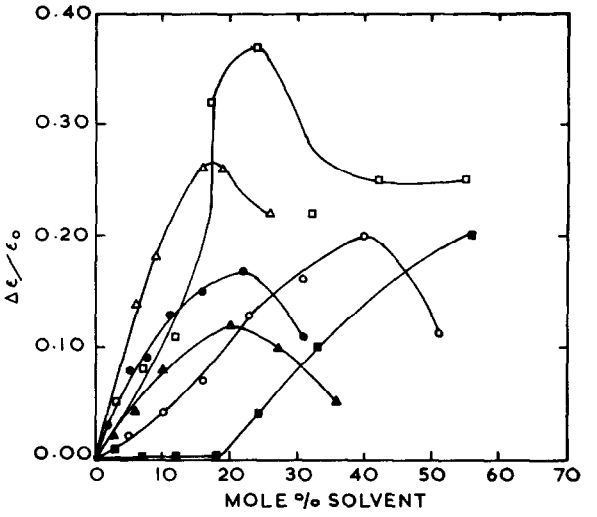

Fig. 1b. Effect of alcohols on the Soret absorbance of haempeptide.

Table 1
Solvent effects on Soret peaks of cytochrome *c* and haemundecapeptide.

	Cytochrome *c*			Haempeptide		
Solvent	S_m	C^*	$\Delta\epsilon/\epsilon_0$	S_m	C^*	$\Delta\epsilon/\epsilon_0$
Methanol	0.007	19.8	0.20	0.013	5.5	0.22
Ethanol	0.020	13.5	0.37	0.050	2.1	0.40
1-Propanol	0.025	5.6	0.27	0.063	2.5	0.43
t-Butanol	0.016	5.5	0.17	0.026	2.0	0.22
Ethylene glycol	0.007	32.5	0.20	0.023	8.0	0.37
Glycerol	0.006	8.0	0.12	0.018	6.9	0.28
Formamide	0.004	22.5	0.16	0.017	9.0	0.33
Dimethyl formamide	0.009	15.0	0.23	0.050	1.2	0.28
Tetrahydrofuran	0.030	4.3	0.23	0.074	2.6	0.42
Dioxane	0.013	7.0	0.17	0.064	2.1	0.29
Methoxy ethanol	0.021	14.5	0.23	0.054	2.0	0.30
Ethoxy ethanol	0.014	9.8	0.26	0.060	1.4	0.24
n-Butoxy ethanol	0.029	1.1	0.09	0.050	0.3	0.17
Methyl digol	0.007	10.0	0.13	0.109	1.0	0.31
n-Butyl digol	0.011	3.5	0.08	0.278	0.5	0.33
Dimethyl digol	0.030	2.7	0.18	0.192	0.8	0.28

See text for definition of C^* and S_m.

in Soret absorbance reflects denaturation, this result implies that the structure of the haempeptide in aqueous solution is not completely random as previously assumed [6]. (b) 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. (c) The presence of urea similarly enhanced the S_m. The decline in Soret peak intensity at higher solvent concentrations was not suppressed by urea, and it is thus unlikely that it reflects a refolding effect of high concentrations of solvent as suggested by Tanford [8]. (d) Increasing the length of the hydrocarbon chain increased S_m. Branching however, produced a decrease. The fact that *t*-butanol 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, cyclisation of a particular chain had negligible effect in the case of ethoxyethanol (compare dioxane). (e) The importance of hydrophobicity is evident from the decrease in S_m which occurs on replacing a carbon by an oxygen (compare *n*-butoxy-ethanol 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). (f) When chain length and oxygen content were simultaneously increased by adding a carbinol group, the above opposing effects balance each other (compare ethylene glycol and glycerol, or methanol and ethylene glycol). (g) That hydrogen bond disrupting capacity is also important, is seen in the comparison of formamide and dimethyl formamide. On a volume percent 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 [2]. The absence of any significant α-helical structure in cytochrome *c* [9] does not negate the importance of hydrogen bonding in stabilizing its native conformation. In the case of the haempeptide 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 haempeptide. Derivation of a satisfactory theoretical or empirical relationship between the solvent sensitivity of a protein and the physical properties of the protein and the attacking solvents will require further data, and further studies are in progress.

Acknowledgments

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