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Biphasic Effects of Alcohols on the Phase Transition of Poly(L-lysine) between α-Helix and β-Sheet Conformations†

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ABSTRACT: Poly(L-lysine) exists as a random-coil at neutral pH, an α-helix at alkaline pH, and a β-sheet when the α-helix poly(L-lysine) is heated. The present Fourier-transform infrared (FTIR) study showed that short-chain alcohols (methanol, ethanol, and 2-propanol) partially transformed α-helix poly(L-lysine) to β-sheet when their concentrations were low. At higher concentrations, however, these alcohols reversed the reaction, and the alcohol-induced β-sheet was transformed back to α-helix structure. The reversal occurred at 1.40 M methanol, 0.96 M ethanol, and 0.55 M 2-propanol. The alcohol effects on the secondary structure were further investigated by circular dichroism (CD) on the thermally induced β-sheet poly(L-lysine). Methanol, ethanol, and 1-propanol, but not 1-butanol, shifted the negative mean-residue ellipticity at 217 nm of the β-sheet poly(L-lysine) to the positive side at low concentrations of the alcohols and to the negative side at high concentrations. With 1-butanol, only the positive-side shift was observed. The positive-side shift at low concentrations of alcohols indicates enhancement of the hydrophobic interactions among the side chains of the polypeptide in the β-sheet conformation. The negative-side shift indicates a partial transformation to α-helix. The shift from the positive to negative side occurred at 7.1 M methanol, 4.6 M ethanol, and 3.1 M 1-propanol. The alcohol concentrations for the β-to-α transition were higher in the CD study than in the IR study. This result suggests the multiplicity of the β-sheet conformations between the heat-induced and solvent-induced structures and also between the intramolecular and intermolecular β-sheets. The poly(L-lysine) concentration in the CD study was 0.5 mM where the β-structure is mainly intramolecular hydrogen bonding, whereas that in the IR study was 0.1 M where the hydrogen bonding is intermolecular as well as intramolecular. The biphasic effects coincide with those of alcohols on the main phase transition of dipalmitoylphosphatidylcholine (DPPC) membranes where methanol, ethanol, and 1-propanol, but not 1-butanol, decreased the transition temperature at low concentrations and increased it at high concentrations. The similarity between the alcohol concentrations that induce biphasic effect in the DPPC phase transition and in the β-to-α transition in the IR study suggests that the intermolecular hydrogen bonds are susceptible to alcohol perturbations. These alcohol actions on macromolecules are essentially solvent effects.

The biphasic effects of short-chain alcohols (methanol, ethanol, and 1-propanol) on macromolecular structures received recent research interest since the discovery of the "interdigitated" state in the phospholipid bilayer structure (McDaniel et al., 1983; Simon & McIntosh, 1984; Rowe, 1985; Nambi et al., 1988; Rowe & Cutrera, 1990). These alcohols depress the temperature of the main phase transition of lipid membranes between the solid-gel and liquid-crystalline phases, and disorder (or fluidize) the membrane. At extremely high concentrations, however, the alcohols shorter than four or five carbon atoms elevate the main transition temperature of the dipalmitoylphosphatidylcholine (DPPC) vesicle membranes (Jain & Wu, 1977).

We (Shibata et al., 1982, 1984, 1991) reported anesthetic effects upon the secondary structure of polypeptides and showed that volatile anesthetics partially transformed α-helix poly(L-lysine) to the β-sheet conformation. The present report deals with alcohol effects on the secondary structure of poly(L-lysine), where short-chain alcohols promoted the β-sheet conformation at low concentrations, but they supported the α-helix conformation when the concentrations exceeded the

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pharmacological level. The study consists of two parts. The alcohol effects on (1) the α-helix poly(L-lysine), which were evaluated by Fourier-transform infrared (FTIR) spectroscopy, and on (2) the β-sheet poly(L-lysine), which were evaluated by circular dichroism (CD) spectroscopy.

