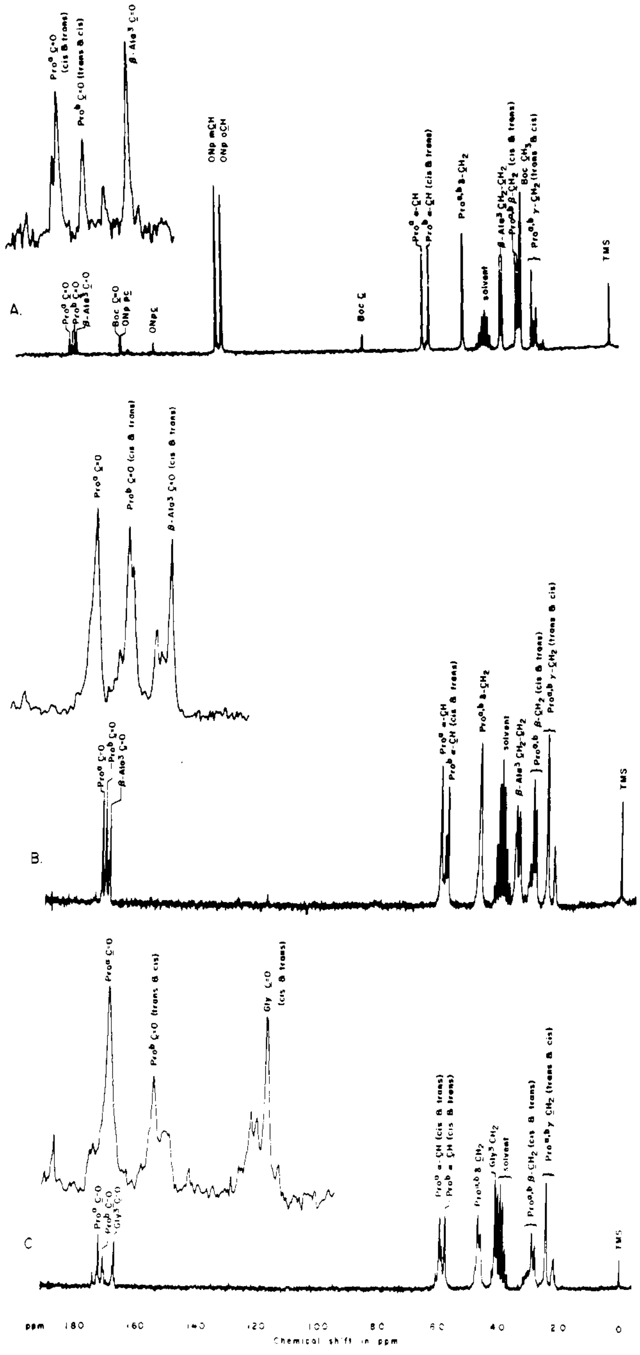

Figure 1. Carbon-13 magnetic resonance at 25 MHz in Me_2SO-d_6 of (A) Boc-Pro_1-Pro_2-β-Ala_3-ONp, (B) (Pro_1-Pro_2-β-Ala_3)$_n$, and (C) (Pro_1-Pro_2-Gly_3)$_n$. Note that the carbonyl signals are shown in the expanded scale at the top of each spectrum.

Table I

Attempt	Monomer	% yield of polymer	Av mol wt of polymer
1	F_3AcOH·H-Pro-Pro-β-Ala-ONp, 415 mg	159 mg, 75%	$n > 40$
2	F_3AcOH·H-Pro-Pro-β-Ala-ONp, 415 mg	149 mg, 70%	$n > 40$
3	F_3AcOH·H-Pro-Pro-β-Ala-ONp, 415 mg	144 mg, 68%	$n > 40$

elimination of the terminal amino acid might be greater. The facile formation of the diketopiperazine could be the reason for obtaining low molecular weight, dialyzable polymers by the pentachlorophenyl ester method.

