The change in the hyperfine coupling constants of the β protons in the series CH_3NHO (I), $CH_2(OH)NHO$ (II), and $CH_3CH(OH)NHO$ (III) is characteristic of hindered internal rotation. The value of a_α^H, the hyperfine coupling constant of the α protons, in these radicals should be a good measure of ρ_N^π, since a McConnell relation like (1) without a θ dependence also apparently holds for a_α^H.[6] In methanol solution the hyperfine coupling constants of all protons and the nitrogen atom in CH_3NHO are 13.8 gauss. The change in a_β^H in the series I → II → III → is not linearly proportional to the change in a_α^H. Consequently, the simplest explanation is that $\langle\cos^2\theta\rangle$ changes in the series I → II → III. Similar effects in alkyl nitro anion radicals have been ascribed to hindered internal rotation.[12]

The low symmetry of II and III prevents their treatment after the fashion of Stone and Maki, who were able to use $1 - \cos 2\theta$ internal rotation potential barriers for the alkyl nitro anion radicals.[12] Radicals II and III should probably be assigned a threefold potential barrier with unequal potential barrier maxima.

Acknowledgments. The author acknowledges with pleasure the many helpful discussions with Dr. J. R. Thomas and the continued encouragement of Dr. R. L LeTourneau and Dr. L. P. Lindeman.

(12) E. W. Stone and A. H. Maki, *J. Chem. Phys.*, 37, 1326 (1962).

Slow Hydrogen–Deuterium Exchange in a Non-α-helical Polyamide

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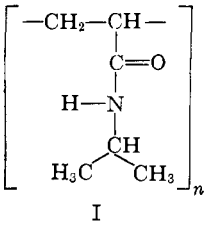
Abstract: The kinetics of hydrogen–deuterium exchange was measured in a polymeric amide, poly-N-isopropylacrylamide, in D_2O solutions. The intrinsic viscosity of the polymer corresponds to that of a swollen, unordered macromolecule. Infrared spectra indicate about one-third of the pendant N–H groups are not hydrogen bonded to carbonyls. Despite the presence of two states of the N–H group only one first-order exchange process is seen. Furthermore the rate constant k is the same whether computed from disappearance of free N–H or bonded N–H $\cdots$O=C, or from appearance of O–H. Exchange behavior of the polymer is similar to that of comparable small amides in regard to catalysis by D^+ and OD^-, activation energy (20 kcal/mole), and pD_{min} of minimum exchange rate. However, the minimum rate constant, k_{min}, is $^1/_{100}$th that of small amides. This markedly reduced exchange rate of an amide group attached to this macromolecule can be interpreted in terms of a change in K_w, the self-dissociation constant of water, in the neighborhood of the pendant residues of the polymer.

One of the techniques that has been widely used to examine protein conformations in solution is hydrogen–deuterium exchange. In native proteins, at least some peptide hydrogens exchange slowly with the aqueous solvent,[1,2] and the rates of exchange are markedly affected by the solvent environment.[3,4] Nevertheless, it is still not clear how much of the decrease in exchange rates is due to locking in of the exchangeable hydrogens in N—H$\cdots$O=C bonds and how much to interactions of side chains with each other or with solvent.[4]

One method of trying to assess the relative importance of different factors in slowing down hydrogen–deuterium exchange is to examine this reaction in a polymeric amide unable to form α-helical conformations but containing fairly large pendant apolar groups. Detailed studies have now been carried out with one such polymer, polyisopropylacrylamide (I), in which the C=O and N–H groups are *not* part of the backbone of the macromolecule. The behavior of this polymer shows some remarkable similarities to that of α-helical polypeptides.



I

Experimental Section

Materials and Methods. N-Isopropylacrylamide was obtained from American Cyanamid Co. and was recrystallized repeatedly from a toluene–hexane mixed solvent. Dioxane was purified by the method of Fieser[5] and was stored over sodium. It was always freshly distilled before use. Chloroform was first washed with water to remove the ethanol impurity and was refluxed over P_2O_5 before distillation. It was kept in a black bottle and used without delay in order to avoid formation of phosgene. Heavy water was purchased from Bio-Rad Laboratories and was warranted to be 99.84% D_2O.

N-Isopropylpropionamide was prepared from N-isopropylacrylamide by dissolving the latter in 95% ethanol and hydrogenating over 10% platinum oxide. When hydrogenation was complete the catalyst was filtered off, the ethanol was removed by rotary evaporation, and the residue was dissolved in ethyl acetate.

(1) H. Lenormant and E. R. Blout, *Nature*, 172, 770 (1953).
(2) A. Hvidt and K. Linderstrom-Lang, *Biochim. Biophys. Acta*, 14, 574 (1954).
(3) A. Hvidt and S. O. Nielsen, *Advan. Protein Chem.*, 21, 287 (1966).
(4) B. H. Leichtling and I. M. Klotz, *Biochemistry*, 5, 4026 (1966).
(5) L. Fieser, "Experiments in Organic Chemistry," D. C. Heath and Co., Boston, Mass., 1957, p 284.