Materials and Methods

The FTIR study used deuterated methanol (CH_3OD), ethanol (C_2H_5OD), 2-propanol (C_3H_7OD) (deuterated 1-propanol was unavailable), and D_2O obtained from Sigma. The CD study used regular methanol, ethanol, 1-propanol, and 1-butanol which were of spectroscopic grade from Wako (Osaka, Japan). Triply distilled water was used throughout.

In the FTIR study, poly(L-lysine) (MW 45000, Sigma) was dissolved in D_2O at 0.11 M lysine residue. The pD was adjusted to 11.8 by sodium deuteroxide (NaOD, Fluka, Ronkonkoma, NY) to form α-helix. Deuterated alcohols were added by a microsyringe, and the added amount was verified by weighing.

Infrared spectra were obtained by a Perkin-Elmer (Norwalk, CT) Model 1750 FTIR spectrophotometer interfaced with a Model 7300 computer. The attenuated total reflectance (ATR) cell was a Spectra-Tech (Stamford, CT) Model 0005-133 CIRCLE liquid system with a sealed window of zinc selenide crystal. A triglycine sulfate detector was used throughout. The cell was equipped with a Spectra-Tech Model 0005-420 heating/cooling jacket to control the cell temperature. A United System (Dayton, OH) Digitec Model 5810 thermistor thermometer was used to monitor the temperature with 0.01 °C resolution. All experiments were performed at 22.0 ± 0.5 °C.

For the circular dichroism study, poly(L-lysine) was dissolved in H_2O at 5.0×10^{-4} M per lysine residue, and the pH was adjusted to 11.4 with NaOH to form the α-helix. It was transformed into β-sheet by heating at 55 °C for 30 min. The β-sheet poly(L-lysine) conformation is metastable. Our differential scanning calorimetry showed no excess heat flow during the cooling scan or the second heating scan after several hours (Chiou et al., 1992). The preparation stayed in the β-sheet structure at 4 °C when measured by FTIR. It has been known that several days are required to transform the β-sheet back into the α-helix conformation in a refrigerator (Carrier et al., 1990). Excess heat flow in a heating scan appeared again after 5 days in a refrigerator (Chiou et al., 1992).

The poly(L-lysine) concentration was kept low to avoid precipitation when thermally transformed into the β-sheet conformation (Chou & Scheraga, 1971). After being heated, poly(L-lysine) was cooled slowly (Pederson et al., 1971), and then an appropriate amount of alcohol was added by a microsyringe. The added amounts of alcohols were verified by weighing. The concentration of poly(L-lysine) was confirmed by a Hitachi Model 1835 amino acid analyzer equipped with a Model 883A data processor (Tokyo, Japan).

Circular dichroism was measured by a JASCO J-600 spectropolarimeter (Tokyo) under a constant nitrogen flush to avoid absorption of the ultraviolet region (shorter than 200 nm) by oxygen. A Teflon-stoppered cuvette—with a light-path length of 1.0 mm—was used. All measurements were conducted at 22.0 ± 0.5 °C within 1 h after preparation of the sample. The details of CD measurements were reported previously (Shibata et al., 1991).

The observed ellipticity, θ_{obs}, was converted to the mean residue ellipticity, $[\theta]$, by

$$[\theta] = 100\theta_{obs}/CL$$

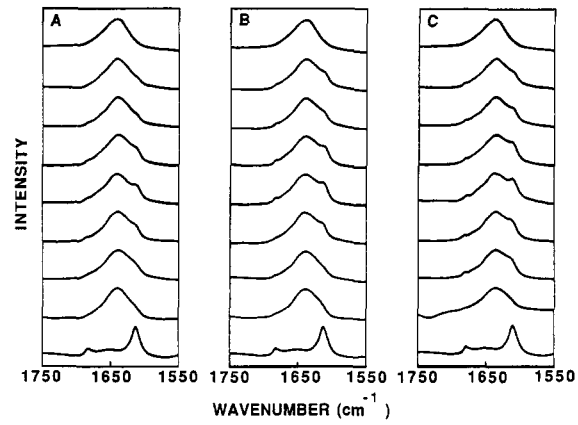

FIGURE 1: Effects of deuterated alcohols on the difference spectra of poly(L-lysine) in D_2O after subtraction of D_2O bands. For comparison, α-helix (top) and β-sheet (bottom) spectra without alcohols are included. Alcohol concentrations from the top downard are the following: (A) deuterated methanol, 0.12, 0.48, 1.06, 1.28, 1.39, 1.82, and 2.24 M; (B) deuterated ethanol, 0.17, 0.49, 0.73, 0.96, 1.26, 1.57, and 1.82 M; (C) deuterated 2-propanol, 0.08, 0.16, 0.32, 0,55, 1.09, 1.42, and 1.70 M.