In the carbonyl region of the ^{13}C spectrum of Boc-Pro_1-Pro_2-β-Ala_3-ONp (shown also in the expanded scale at the top of each spectrum), the two prolyl carbonyls are not assigned and only referred to as Pro^a C=O and Pro^b C=O, Pro^a C=O being the one that appears at the lowest field of the carbonyl region. Comparison of ^{13}C spectra of (Pro_1-Pro_2-Gly_3)$_n$ and (Pro_1-Pro_2-β-Ala_3)$_n$ shows that Gly_3 C=O and β-Ala_3 C=O appear farthest upfield in the carbonyl region (Figures 1B and 1C). By decoupling procedures it appears that Pro^a is Pro_2 and Pro_b is Pro_1.

It can be pointed out from Figures 1B and 1C that the cis and trans isomeric behaviors of both (Pro_1-Pro_2-β-Ala_3)$_n$ and (Pro_1-Pro_2-Gly_3)$_n$ are almost identical. Since (Pro-Pro-Gly)$_n$ has been shown by earlier studies to form triple helical structures[10-14] and (Pro-Pro-β-Ala)$_n$ was shown to have a similar ORD and CD spectra[2a] to (Pro-Pro-Gly)$_n$, it is possible that (Pro-Pro-β-Ala)$_n$ forms a similar helical structure. Further work is in progress in the assignment of ^{13}C signals of these compounds by isotopic labeling and in deriving the preferred conformations in solution of peptide analogues of collagen.

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Preferential Adsorption to Poly[N^5-(2-hydroxyethyl)-L-glutamine] in Water/2-Chloroethanol Mixtures

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The helix–coil transition in proteins and synthetic polypeptides is accompanied by changes in preferential adsorption to the polymer in water/organic solvent mixtures.[1] The interactions of proteins with solvent components of mixed water/2-chloroethanol have been extensively studied.[1,2] 2-Chloroethanol was shown to be a structure forming denaturant solvent and capable of interacting with proteins, probably by way of hydrophobic interactions with the aliphatic side chains. In the case of poly[N^5-(3-hydroxypropyl)-L-glutamine] (PHPG), it was shown that, at low 2-chloroethanol concen-

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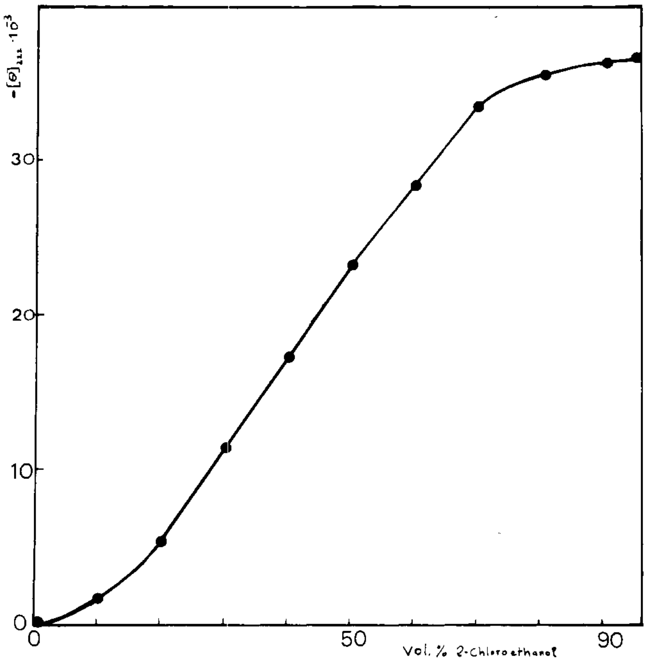

Figure 1. Conformational transition of PHEG in mixed water/2-chloroethanol. Residue ellipticity at 222 nm $[\theta]_{222}$ vs. 2-chloroethanol concentration (v/v %).

trations, a preferential solvation by the organic solvent takes place.[3] At higher concentrations, preferential hydration is observed. Preferential adsorption parallels the solvent-induced coil-to-helix conformational transition. In the present note, the interaction of poly[N^5-(2-hydroxyethyl)-L-glutamine] (PHEG) with 2-chloroethanol/water mixtures is reported. The hydrophilic character of PHEG, as compared to PHPG, was expected to lead to important changes in the preferential adsorption phenomenon.