This solution was washed twice with ice-cold aqueous 5% sodium carbonate and then dried with $MgSO_4$. The ethyl acetate was then removed. The crystalline residue was dissolved in dry benzene and then the benzene removed by rotary evaporation. The last procedure was repeated to ensure removal of traces of water. The residue was sublimed twice at 64–66° (1 mm). The compound is extremely hygroscopic and must be protected accordingly. The nmr spectrum of the compound, dissolved in CCl_4, showed an N–H resonance at 445 cps with indications of two peaks at 442 and 448 cps; a C–H multiplet at 240 cps with coupling constant $J = 7$ cps; a CH_2 quartet at 131 cps with $J = 7.5$ cps; a doublet at 73.5 cps with $J = 7.5$ cps, due to the CH_3 groups of the isopropyl substituent; and a triplet at 67 cps, with $J = 7$ cps due to the remaining CH_3 group of the propionic chain.

Infrared spectra in the fundamental region were scanned with a Beckman Model IR-10 spectrophotometer. In the overtone range, a Cary Model 14R spectrophotometer was used. The temperature of solutions in the optical cells was measured with a thermistor probe attached to a telethermometer of the Yellow Springs Instrument Co. The Varian A60 instrument was used for all nmr work. Tetramethylsilane served as internal reference compound.

Viscosities were measured at 15.2° with an Ostwald viscometer having a capillary path of 23.5 cm. With water as solvent the flow time was 108.2 sec.

A Radiometer pH meter, Model TTT1b, with an external combination electrode from Sargent and Co. was used for pH measurements. The pH readings were changed to pD by means of the conversion equation of Glasoe and Long.[6]

$$\text{pD} = \text{pH (meter reading)} + 0.40$$

Preparation of the Polymer and Its Solutions. Polymerization was carried out in bulk as well as in solution. In bulk, thermal or catalytic procedures were used. For the thermal polymerization, 2.25 g of monomer was placed in a 25-ml, pear-shaped flask fitted with a side arm for the introduction of nitrogen. The flask was heated under nitrogen to 153° in an oil bath. The contents became progressively more viscous and after 15 min N_2 could no longer be bubbled through. Heating was continued for an additional 15 min. The contents was cooled, the solid dissolved, insofar as possible, in chloroform, and the solution poured into a Waring blender containing hexane. A white powdery precipitate was recovered that weighed 1.15 g.

An alternative preparation, with catalyst, started with 2.91 g of N-isopropylacrylamide in a 25-ml, three-necked flask. The molten monomer was deaerated with a slow stream of nitrogen. The liquid was maintained at 70° and 0.05% (by weight) of benzoyl peroxide was added. This initiator set off a vigorous reaction, the temperature of the flask rose to 148°, and the contents solidified within 1 min. The product was cooled, dissolved in chloroform, and poured into a Waring blender containing hexane. The dried polymer weighed 2.61 g.

Solution polymerization was carried out in a 1-l. flask equipped with a stirrer, thermometer, and an inlet for nitrogen. Thirty grams of monomer was dissolved in 450 g of deaerated water at 10°. Thereafter the following were added,[7] each solution having been freshly prepared in deaerated water: 1.2 ml of 1% $NaBrO_3$, 1.45 ml of 1% $Na_2S_2O_3$, 1.5 ml of 1% H_2SO_4. The solution soon became viscous. After 40 min 2.5 ml of 1% $(NH_4)_2S_2O_8$ was added,[7] and stirring was continued for an additional 3 hr. The temperature was then raised to 50°, since the polymer precipitates in warm water. The polymer was redissolved and reprecipitated twice in water. It was then redissolved and lyophilized. To remove more tightly bound water, the solid was dissolved in dioxane and lyophilized again. The solid was then dissolved in chloroform and forced through a pressure filter in a fine stream into a Waring blender containing hexane. The yield of dry white fibrous solid was 26.5 g.

This preparation from solution polymerization was used in quantitative rate measurements, because any residual water-soluble monomer and catalysts should have been separated from the polymer when it was precipitated. It was converted to a fluffy and porous material which facilitated rapid dissolution in D_2O for kinetic measurements, by dissolving again in dioxane and lyophilizing. For rate measurements at 25°, about 0.6 g of fluffy solid was mixed with 35 ml of D_2O at room temperature in a polypropylene flask containing small pieces of Teflon. The pH was then adjusted, and the flask was agitated vigorously on a mechanical shaker. The polymer dissolved in 5–6 min and gave a solution of about 0.15 residue *M*. Bubbles were then removed by centrifugation for 1 min at 2000 rpm in an International Refrigerated Centrifuge, Model PR-I. The clear solution was placed in an optical cell (10-cm path), and absorbance readings were initiated. In most of the rate measurements the appearance of the H–OD at 1.44 μ was recorded with time. The pH of the water used as solvent was set between 4.6 and 5.0 by addition of 0.26 *M* DCl, since this is the region of minimum rate of hydrogen–deuterium exchange. If after the polymer had dissolved a more basic pH was desired, then 0.2 *M* sodium cacodylate was added. More acidic pH's were obtained with additional DCl. After the exchange reaction had reached completion the pH was checked precisely by insertion of a Sargent combination electrode directly into the optical cell. A small magnetic stirring bar was also inserted into the vessel to keep liquid moving around the electrode and thus to avoid the clinging of a film of polymer around the glass. In measuring rates, the time of introduction of added cacodylate or of added DCl, which provided basic or acidic catalysts, was taken as t_0. For solutions at pH 4.6–4.65 the beginning of shaking was regarded as the initial time.