where C is the molarity of the poly(L-lysine) residue and L is the cuvette light-path length in centimeters. Calculation of the secondary structure of poly(L-lysine) from CD data is based on the assumption that at each wavelength, the CD spectrum of poly(L-lysine) can be described as a linear arithmetic sum of contribution from α-helix, β-sheet, and random-coil as follows (Greenfield & Fasman, 1969; Chang et al., 1978; Takeda et al., 1987):

$$[\theta] = f_\alpha[\theta]_\alpha + f_\beta[\theta]_\beta + f_c[\theta]_c$$

where $[\theta]$ is the experimentally obtained mean residue ellipticity and $[\theta]_\alpha$, $[\theta]_\beta$, and $[\theta]_c$ are the mean residue ellipticities of the reference spectra of α-helix, β-sheet, and random-coil, respectively; f is the relative proportion, and $\sum f_i = 1$. The mean residue ellipticity would then be the sum of the contributions from each form.

Results

The FTIR study showed that the addition of alcohols to α-helix poly(L-lysine) induced a biphasic response in the secondary structure: an α-to-β transformation at low alcohol concentrations and a reverse (β-to-α) transformation at high alcohol concentrations. Figure 1 shows the IR spectra for the effects of deuterated alcohols on the α-helix poly(L-lysine) in D_2O. For comparison, the control α-helix and β-sheet spectra are shown at the top and bottom of each figure, respectively. It is seen that the β-characteristic 1614-cm^{-1} peak appeared as alcohols were added to the α-helix poly(L-lysine). Coincident with further increases in the alcohol concentration, the β-peak started to decrease and the α-characteristic 1638-cm^{-1} became dominant again. In Figure 2, the relative α-to-β ratio is plotted against the alcohol concentrations. The transition points in the biphasic response were 1.40, 0.96, and 0.55 M for deuterated methanol, ethanol, and 2-propanol, respectively.

The alcohol effects on the thermally induced β-sheet poly(L-lysine) were analyzed by circular dichroism. Figure 3 shows the reference CD spectra of α-helix, β-sheet, and random-coil in the absence of alcohols. These conformations show negative extrema at 206 and 221 nm for α-helix, 217 nm for β-sheet, and 194 nm for random-coil. These spectra were used as a reference to estimate each component in the secondary structure of poly(L-lysine) after the addition of alcohols.

Figure 4 shows CD spectra of β-sheet poly(L-lysine) in the presence of various concentrations of 1-propanol. The β-

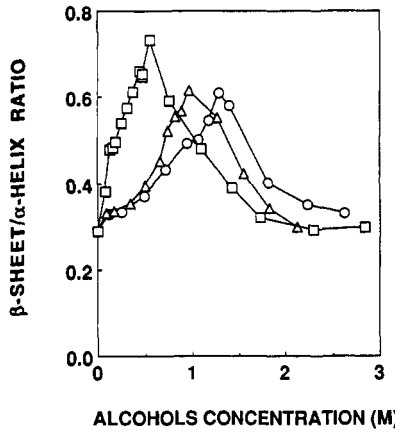

FIGURE 2: Effects of deuterated alcohols on α-helix poly(L-lysine). Ordinate: β-to-α ratio. Abscissa: deuterated alcohol concentrations. Circles, deuterated methanol; triangles, deuterated ethanol; squares, deuterated 2-propanol.