PHEG was prepared by aminolysis[4] of a poly(γ-benzyl L-glutamate) sample with 2-amino-1-ethanol. Viscosity measurements[5] indicate a weight-average molecular weight of 110 000.

Circular dichroism spectra were recorded on a CNRS-Jobin-Yvon Dichrograph III. The residue ellipticity at 222 nm $[\theta]_{222}$ is expressed in deg cm^2 dmol^{-1}.

The preferential adsorption coefficient was determined by the density increment method:[6-8]

$$(\partial g_3/\partial g_2)_{\mu_1,\mu_3} = \frac{(\partial\rho/\partial c_2)_{\mu_3,\mu_1} - (\partial\rho/\partial c_2)_{m_3}}{1 - \bar{V}_3\rho_0}$$

$(\partial g_3/\partial g_2)_{\mu_1,\mu_3}$ represents the preferential binding of the organic solvent (refered to as component 3), given in grams of component 3 per gram of polypeptide (refered to as component 2); $\bar{V}_3$ is the partial specific volume of component 3 and ρ_0 is the density of the mixed solvents; $(\partial\rho/\partial c_2)_{m_3}$ and $(\partial\rho/\partial c_2)_{\mu_3,\mu_1}$ represent, respectively, the density increment at constant concentration of component 3 and at constant chemical potential of 2-chloroethanol and water (component 1) (which is achieved by dialysis of the polypeptide solutions against the pure mixed solvents).

The density increments were obtained by measuring the density of polypeptide solution at 25 °C on a DMA 02 D precision density meter (Anton Paar). Experimental details on the procedure can be found elsewhere.[3]

Conformational Transition of PHEG

The conformational state of PHEG is given by the value of residue ellipticity at 222 nm, $[\theta]_{222}$. In pure water $[\theta]_{222} = 0$. Therefore the conformation of PHEG is completely disordered. When adding 2-chloroethanol, the helical content of the polypeptide increases rapidly (Figure 1). The transition midpoint is obtained for 40% vol of 2-chloroethanol. The maximum value, $[\theta]_{222} = -36\,600$, is attained in a 95% 2-chloroethanol aqueous mixtures. This value is close to that measured for PHEG in tetrafluoroethanol[9] and in a 95% methanol aqueous mixture.[10]

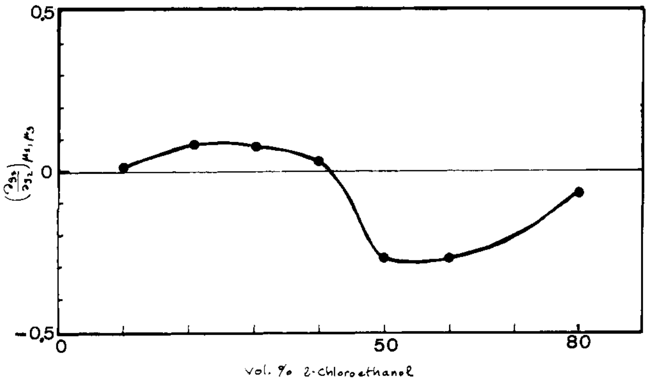

Figure 2. Preferential adsorption $(\partial g_3/\partial g_2)_{\mu_1,\mu_3}$ (g/g) to PHEG vs. 2-chloroethanol concentration (v/v %) in mixed water/2-chloroethanol at 25 °C. Relative error ±5%.

As a comparison, the transition midpoint is located at 20% of 2-chloroethanol for PHPG,[3] which confirms the more hydrophobic character of PHPG.

Preferential Adsorption to PHEG

Figure 2 shows the variation of preferential adsorption to PHEG when 2-chloroethanol concentration is increased. One can notice the following features: a weak preferential adsorption by the organic solvent is followed by preferential hydration; the cross-over point is located at a 2-chloroethanol concentration (~40% v/v) which corresponds to the conformational transition midpoint.