For the experiments at 15° precautions were taken to keep the solutions and containers at or below this temperature until the rate measurements were to be made. Spectrophotometer cells (5 cm) were filled with D_2O and placed in the cell compartment maintained at 15° to cool down to the ambient temperature. The polymer was then dissolved in the cooled D_2O with the aid of a modified Cole-Palmer Supermixer placed in a cold room at 5°. The 50-ml polyethylene flask containing dissolved polymer was centrifuged under refrigeration to remove air bubbles, and then the solution was transferred through a glass-wool plug into the spectrophotometer cell. The temperature of the solution at this stage was generally 15 ± 0.5°.

At the conclusion of a kinetic experiment the N-deuterated polymer was converted back to the N–H form usually by preliminary dialysis against acidified H_2O (pH 3) for 3–4 hr. The acid was then removed by dialysis against pure water and the polymer returned to its initial fluffy state by lyophilization from water, dissolution in dry dioxane, and lyophilization again.

Results

Characterization of Polymer. Poly-N-isopropylacrylamide is evidently highly stable in water; *i.e.*, the amide groups are not hydrolyzed. Infrared spectra after 3 weeks of dialysis against water showed no evidence of loss of amide groups.

The polymer is soluble in water, dioxane, dimethyl sulfoxide, dimethylformamide, chloroform, and trifluoroacetic acid. In water it shows an inverse temperature coefficient of solubility and precipitates sharply when the temperature of a 2% aqueous solution reaches 31°. At room temperature 15% solutions can be prepared in water, very high viscosity being encountered above 7%. The polymer is insoluble in hexane, cyclohexane, benzene, toluene, or carbon tetrachloride.

Intrinsic viscosities $[\eta]$ in aqueous solution were measured at 15.2°. These ranged as follows (in dl/g): bulk thermal product, 0.47; bulk catalyst, 0.78; solution product, 3.0. Thus the material produced by solution polymerization has the higher molecular weight. Its intrinsic viscosity is consistent with a random conformation of the macromolecule since its molecular weight is about 200,000 (see below). The viscosity did not change appreciably when the polymer was dialyzed in water to remove any residual low molecular weight material.

Sedimentation measurements,[8] over a concentration range of 0.025–1.0% polymer (from the solution polymerization), were carried out with a Spinco Model E

(6) P. K. Glasoe and F. A. Long, *J. Phys. Chem.*, **64**, 188 (1960).

(7) Catalysts were added to the flask through a rubber plug by means of a hypodermic syringe.

(8) These were carried out by Mr. Neal Langerman, to whom we wish to express our appreciation.

