Table 2 Conditions: 1-napthol, 0.2 M; temperature, 40°C

CuCl (M)	Ligand ratio	Fractional yield		Intrinsic viscosity (dl/g)
		C – O	C – C	
0.005	1	0.260	0.643	0.045
0.010	1	0.505	0.385	0.063
0.050	1	0.765	0.142	0.072

The catalyst complex formed at low ligand ratio is found to favour the formation of C–C coupled product. The higher yield of the C–C coupled product at ligand ratios above ten indicates that theoretical molar ratio of pyridine to copper is not the actual molar proportion so far as the active catalyst complex is concerned. At higher ligand ratios, when pyridine is the solvent there seems to be no significant change in the C–O coupling reaction rate. The viscosity data indicate that the degree of polymerization is also high at higher ligand ratios. This observation is also in conformity with the observation of Enders et al.[4] in case of oxidative polymerization of 2,6-dimethyl phenol.

Under identical conditions the increase in molar proportion of 1-napthol has little or no effect over the relative rate of C–O and C–C coupling reactions.

From Table 2 it is observed that varying catalyst concentrations at constant ligand ratio has got also an important effect on the rate of C–O and C–C coupling reactions similar to those observed in case of varying ligand ratio. The formation of polymeric product (C–O coupling reaction) is favoured with increasing catalyst concentration keeping the ligand ratio constant.

Table 3 Conditions: 1-naphthol, 0.2 M; pyridine, 0.05 M; temperature, 40°C; polymerization, 2 h

CuCl (M)	Fractional yield		Intrinsic viscosity (dl/g)
	C – O	C – C	
0.005	0.965	0.0	0.065
0.010	0.872	0.125	0.068
0.020	0.833	0.138	0.074
0.030	0.754	0.172	0.078
0.040	0.737	0.195	0.075
0.050	0.765	0.142	0.072

Table 4 Conditions: cuprious chloride, 0.05 mol; ligand ratio, 1; temperature, 40°C; time, 2 h

1-napthol (M)	Fractional yield		Intrinsic viscosity (dl/g)
	C – O	C – C	
0.10	0.725	0.136	0.068
0.20	0.775	0.142	0.074
0.40	0.823	0.139	0.072
0.80	0.784	0.147	0.075

However the yield of C–O coupled product has been observed to decrease by increasing copper concentration while keeping the ligand ratio constant at 10 where maximum carbon oxygen coupling reaction occurs as evident in the earlier set of experiments.

According to these observations the catalytic complex that is formed at low ligand ratios favours the formation of C–C coupled product whereas the complex formed at high ligand ratio favours the formation of polyether. A plausible mechanism involving chain reaction with the catalyst complex at low ligand ratio may be suggested as follows. A C–C coupled quinine that is obtained at low ligand ratios, has been confirmed by i.r. analysis. The tetra coordinated complex formed predominantly at higher ratios of pyridine to copper appears to catalyse step reaction involving dehydration leading to the formation of a polyether.

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Effect of organic solvents on the stability of poly (L-glutamic acid) α-helix

M. Morcellet and C. Loucheux
Laboratoire de Chimie Macromoléculaire, Université des Sciences et Techniques de Lille, B. P. 36 59650 Villeneuve d'Ascq, France
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INTRODUCTION

The stability of the α-helical conformation of poly(L-glutamic acid) (PLGA) has been extensively studied especially in aqueous solutions. It is usually expressed as $\Delta F°$, the free energy of the helix to coil transition between the uncharged conformers. This value can be obtained from the modified titration curve of the polyacid, according to the method of Zimm and Rice[1].

Some potentiometric studies have also been carried out in water–organic solvent mixtures and the main result is that the addition of organic solvent increases the stability of the helical conformation of PLGA[2-5]. Conio et al.[3] have studied the helical stability of PLGA in mixtures of water with aliphatic primary alcohols and found that, at constant molarity of the organic solvent, $\Delta F°$ increases with the length of the aliphatic alcohol chain. From their results, Dubin[5] has shown that the free energy $\Delta F°$ depends mainly on the dielectric constant of

the solvent, irrespective of the length of the alcohol. In the following are presented some results on other water—organic solvent mixtures.

EXPERIMENTAL

The PLGA sample (in the acid form) was prepared by debenzylation of a poly(γ-benzyl-L-glutamate) sample according to the method of Idelson and Blout[6]. Its molecular weight, deduced from the intrinsic viscosity—molecular weight relationship of Wada[7] is $\overline{M}_w$ = 33 000. pH measurements were made with a Radiometer pH M 26 pH meter equipped with a G202C glass electrode and a K101 electrode. The temperature was controlled at 25° ± 0.01°C.