This shows that no strong affinity occurs between PHEG and 2-chloroethanol. The hydrophilic character of the polypeptide is pointed out; even at low water content, preferential hydration is observed. As in PHPG[3] the amide and alcohol side-chain group must be responsible for preferential hydration. The preferential adsorption by 2-chloroethanol to PHEG is weaker than to PHPG where it attained 1.14 g/g.[3] An explanation can be found in the more hydrophobic character of PHPG, which can enhance interactions with the organic solvent. This is consistent with the assumption[1,2] that 2-chloroethanol interacts with hydrophobic sites in globular proteins. When 2-chloroethanol is added to an aqueous mixture, the activity of water is lowered. Thus, the interactions of the active solvent (water) with the polypeptide are weakened. This is marked by a deficiency of water in the solvation layer and preferential adsorption of organic solvent is observed. Some portions on the polypeptide are less solvated by water and can undergo a coil to α-helix transition. Beyond the transition midpoint, where PHEG is essentially α helical, water is excluded from the sphere of influence of peptide linkages; nevertheless, preferential hydration is observed. Hydrophilic sites, such as amide or alcohol group side chain, must be responsible for strong interactions with the aqueous component, even at high 2-chloroethanol content.

In conclusion, the preferential adsorption of solvent components to PHEG in mixed water/2-chloroethanol, which parallels the coil-to-helix transition, gives further evidence for the hydrophilic character of PHEG, when compared to PHPG; less preferential adsorption of the nonpolar solvent component is observed. In both cases, polar side-chain groups induce a preferential hydration of the polypeptide in helical conformation.

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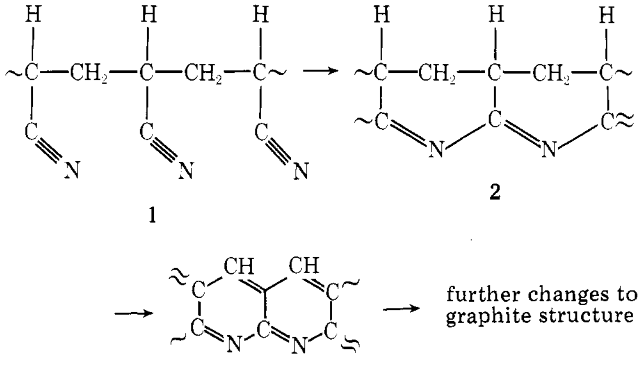
Communications to the Editor

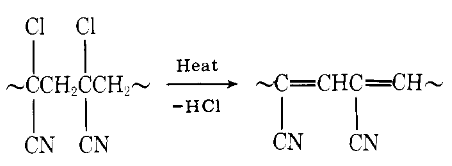
ESCA Studies of Structural Changes of Poly(acrylonitrile) and Poly(chloroacrylonitrile). High-Temperature-, Electron-Impact-, and Ultraviolet-Light-Induced Changes

Poly(acrylonitrile) is well known to undergo structural changes in the presence of oxygen at high temperatures as shown in the following,[1]



Coloration, an increased electrical conductivity, and gas evolutions have been taken as evidence for the above structural changes, together with reduced intensity of the cyano group infrared band after heat treatment.[2]

Thermal degradation of poly(α-chloroacrylonitrile) has been studied up to 200 °C.[3] The result showed a facile elimination of hydrogen chloride, far easier than in poly(vinyl chloride), as shown below:



$$\sim\underset{\mathrm{CN}}{\overset{\mathrm{Cl}}{\mathrm{C}}}\mathrm{CH_2}\underset{\mathrm{CN}}{\overset{\mathrm{Cl}}{\mathrm{C}}}\mathrm{CH_2}\sim \xrightarrow[-\mathrm{HCl}]{\mathrm{Heat}} \sim\underset{\mathrm{CN}}{\mathrm{C}}{=}\mathrm{CH}\underset{\mathrm{CN}}{\mathrm{C}}{=}\mathrm{CH}\sim$$

4 5

With no other observable gaseous products except hydrogen chloride, the black coloration, the disappearance of the "quenching effect" of chlorine on the infrared absorption intensity of the cyano group, and the appearance of the ethylenic infrared bands are taken as the evidences for structure **5**.[3] A pyrolysis of poly(cyanoacetylene), prepared in a catalytic polymerization of cyanoacetylene, was reported to yield an infrared spectrum similar to the poly(acrylonitrile) baked at high temperature.[4] The changes of the infrared bands alone, however, cannot be taken as evidence for the ladder-type structure, because hydrogen cyanide and other cyano compounds were reported as products in the pyrolysis of poly(acrylonitrile) at high temperature.[5]

We wish to report here our ESCA studies of structural changes of poly(acrylonitrile) and poly(α-chloroacrylonitrile) induced by high temperature, electron impact, and UV-light exposure.