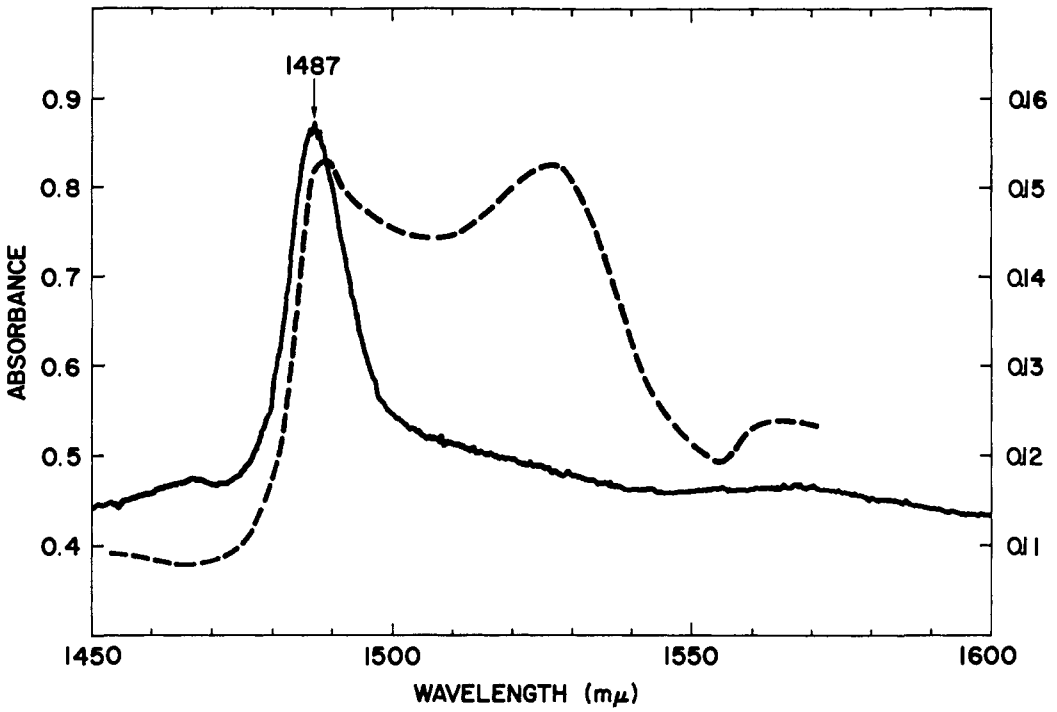


Figure 1. Near infrared spectra of poly-N-isopropylacrylamide: in chloroform (left ordinate scale), in D_2O (right ordinate scale).

analytical ultracentrifuge using both Schlieren and Rayleigh optics. Extrapolation to infinite dilution gave 3.57 S for $s^0_{20,w}$.

A diffusion coefficient, $D_{20,w}$, was measured at 0.67% polymer and found to be 3.02×10^{-7} cm²/sec. These hydrodynamic parameters combined with a measured partial specific volume of 0.85 ml/g lead to a molecular weight of 192,000. Low-speed equilibrium sedimentation of a 0.47% solution in 0.1 *M* NaCl gave a weight-average molecular weight of 230,000. It seems evident, therefore, that the material from solution polymerization has a molecular weight near 200,000.

Infrared Spectrum and Hydrogen Bonding of Polymer. In the overtone region near 1.5 μ (6700 cm^{-1}) the polymer in chloroform shows essentially one N–H peak, at 1.487 μ (6725 cm^{-1}) (Figure 1). The position of this peak is shifted only slightly if the solvent is changed from chloroform (to dioxane) to water. In dioxane and water, however, additional bands appear at 1.53 (6536 cm^{-1}) and 1.57 μ (6370 cm^{-1}). It has been shown[9-11] previously in extensive studies with simple amides in the same solvents that the 1.487-μ absorbance is due to N–H groups *not* hydrogen bonded to C=O, whereas the 1.53–1.57-μ peaks are seen under conditions where N—H···O=C bonds are present. In addition, spectra of N-isopropylpropionamide, the monomeric equivalent of the residues in the polymeric acrylamide, show a single peak at 1.487 μ in dilute solutions (0.018–0.4 *M*) in chloroform and the N—H···O=C double peak at higher concentrations.

The extent of hydrogen bonding in the polymer cannot be stated with confidence since it has not been possible to measure the extinction coefficients of the bonded and nonbonded N–H bands for this polymer.[12] On the other hand, if it is assumed that N–H on the polymer has an extinction coefficient near that of small amides,[9] then the polymer absorbances at 1.487 and 1.53 μ correspond to about 35% nonbonded N–H and 65% N—H···O=C.

In the fundamental region a chloroform solution of the polymer showed bands at 2.95 (3390 cm^{-1}) and 3.05 μ (3279 cm^{-1}), an "amide I" doublet at 6.04–6.09 μ (1656–1642 cm^{-1}), an "amide II" band at 6.48 μ (1543 cm^{-1}), and absorbance peaks at 8.53 (1172 cm^{-1}) and 8.82 μ (1134 cm^{-1}) that can be assigned to the isopropyl group.[13] N-Isopropylpropionamide, dissolved in chloroform at a concentration of 0.45 *M*, absorbs at 2.92 (3425 cm^{-1}) and 3.08 μ (3246 cm^{-1}). At this concentration, the overtone spectrum indicates some free N–H and some N—H···O=C; hence it seems reasonable to assign the 2.92-μ band to the former and the 3.08-μ to the latter. On this basis the polymer band in chloroform solution at 2.95 μ should probably be assigned to free N–H and that at 3.05 μ to N—H···O=C.

The fact that the observed absorbancies near 3 μ in the fundamental are nearly equal, whereas in the overtone the free N–H peak in chloroform solution, at 1.487 μ, is overwhelmingly greater than that of N—H···O=C, is consistent with the known tendency of peaks due to hydrogen-bonded species to be of higher relative intensity in the fundamental and of lower relative intensity in the overtone region, as compared to the nonhydrogen-bonded peak.[14]

If a segment of the polymer chain is constructed from space-filling atomic models,[15] two features become

(9) I. M. Klotz and J. S. Franzen, *J. Am. Chem. Soc.*, **84**, 3461 (1962).
(10) S. Hanlon and I. M. Klotz, *Biochemistry*, **4**, 37 (1965).
(11) S. Hanlon, *ibid.*, **5**, 2049 (1966).
(12) With simple amides involved in intermolecular hydrogen bonding this can be done by extrapolating apparent extinction coefficients to zero concentration of solute and to pure solute, respectively. However, with this polymer, dilution does not change the relative intensities of the two bands in either the fundamental or overtone regions. Thus, the N—H···O=C bonds in polyisopropylacrylamide evidently are intramolecular.
(13) J. C. Bellamy, "The Infrared Spectra of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1954, p 25.
(14) G. C. Pimentel and A. L. McClellan, "The Hydrogen Bond," W. H. Freeman and Co., San Francisco, Calif., 1960, p 70.