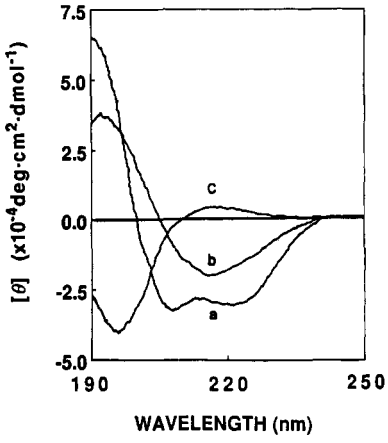

FIGURE 3: Reference CD spectra of poly(L-lysine) in (a) α-helix, (b) β-sheet, and (c) random-coil in aqueous solution.

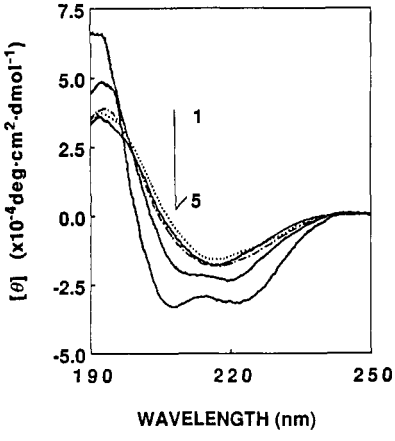

FIGURE 4: CD spectra of β-sheet poly(L-lysine) with 3.0 M (trace 1, dotted line), 4.0 M (trace 3, dashed line), and 6.0 M (trace 4) 1-propanol. The spectra of the β-sheet trace (trace 2) and α-helix (trace 5) are shown as references.

characteristic 217-nm negative ellipticity (Townend et al., 1966) was decreased (shifted to the positive side) by dose-dependent form up to 3 M 1-propanol, but started to increase (shift to the negative side) above 4 M.

To analyze the biphasic behavior, the 217-nm ellipticity, θ_{217}, was plotted against the alcohol concentrations (Figure 5). All four alcohols decreased $[\theta]_{217}$ linearly at low concentrations. When their concentrations are increased, however, the plot became nonlinear and started to increase except for 1-butanol. The concentrations, where the decrease to increase occurred, were 7.1 M methanol, 4.6 M ethanol, and 3.1 M 1-propanol.

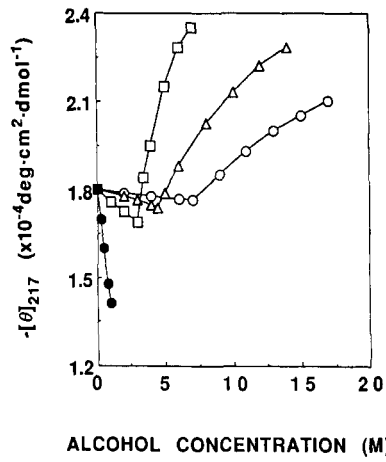

FIGURE 5: Negative mean-residue ellipticity of poly(L-lysine) at 217 nm (θ_{217} as a function of alcohol concentrations). Open circles, methanol; open triangles, ethanol; open squares, 1-propanol; closed circles, 1-butanol.

1-Butanol displayed only a linear decrease in negative ellipticity.

Discussion

The IR results showed that the response of the α-helix poly(L-lysine) to short-chain alcohols is clearly biphasic: α-to-β at low concentrations and its reverse at high concentrations. The alcohol-induced β-peak, however, was observed at 1614 cm^{-1} and was not identical to the thermally induced β-sheet control peak, which was located at 1610 cm^{-1}. It suggests that there may be structural differences between the β-sheet conformations induced by alcohols and heat. Carrier et al. (1990a,b) demonstrated that a hydrostatic pressure of 19 200 atm was required to completely reverse the thermally induced β-sheet poly(L-lysine) to α-helix. Our high-pressure studies (Chiou et al., 1992) revealed that volatile anesthetics partially transformed α-helix poly(L-lysine) into β-sheet and that 600 atm hydrostatic pressure reverted the anesthetic-induced β-sheet structure to α-helix. The mechanism for the difference between the hydrostatic pressures required to reverse the solute-induced and heat-induced α-to-β transition is unclear at present. It may be caused by the structural difference in the solute-induced and heat-induced β-sheets as suggested by the IR spectra and by the possible difference in the activation energy for the heat-induced and solute-induced transitions.