Poly(acrylonitrile) used was obtained from Polysciences Inc., and its thin films were cast from a dimethylformamide solution on substrates such as aluminum, silicon, or gold by spin-coating. Poly(α-chloroacrylonitrile) was prepared from its monomer obtained from Polysciences in a similar way as described in the literature.[2] Its thin films were cast from a dimethylformamide solution in the same way as for poly(acrylonitrile). Surface photopolymerization from gaseous 2-chloroacrylonitrile on proper substrates was also carried out with low-pressure mercury lamps; our technique has already been reported with methacrylic anhydride.[6]

Heating of the polymer films was carried out in a sealed tube under vacuum. Electron beam exposures were performed with an Auger electron gun made by Varian in a dosage of 10^{-4} to 10^{-3} C/cm^2 at 3 keV. The UV light exposure was made under vacuum with low-pressure mercury lamps for 6 h. ESCA measurements were conducted at room temperature under a vacuum better than 2×10^{-9} Torr with a Hewlett-Packard Model 5650A ESCA spectrometer, which had resolution better than 1 eV. The samples were flooded with thermal electrons during the data collections to neutralize the charge effect. The reported binding energies were referred to $\mathrm{Au_{4f_{7/2}}}$ transition at 83.9 eV.

Poly(acrylonitrile). The $\mathrm{C_{1s}}$ and $\mathrm{N_{1s}}$ core level spectra of poly(acrylonitrile) before and after thermal treatment at 500 °C are shown in Figure 1. The maximum peak of the $\mathrm{C_{1s}}$ core level signal shifts by the thermal treatment from 285.2 to 284 eV in the binding energy with a pronounced shoulder at 286 eV. The shift of the carbon signal by heat is caused by the structural changes from **1** to **2** and **3**. It is known that the conjugated carbon has a lower binding energy than those in a saturated structure; the carbon core level of a $-\mathrm{C{\equiv}N}$ is also known to have a higher binding energy than those of a $>\mathrm{C{=}N}-$ group.[7] A partial conversion of the $-\mathrm{C{\equiv}N}$ group to the $>\mathrm{C{=}N}-$ group of **3** is also shown in the change of the $\mathrm{N_{1s}}$ core level signal. After the heat treatment, a symmetrical Gaussian-type $\mathrm{N_{1s}}$ core level signal with the maximum peak at 399.0 eV becomes broad and asymmetrical with a newly formed shoulder peak at 400.5 eV in the binding energy; the shoulder peak belongs to the nitrogen of the $>\mathrm{C{=}N}-$ group of **3**. A positive shift of 1.5 eV of the $\mathrm{N_{1s}}$ from **1** to **3** is consistent with a loss of a negative charge in the nitrogen of the $>\mathrm{C{=}N}-$ group from the $-\mathrm{C{\equiv}N}$ group.[7] A partial conversion from **1** to **2** and **3** is in agreement with the infrared absorption spectra which reveals that the $-\mathrm{C{\equiv}N}$ group is still observable at 2220 cm^{-1} after heating at 500 °C for 15 min, although the cyano group completely converts to the $>\mathrm{C{=}N}-$ group in a prolonged heating at this temperature.

Poly(α-chloroacrylonitrile). The $\mathrm{C_{1s}}$, $\mathrm{N_{1s}}$, and $\mathrm{Cl_{2p}}$ core level spectra of poly(α-chloroacrylonitrile) films which were cast from a dimethylformamide solution of the polymer prepared in an emulsion polymerization of 2-chloroacrylonitrile

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