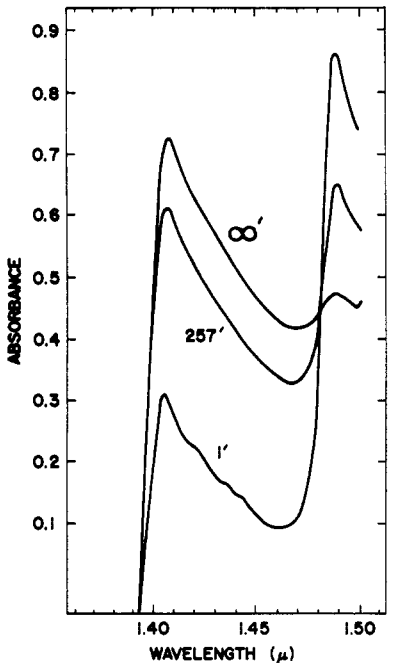
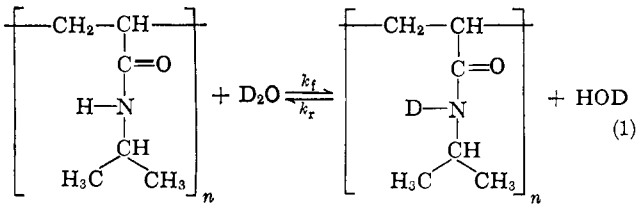


Figure 2. Variation of absorbance (×10) with time in region 1.4–1.5 μ for solutions of polyisopropylacrylamide in D_2O.

apparent. Intermacromolecular N—H···O=C bonds between different chains are essentially impossible because of steric interference from the bulky isopropyl groups. Intramacromolecular bonds are also subject to steric hindrance but some evidently could be formed.

We conclude, therefore, from infrared observations and model building, that polyisopropylacrylamide[16] has some nonbonded N–H and some N—H···O=C groups in all solvents examined. In any event, since the nonbonded N–H shows a discrete absorption band in the overtone infrared, we can follow its rate of exchange separately from the over-all kinetics.

Calculation of Rate Constants. The exchange reaction may be represented as



(1)

In practice the initial D_2O is present in such overwhelming concentration that the reaction is essentially pseudo first order in the forward direction. Thus we may write

$$-\mathrm{d(NH)}/\mathrm{d}t = \mathrm{d(OH)}/\mathrm{d}t = k(\mathrm{NH}) \qquad (2)$$

Absorbances were measured as a function of time in the wavelength region of 1.4–1.5 μ (7100–6700 cm^{-1}) (Figure 2). Since there is some light scattering due to the macromolecular character of the polymer, all absorbances were scaled with reference to that observed between 1.20 and 1.26 μ (8330–8000 cm^{-1}), a relatively flat region devoid of overtone bands.

(15) W. L. Koltun, *Biopolymers*, 3, 665 (1965).

(16) It should be noted also that nmr studies of related polyacrylamides [F. A. Bovey and G. V. D. Tiers, *J. Polymer Sci.*, **A1**, 849 (1963)] indicate that extensive intramolecular hydrogen bonding does not occur.

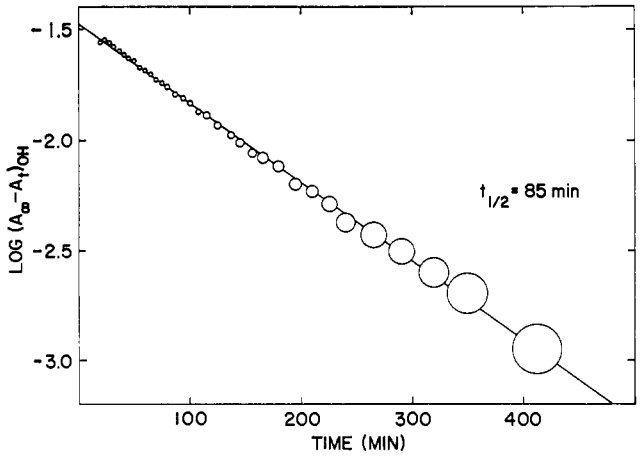


Figure 3. First-order exchange kinetics for polyisopropylacrylamide in D_2O.