Multiplicity of the β-sheet structure is well-known, especially for multidomain macromolecules (Blout & Lenormant, 1957; Wasacz et al., 1987). Wasacz et al. (1987) used IgG to estimate the effect of amphiphiles on the β-sheet structures of protein macromolecules, because a large part of this protein is in the β-sheet conformation. From the shift of the IR peak, they reported that ethylene glycol induced a new β-sheet structure in the IgG segments by first unfolding and then refolding the macromolecule. The refolded structure may involve different parts of the molecule interacting with each other, and is not identical with the original β-sheet.

The present CD study on the heat-induced β-sheet poly(L-lysine) showed that low concentrations of alcohols decreased the negative ellipticity at 217 nm. This result appears to indicate a transition from β-sheet to random-coil. When the alcohol concentrations were increased, however, the negative ellipticity started to increase. This indicates a transition from β-sheet to α-helix. To quantitate the shift in the CD spectra of poly(L-lysine), we analyzed the alcohol effects on the secondary structure of poly(L-lysine) by the curve-fitting procedure (Greenfield & Fasman, 1969; Chang et al., 1978; Takeda

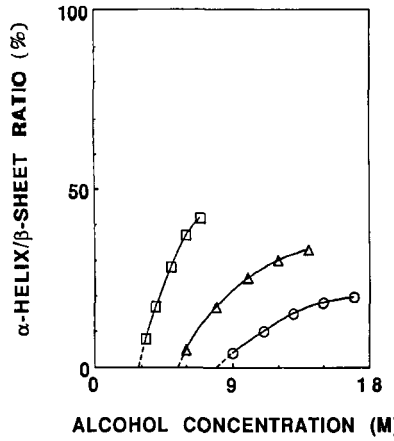

FIGURE 6: Relative proportions of α-helix and β-sheet as a function of high alcohol concentrations. Circles, methanol; triangles, ethanol; squares, 1-propanol.

et al., 1987). When the alcohol concentrations are high, the curve-fitting program computed the relative proportions of the secondary conformations without any difficulty. The relative ratio in the secondary structure of the heat-induced β-sheet poly(L-lysine) in the presence of high concentrations of alcohols is shown in Figure 6. The increases in the concentrations of methanol, ethanol, and 1-propanol, but not 1-butanol, increased the relative proportion of the α-helix conformation. The random-coil structure was not observed. The alcohol concentrations that transformed the heat-induced β-sheet poly(L-lysine) 15% into α-helix were 13.7 M methanol, 7.8 M ethanol, and 4.2 M 1-propanol (Figure 6).

At low alcohol concentration ranges, however, the curve-fitting program could not compute the relative proportions. This failure in computation of the secondary structure of poly(L-lysine) may have originated from the assumption in the curve-fitting procedure. A restriction for applying the computer simulation to determine the secondary structure of poly(L-lysine) is the condition that poly(L-lysine) exists in α-helix, β-sheet, random-coil, or a combination of these three conformations represented only by the reference spectra. If the present results in the decrease (shift to the positive side) of negative ellipticity at 217 nm, $[\theta]_{217}$, indicate β-to-random transition, then the decrease in $[\theta]_{217}$ should be accompanied by an increase (shift to the negative side) in $[\theta]_{194}$ (random-coil-characteristic negative ellipticity at 194 nm) (Greenfield & Fasman, 1969). Instead, $[\theta]_{194}$ shifted to the positive side (Figure 4). The decrease of negative ellipticity by low concentrations of alcohols was not caused by the β-to-random transition. This failure of the curve-fitting analysis indicates that the structure of poly(L-lysine) was not a simple mixture of the three conformations represented by the reference spectra. Conformations other than the reference spectra must have evolved in the presence of low concentrations of alcohol.