Titrations were carried out on solutions containing ~40 mg of polymer in 20 ml of solvent. Mixed solvents were made from aqueous 0.2 M MaCl and pure organic solvents. Solvent compositions are expressed in vol %. In all cases, appropriate blank titration of the solvent was also carried out.

From the experimental data, a plot of pH − log [α/(1 − α)] *versus* α was drawn. The area A between the modified titration curve and the extrapolation of the titration curve of the coil is related to the free energy of transition $\Delta F°$ by the relation[1]:

$$\Delta F° = -2.3\,RT \times A$$

in which R is the gas constant and T the absolute temperature.

Dielectric constant

The dielectric constant of the solvent was assumed to be linearly dependent on the composition. The following values were used for pure solvents: water, ϵ = 78.5; dioxane, ϵ = 2.2; ethanol, ϵ = 24.3; 2-chloroethanol, ϵ = 25.8; acetone, ϵ = 20.7; methyl ethyl ketone, ϵ = 18.5, at 25°C.

RESULTS AND DISCUSSION

The variation of $\Delta F°$ with the organic solvent concentration is plotted in *Figure 1* for some water—organic solvent mixtures. In all cases the addition of the organic solvent increases the absolute value of $\Delta F°$. This indicates an increase in the stability of the helical conformation of PLGA. Moreover the stability increases with the nature of organic solvent in the following order: dioxane; ethanol; acetone; methyl ethyl ketone; 2-chloroethanol.
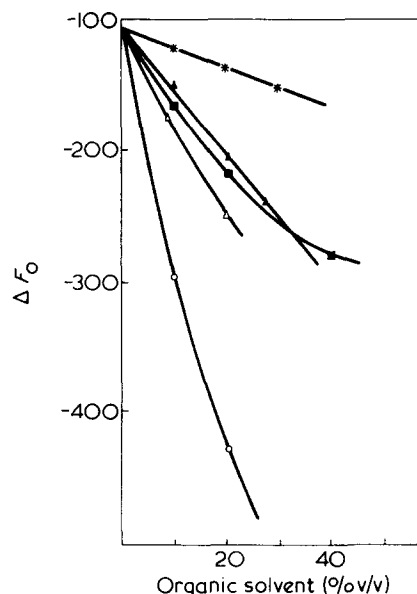

Figure 1 The variation of the free energy of transition of PLGA *versus* the solvent composition: *, NaCl 0.2 M/dioxane; ■, NaCl 0.2 M/acetone; △, NaCl 0.2 M/methyl ethyl ketone; ○, NaCl 0.2 M/chloroethanol; ▲, NaCl 0.2 M/ethanol[2]

Rather than in terms of organic solvent concentration, the experimental data may be treated, according to von Hippel and Wong[8] by considering the dependence on the methylene's molarity. This method takes into account for the hydrophobic nature of the organic solvent. The concentration C expressed in methylene molarity is related to the vol % of organic solvent P by the relation:

$$C = 10\,\frac{nd}{M}\,P \tag{1}$$

where d and M are the density and molecular weight of the organic solvent, n is the number of methylene groups in the molecule (n = 2 for ethanol and n = 4 for dioxane). If $\Delta(\Delta F°)$ is the increase of the free energy of transition in the mixed solvent with

Table 1 Values of the increment of the free energy of transition with respect to water for PLGA in some water—organic solvent mixtures

Solvent		$\Delta(\Delta F°)$ (cal/mmol)
NaCl (0.2 M)/dioxane[a]		− 3
KC1 (0.1 M)	methanol[b]	−13
	ethanol	
	propanol	
	butanol	
NaCl (0.2 M)/methyl ethyl-ketone[c]		−32
NaCl (0.2 M)/acetone[c]		−17
NaCl (0.2 M)/chloroethanol[c]		−53
H₂O/t-butanol[d]		−57

a Ref 9; b ref 3; c this work; d ref 5

respect to water, then we can define the increment of the free energy per unit of methylene molarity, $\Delta(\Delta F°)$ cal/mmol. Conio *et al.*[3] have shown that for PLGA in water—alcohol mixtures, this value is constant, irrespective of the nature of the alcohol and equal to −13 cal/mmol. This means that in water—alcohol mixtures the stability of the α-helix depends only on the length of the aliphatic chain of the organic solvent. It is interesting to note that, in water, the stability of the helical conformation of polypeptides such as poly(N_5-(3-hydroxyalkyl-L-glutamine) depends strongly on the length of the aliphatic part of the side chain. This emphasizes the important role played by hydrophobic interactions on the helix stability.