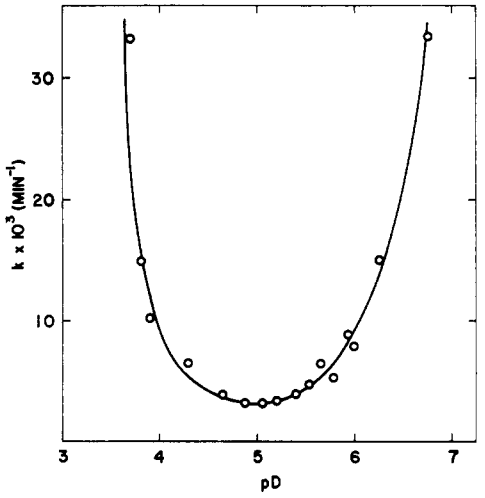


Figure 4. Rate–pH profile for hydrogen–deuterium exchange of polyisopropylacrylamide in D_2O at 25°.

The observed rates were the same no matter whether the appearance of O–H (1.40–1.45 μ (7200–7000 cm^{-1})), disappearance of N–H (1.49 μ (6725 cm^{-1})), or disappearance of N—H···O=C (1.53 μ (6536 cm^{-1})) was followed (Table I). For example, in one set of

Table I. Comparison of Rates Following Infrared Bands for HOD Production, Free N–H Diminution, and N—H···O=C Diminution

		$k \times 10^3$, min^{-1}		
pD	Temp, °C	1.407 μ (7107 cm^{-1})	1.489 μ (6705 cm^{-1})	1.53 μ (6536 cm^{-1})
3.78	15	10.2	8.9	9.2
4.54	15	1.96	1.77	2.06
6.06	15	3.80	3.85	3.62
3.78	25	35.2	30.6	30.8

experiments at 15° the respective rate constants k were 10.2×10^{-3}, 8.9×10^{-3}, and 9.2×10^{-3} min^{-1}. In all cases the rate constants were computed from a graph of log $(A_\infty - A_t)$ *vs.* time (Figure 3), where A is the absorbance. The infinity reading, A_∞, was taken after eight half-lives. Since the exchange reaction was generally very slow, A_∞ was sometimes checked by

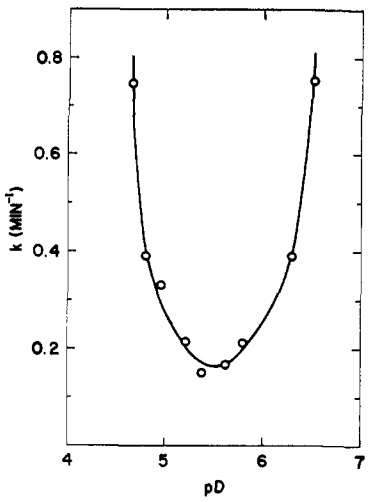
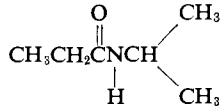


Figure 5. Rate–pH profile for hydrogen–deuterium exchange of N-isopropylpropionamide, monomeric analog of residue of polymer, in D_2O at 25°.

addition of DCl to pH 1.5 or of NaOD to pH 9.5, at which pH's the rates are very fast.

The observed rate constants as a function of pD, at 25° and at a polymer concentration of 0.15 residue M, are summarized in Figure 4. A similar curve was obtained at 15°. The rate–pD profile is analogous to that observed in other amide molecules, small or large.[4,17] A few experiments were also carried out at a higher concentration of polymer, 0.3 residue M, but the same rate constants were observed. Still higher concentrations could not be tried because the solutions became very viscous and unwieldy.

For comparison k was also measured over a range of pD for the simple compound



which corresponds in structure to a single residue unit of the polymer (see structure I). Again a parabolic curve is obtained (Figure 5). The pD_{min} of minimum exchange is shifted only slightly with respect to polymer but k_{min} is strikingly larger.

From experiments at 5, 15, and 25°, an activation energy, E_D^*, in the acid range of the rate–pH curve was computed and found to be near 20 kcal/mole.

Discussion

There are four features of the exchange reaction in polyisopropylacrylamide that need to be compared with corresponding observations in other amides and peptides: (1) k–pD profile; (2) E_D^*; (3) pD_{min}, the pD of minimum exchange rate; (4) k_{min}.

The parabolic pD profile demonstrates that exchange in the polymer, as in other amides, is catalyzed by both D^+ and OD^-. Thus one may write, as before[4]

$$k = k_0 + k_D(D^+) + k_{OD}(OD^-) \quad (3)$$

where k_0 is the rate constant for the spontaneous reaction, k_D that for the acid-catalyzed reaction, and k_{OD} that for the base-catalyzed exchange. It has also been shown previously[4] that