The addition of alcohols to heat-induced β-sheet poly(L-lysine) might have formed the β-sheet conformation represented by the 1614-cm^{-1} IR peak in addition to the heat-induced 1610-cm^{-1} peak. The CD spectra computation is programmed to read only the structure represented by the IR peak at 1610 cm^{-1} and may have difficulty in recognizing the 1614-cm^{-1} peak structure. This may be a reason that the computer program failed to estimate the secondary structure in the low concentration range of alcohols.

The alcohol concentrations for the β-to-α change in the CD study were significantly higher than those in the IR study. The poly(L-lysine) concentration in the IR study (0.11 M per lysine residue) was much higher than in the CD study (0.5 mM). Wooley and Holzwarth (1968) showed that when the poly(L-lysine) concentration was less than 1.0 wt %, the β-sheet was formed by the intramolecular hydrogen bonds whereas above this concentration both inter- and intramolecular forms were generated. The low alcohol concentrations in the IR study that induced the β-to-α transition appear to indicate that the intermolecular hydrogen bonds are more vulnerable to solvent perturbations.

The effects of amphiphiles and nonpolar additives on proteins in an aqueous solution are often biphasic and vary between high and low concentration ranges. The effects of alcohols on the conformation of human serum albumin, human transferrin, and lysozyme (Abu-Hamdiyyah, 1991), or the effects of surfactants (sodium octyl sulfate, sodium decyl sulfate, and sodium dodecyl sulfate) on the conformation of human serum albumin (Reynold et al., 1967), are biphasic. In each case, addition of low concentrations of amphiphiles to aqueous protein solutions shifted optical rotation to the positive side (less levorotatory). The addition of nonpolar additives (methane, ethane, propane, and butane) to the aqueous solutions of lactoglobulin and bovine serum albumin shifted optical rotation also to the positive side (Balasubramanian & Wetlaufer, 1966). These shifts of optical rotation to a less levorotatory side by nonpolar additives and low concentrations of amphiphiles are the reverse of the effects of protein denaturants, such as urea. Considering that urea shifts optical rotation of proteins to the negative side (more levorotatory) and weakens the hydrophobic interaction of proteins in aqueous solution (Tanford et al., 1960; Brandts & Hunt, 1967; Dubin & Strauss, 1973), the amphiphiles and nonpolar additives enhance hydrophobic interactions. Thus, it is generally accepted that their effect is attributable to the enhancement of hydrophobic interactions among side chains (Tanford & De, 1961; Herskovitz et al., 1970).

In surfactant solutions, the enhancement of the hydrophobic interaction is expressed by a decrease in the critical micelle concentration (cmc). When amphiphiles or nonpolar molecules are present in a micellar solution, the ability to enhance the hydrophobic interaction is expressed by the initial rate of the depression of the cmc at infinite dilution of the additive molecules, $-\ln [d(\text{cmc})/dC]_{C\to 0}$, where C is the additive concentration (Kaneshina et al., 1981; Abu-Hamdiyyah & Rahman, 1985). In aqueous solutions of proteins, the ability to enhance the hydrophobic interaction between proteins and additives can be estimated by the initial rate of change in the positive-side shift of the optical rotation, coincident with the increase in additive concentrations. In the CD spectra, the initial rate of change in the negative ellipticity at infinite dilution of alcohols, $\ln (d\theta/dC)_{C\to 0}$, represents the hydrophobic interaction. A high correlation has been recognized between the two systems in evaluating the change in hydrophobic interactions.

From the data of $[\theta]_{217}$ at low alcohol concentrations, we estimated the hydrophobicity parameter, $\ln (d\theta_{217}/dC)_{C\to 0}$. The relation between the parameter and the alcohol carbon chain lengths was linear ($r^2 = 0.99$). The slope is comparable to that of the chain-length dependence of the standard free energy change (per CH_2) for the transfer of alcohols from an aqueous solution to a hydrophobic environment (Lin & Somasundaran, 1971). This linear relation between the alcohol carbon chain lengths and the $\ln (d\theta_{217}/dC)_{C\to 0}$ values suggests that the alcohol-induced decrease in the negative ellipticity is related to the increase in the hydrophobic interaction among side chains. Alcohols induced greater structuring of the β-conformation with the enhancement of hydrophobic interaction

among the un-ionized side chains of poly(L-lysine). The new structure may be viewed as an enhanced hydrophobic β-sheet conformation.