It is also important to point out that the increment $\Delta(\Delta F°)$ cal/mmol is exactly the same for poly(L-glutamic acid) and poly(L-ornithine) in water—alcohol mixtures[3]. This fact shows that in this case the nature of the organic solvent is more important than the nature of the side chain of the polypeptide.

Table 1 gives the values of $\Delta(\Delta F°)$ cal/mmol for PLGA in some water—organic solvent mixtures. The stabilizing effect increases from dioxane to 2-chloroethanol, corresponding to the features of *Figure 1*.

The value for the water—t-butanol mixture was obtained in the absence of salt[5]; nevertheless we have shown[9] that $\Delta(\Delta F°)$ cal/mmol is nearly the same in the presence or in the absence of salt. Thus, this result can be compared to those obtained in the presence of salt. The value of the increment is very different for acetone and methyl ethyl ketone whereas there is a unique value (−13 cal/mmol) for methanol, ethanol, propanol and butanol[3]. The reason for this may be that these alcohols are primary and linear ones whereas the ketones cannot be considered as homologous. The value reported for t-butanol is also very different from the one given for n-butanol (respectively −57 and −13 cal/mm).

Figure 2 shows the variation of $\Delta F°$ *versus* the dielectric constant of the solvent. Dubin[5] has shown that a unique curve is obtained for all the mixtures of water with alcohols[5]. This is in good agreement with the fact that the increment $\Delta(\Delta F°)$ cal/mmol is the same for all the water—alcohol mixtures.

From the *Figure 2* it is clear that the variation of the dielectric constant is not sufficient to explain the variation
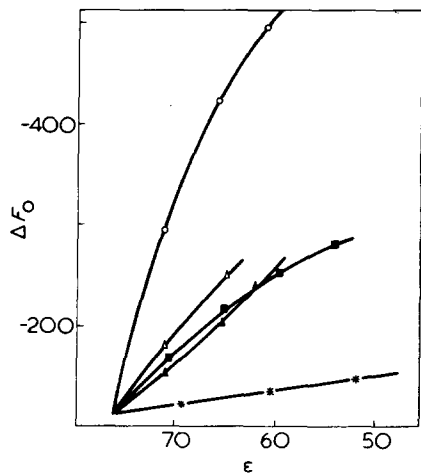
Figure 2 The variation of ΔF° *versus* the dielectric constant of the solvent: *, NaCl 0.2 M/dioxane; ■, NaCl 0.2 M/acetone; △, NaCl 0.2 M/methyl ethyl ketone; ○, NaCl 0.2 M/chloroethanol; ▲, NaCl 0.2 M/ethanol[2]

of ΔF°. The comparison of ethanol and 2-chloroethanol is interesting. These two solvents have about the same dielectric constant ($\epsilon = 24.5$ for ethanol and $\epsilon = 25.8$ for 2-chloroethanol), thus similar values of $\Delta(\Delta F^{\circ})$ cal/mmol should be expected. On the contrary, the increase in ΔF° is much more important in the case of 2-chloroethanol and the increment $\Delta(\Delta F^{\circ})$ cal/mmol is about four times greater than the corresponding value for ethanol.

A number of facts suggest that the increased stability of the helical con-

formation in water–organic solvent mixtures depends on the state of solvation by the organic solvent. The determination of the thermodynamic quantities ΔH° and ΔS° in water–alcohol[2,3] and water–dioxane[9] mixtures shows that, in both cases, the stabilization of the helical conformation has an entropic origin. An increase in ΔS° when changing from water to the mixed solvent indicates a relative increase of the entropy of the helix S_h and/or a relative decrease of the entropy of the coil S_c. It has been shown[10] that in NaCl (0.2 M/dioxane mixtures containing 10 to 30% of dioxane, the PLGA molecule, which has a non-ordered conformation, exhibits a strong preferential absorption of dioxane. This could account for the observed changes of the entropy ΔS°.

Moreover, some experimental facts found in the literature should also be emphasized. In the case of β-lactoglobulin, it has been shown by Inoue and Timasheff[11] that the preferential solvation by 2-chloroethanol is about four times greater than the preferential solvation by other alcohols in water–alcohol mixed solvents. *Table 1* shows that, except for t-butanol, 2-chloroethanol has the greater stabilizing power. In addition, though it is difficult to draw a close comparison between results obtained on proteins and model polypeptides, it should be point-

ed out that the preferential solvation of chloroethanol on β-lactoglobulin[11] is about four times greater than the preferential solvation of dioxane on poly(glutamic acid)[10]. *Table 1* shows that the stabilizing power of chloroethanol is also greater than that of dioxane.

Thus, there seems to be at least a qualitative correlation between the stabilizing power of organic solvent towards the helical conformation and the extent of the preferential solvation of the organic solvent onto the polymer under its disordered conformation.

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