$$(D^+_{min}) = \left(\frac{k_{OD}}{k_D} K_w\right)^{1/2} \quad (4)$$

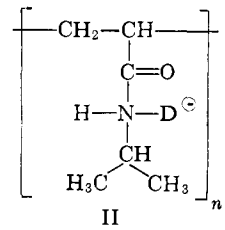
and

$$k_{min} = k_0 + 2k_D(D^+_{min}) \quad (5)$$

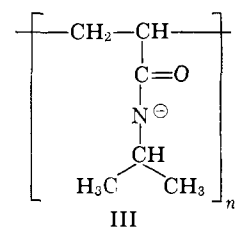
where K_w is the self-dissociation constant of water. Equation 5 may also be converted to

$$k_{min} = k_0 + 2(k_D k_{OD} K_w)^{1/2} \quad (6)$$

Since the acid and base catalysis in the polymer is similar to that in other amides and peptides,[3,4,17] it seems reasonable to assume that the mechanism of the exchange reaction is the same. This conclusion is supported by the observation that E_D^* for the polymer is 20 kcal/mole, very close to that found in other amides and peptides.[4] Thus we may presume that the rate-controlling step in acid solution is[3,4] that leading to the amide cation II and in basic solution that leading to the anion III.



II



III

On this basis one can understand why pD_{min} for the polymer, 5.0, is only slightly lower than that, 5.38, for the monomeric isopropylpropionamide.

It has been shown previously[4] that pD_{min} is determined largely by inductive effects of groups on both sides of the –CONH–. In D_2O solvent, $CH_3CONHCH_3$ has a pD_{min} of 5.45. An electron-withdrawing substituent such as $ClCH_2$– in place of C-methyl, or $-CH_2COC_2H_5$ in place of N-methyl, lowers pD_{min} by 1.2–1.4 pH units. The small difference, 0.4 unit, between polymer and monomer can thus be rationalized in terms of a small inductive effect of neighboring amide residues in the polymer.

The striking distinction between the polymeric amide and corresponding small molecules is the very much lower value of k_{min} for the former.

This cannot be due to the locking in of all N–H groups in N—H···O=C hydrogen bonds, for the infrared spectra show that about one-third of the amide N–H's are not bonded to carbonyl groups. Despite the presence of comparable concentrations of N–H and N—H···O=C groups, only a single first-order rate is observed (Figure 3). Clearly the free N–H's and bonded N–H's are not kinetically segregated into classes of exchangeable amides. Evidently these two states of amide group are in a mobile equilibrium which must be fast compared to the rate of exchange. This is also obvious from the comparable values of k obtained from the decrease in intensity of the 1.49-(free N–H) or 1.53-μ (N—H···O=C) peaks (Table I).

Under these circumstances one might be inclined to describe the exchange kinetics in terms of a "motility" mechanism[3]

$$\text{N—H}\cdots\text{O=C} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{N—H} + \text{O=C} \xrightarrow[\text{in } D_2O]{k_3} \text{N—D} + \text{O=C} \quad (7)$$

(17) I. M. Klotz and B. H. Frank, *J. Am. Chem. Soc.*, 87, 2721 (1965).

in which the bonded form represents a completely nonexchanging state and the free N–H represents a state in which amide groups are fully exposed to the bulk solvent. Since the infrared spectra show that bonded amide and nonbonded amide exchange H for D at the same rate, and hence that these two states of the amide are in rapid equilibrium compared to step k_3, we may write the following relationships.

$$\mathrm{d(N\text{–}D)}/\mathrm{d}t = k_3(\mathrm{N\text{–}H}) \tag{8}$$

$$\frac{(\mathrm{N\text{–}H})}{(\mathrm{N{-}H\cdots O{=}C})} = \frac{k_1}{k_2} = K \tag{9}$$

$$(\mathrm{N\text{–}H}) = (\mathrm{NH_{total}})\frac{K}{K+1} \tag{10}$$

$$\frac{-\mathrm{d(NH_{total})}}{\mathrm{d}t} = \frac{\mathrm{d(N\text{–}D)}}{\mathrm{d}t} = k_3(\mathrm{NH_{total}})\frac{K}{K+1} \tag{11}$$

Therefore

$$k = [K/(K+1)]k_3 \tag{12}$$

In the present circumstances we can estimate K, the equilibrium constant for bonded $\rightleftarrows$ nonbonded amide, from the absorbances of each group in the infrared. Using[9] 200 for $\epsilon_{1.489\mu}$ and 100 for $\epsilon_{1.53\mu}$ we find $K \simeq 0.5$. If k_3 in the polymer were the same as in simple amides, then the observed k should be reduced by only a factor of about 3. In actual observation k_{min} for the polymer is 100 times less than that of the corresponding monomer residue N-isopropylpropionamide. It is thus evident that the reduced rate in the polymer cannot be attributed to a shift in state of the amide to the nonexchanging, bonded N—H···O=C form.

We are forced to conclude, therefore, that the exchange of the *nonbonded* N–H groups themselves is being slowed down by their environment.