Amphiphiles induce transformation of the secondary structure of poly(L-lysine) and favor the α-helix structure when their concentrations are very high (Epand & Scheraga, 1968; Satake & Yang, 1975; Takeda, 1985). From the optical rotatory dispersion and CD studies, Epand and Scheraga (1968) reported that the addition of methanol to random-coil poly(L-lysine) at neutral pH transformed it into α-helix when the methanol concentration reached 87–90 vol %. According to Raman spectroscopy, when the random-coil poly(L-lysine) was incorporated into the hydrophobic domain of phosphatidylglycerol bilayer membranes, it transformed into α-helix (Carrier & Pézolet, 1984). Takeda (1985) reported that when sodium octyl sulfate was added to random-coil poly(L-lysine), the α-helix structure dominated at the surfactant concentration between 4 and 6 mM but β-sheet appeared above this surfactant concentration.

The stability of the secondary structure of polypeptides is maintained primarily by the hydrogen bonding between the peptide bonds NH···O=C and the non-hydrogen-bonded interactions. Because both α and β structures contain a similar number of hydrogen bonds among the peptide skeletons, the difference in stability is attributable to non-hydrogen-bonded interactions. The hydrophilicity of the peptide surface decreases in the order of random-coil, α-helix, and β-sheet. The random-coil conformation with ionized side chains is highly hydrated. Between the α and β conformations, Blout and Lenormant (1957) reported that the α-helix surface of poly(L-lysine) is hydrated by four to six water molecules per lysine residue, whereas the β-sheet surface has less than two water molecules per lysine residue. Addition of amphiphiles appears to favor hydrophobic structures in macromolecules, but the effects are not uniform. It is unclear why the final conformation of poly(L-lysine) is α-helix with alcohols shorter than three carbon atoms, whereas it is β-sheet with longer chain alcohols.

When the minimum conformational free energy is compared between α-helix and β-sheet in poly(L-alanine), where the hydrophobic contribution to the secondary structure can be neglected, the free energy of the α-helix structure is lower than that of the β-sheet by only 0–4 kJ residue^{-1} (Pederson et al., 1971; Scheraga et al., 1967). The greater stability of α-helix over β-sheet in poly(L-alanine) at very high concentrations of short-chain alcohols may be caused by the less favorable packing of the β-sheet conformation.

The effect of high alcohol concentrations on the β-to-α transition in poly(L-lysine) appears to depend upon a similar mechanism. In the presence of very high concentrations of alcohols, the excessive enhancement of hydrophobic interactions may worsen the packing, and initiate the β-to-α transition. In this context, the partial molar volume at infinite dilution of β-sheet in aqueous phase is about 2.7 cm^3 (mol of residue)$^{-1}$ larger than α-helix (unpublished data).

Another possibility is the validity of the assumption that the β-sheet conformation of poly(L-lysine) is stabilized only by the hydrophobic interactions among the side chains. The hydrophobic effect alone may not explain the enhancement in the α-helical structure at very high alcohol concentrations (Hermans, 1966; Epand & Scheraga, 1968; Conio et al., 1970; Mishra & Ahluwalia, 1983). At higher alcohol concentrations, the change in the solvent property around the alkyl side chains of poly(L-lysine) can be expected. This would rearrange the interaction among water, alcohol, and poly(L-lysine) molecules (Franks & Smith, 1968). It was suggested that the entropy change of the hydrogen-bonding or dipole interaction between the peptide groups (CONH) and alcohol molecules is more negative than the similar interaction between the peptide and water (Conio et al., 1970; Mishra & Ahluwalia, 1983). The negative entropy of mixing of the CONH group with alcohols may attribute to the enhanced stability of the α-helix structure under higher alcohol concentrations.

The biphasic response of poly(L-lysine) to alcohols resembles the biphasic response of the phase transition of DPPC vesicle membranes. In DPPC vesicles, alcohols shorter than three carbon atoms favored the high-entropy liquid phase at low concentrations, but they supported the low-entropy solid phase at high concentrations. 1-Butanol did not show this biphasic effect (Jain & Wu, 1977). The change from solid→liquid to liquid→solid was later explained by interdigitation, where apposing leaflets of lipid monolayers penetrate each other to form an all-trans solid phase, now known as the $L_{\beta I}$ phase, and $L_\alpha \rightarrow L_{\beta I}$ transition. The thermotropic phase transition of phospholipid membranes from solid (rippled $P_{\beta'}$) to liquid (L_α) is endothermic and is comparable with the endothermic transition of poly(L-lysine) from α-helix to β-sheet. Here, the $P_{\beta'}$ (low-entropy) phase corresponds to α-helix and the L_α (high-entropy) phase to β-sheet. The change in the DPPC phase transition occurred at 3.0 M for methanol, 1.0 M for ethanol, and 0.4 M for 1-propanol. The concentrations at the reverse reaction in the present IR study were 1.40 M methanol, 0.96 M ethanol, and 0.55 M 2-propanol. This similarity in their concentration range in inducing the biphasic response between DPPC and poly(L-lysine) and the three-carbon limit are intriguing.

Lipid membranes cannot be formed without water. The structure is maintained by the balance between the lipid–lipid cohesive force and the lipid–water adhesive force. When the lipid–water adhesive force is overtly attenuated, the apposing monolayers penetrate each other, and the lipid tails form the low-entropy all-trans conformation to improve molecular packing in the membrane. The alkyl chain of longer alcohols apparently interacts with the lipid tails of phospholipids and may prevent interdigitation. Protein structures in water are also maintained by the hydrophobic effect. The agreement on the biphasic effect suggests that the alcohol effects on macromolecular structures may be similar between DPPC and poly(L-lysine). These conformational changes are essentially caused by solvent effects. However, the exact mechanism of the biphasic response in polypeptides remains to be elucidated.

Registry No. DPPC, 2644-64-6; poly(Lys) homopolymer, 25104-18-1; poly(Lys) SRU, 38000-06-5; methanol, 67-56-1; ethanol, 64-17-5; 1-propanol, 71-23-8; 1-butanol, 71-36-3; 2-propanol, 67-63-0.

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Cement Precursor Proteins of the Reef-Building Polychaete *Phragmatopoma californica* (Fewkes)

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ABSTRACT: Two distinctive 3,4-dihydroxyphenyl-L-alanine- (DOPA-) containing proteins (Pc-1 and Pc-2) have been isolated and partially characterized from the thorax of the reef-building sabellariid *Phragmatopoma californica*. They are the first such reported from the phylum Annelida. The proteins are presumed to be soluble precursors of the quinone-tanned cement used to bind particulate materials in the construction of the tubes that serve as habitats for the worms. The proteins have apparent molecular weights ranging from 18 000 to 20 000 and isoelectric point ≥ 8.0. Both proteins consist of repeated sequence motifs in their primary structure. Pc-1 has repeats of {XGGY*GY*GAK} where X = V, L, I, AA, or KV, and Y* is DOPA or tyrosine. Pc-2, in contrast, appears to have repeats of $\{X_1\text{-}[GGY^*]_n\text{-}[GA]_m\text{-}X_2\text{-}[HP(A)V]_p\text{-}HK\}$ where X_1 can be AL, A, or F; X_2 can be WG or absent; n and m can be 1 or 2, and $p = 0$–2. Both protein families appear to share the same C-terminal sequence ALGGY*GAGA. Of the DOPA-containing proteins characterized from other phyla, *Phragmatopoma* cement precursors most resemble those from the liver fluke *Fasciola hepatica* and the mussel *Trichomya hirsuta*.

The gregarious marine sabellariid polychaetes build massive reef-like mounds which consist of a honeycomb of contiguous tubes each with a permanently resident polychaete (Hartman, 1944; Sisson, 1986). The tube walls are constructed in a manner that closely resembles stone masonry. The polychaete collects passing particulate material and debris (average diameter 500 μm in *Sabellaria alveolata*) from the water column

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