In most systems examined previously k_0 (eq 3) is small or negligible, and hence changes in the second term on the right-hand side of eq 6 dominate k_{min}. The marked drop in exchange rate in the polymer implies that k_D, k_{OD}, or K_w, or any combination of these, is smaller in the macromolecule than in the monomer. Something in the vicinity of the polymer amide residue, therefore, must have a pronounced effect on one of the rate constants and/or on the dissociation constant of water.

The polymer itself is almost certainly atactic in conformation. It was prepared in aqueous solution, a good solvent, at 10°, with free radicals as initiators of the polymerization. Syndiotactic N-isopropylacrylamide has been found insoluble in all common solvents,[18] whereas our preparation is soluble in many solvents. Space-filling models of isopropylacrylamide indicate that the bulkiness of the isopropyl group interferes with regular conformations. The irregular conformation suggested by the models is in accord with the high intrinsic viscosity observed for the polymer in water. Furthermore the high intrinsic viscosity indicates a very loose swollen conformation for the macromolecule.

It is unlikely that increases in bulk viscosity of the solution in the presence of isopropylacrylamide are responsible for the decrease in exchange rate. First the rate does not change appreciably when the concentration of polymer is doubled. Furthermore, it has been observed in separate experiments[19] that the addition of (15%) polyvinylpyrrolidone to water to increase solvent viscosity about tenfold produces only a minor drop, 20%, in the rate of hydrogen–deuterium exchange in N-methylacetamide. It is thus apparent that the local molecular environment around the *small* amide CONH is not strongly affected despite the enormous increase in viscosity as measured by a macroscopic technique.

On the other hand, if a $-\mathrm{CONHCH(CH_3)_2}$ group is attached to a polymeric backbone its local molecular environment is obviously not the same as when it is attached to a small substituent, such as $\mathrm{CH_3CH_2}-$ in N-isopropylpropionamide. The N–H groups must be, therefore, in an environment which can decrease k_D, k_{OD}, or K_w, or all three parameters that can affect k_{min}.

It has been found previously[20,21] that the equilibrium acid–base behavior of $-\mathrm{N(CH_3)_2}$ groups conjugated covalently to polyvinylpyrrolidone, is markedly affected by the macromolecular environment. Thus pK_a's are shifted 1–2 pH units compared to a corresponding small molecule. Furthermore, these shifts are abolished if urea is added to the solvent. Since polyvinylpyrrolidone can form no intramacromolecular hydrogen bonds (because it has no hydrogen-donor groups), the effect of urea cannot be the disruption of such hydrogen bonds. The intrinsic viscosity[22] of polyvinylpyrrolidone is high (0.22 dl/gm) for a molecule of 40,000 molecular weight and indicates that the polymer in solution is in a loose unordered conformation. Furthermore, urea does not change the intrinsic viscosity appreciably and hence the polymer conformation must remain loose. On the other hand, urea molecules presumably can form strong hydrogen bonds, and in an aqueous solvent they are likely to form such bonds with water molecules. The natural structure of water would thus be disrupted by high concentrations of urea. It seems, therefore, that urea abolishes the effect of the polymer on the pendant $-\mathrm{N(CH_3)_2}$ groups by interfering with the water structure in the local environment of the macromolecule. These effects of polyvinylpyrrolidone on attached groups thus seem to be intimately linked with the nature of the solvent in the neighborhood of the polymer.

It is unlikely that such phenomena are limited to polyvinylpyrrolidone. Recent studies[23] of ultrasonic attenuation in aqueous solutions of polyethylene glycol also reveal a cooperative breakdown in the local water structure in the neighborhood of the polymer. In addition it has been observed that the cooperative water structure around the polymer increases with molecular weight until a critical size is reached; thereafter a plateau is maintained.

Similar polymer–solvent interactions would be expected for polyisopropylacrylamide. Since the absorbance of the free N–H at 1.487 μ changes so markedly when the solvent is changed from chloroform to water,

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it is evident that this group is exposed to solvent. Hence the apolar substituents attached to the amide must also be in contact with solvent. In aqueous solution, in the local environment of the macromolecule with its many apolar groups, K_w should be significantly affected. If K_w is reduced in the neighborhood of the polymer, then k_{min} would be definitely decreased, as is indicated by eq 6 as well as by experiments in simpler systems.[4] In addition if the solvent character is changed at the polymer surface, the concentrations of the charged intermediates II and III would be reduced, and hence k_D and k_{OD} would also be lowered. In consequence the net, observed rate would be decreased even further.

Thus from these experiments with a synthetic polymer it seems apparent that the nature of the solvent in the neighborhood of a residue of a macromolecule is different from bulk solvent and that this difference may have a profound effect on the reactivity of constituent residues. It seems likely, therefore, that similar phenomena would be encountered in protein solutions and hence that hydrogen–deuterium exchange in these biopolymers would also be affected by the state of the solvent in the local environment.

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Very Low Pressure Pyrolysis. II. Decomposition of Nitropropanes[1]

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Abstract: The technique of very low pressure pyrolysis (vlpp) has been applied to 1- and 2-nitropropane. Pyrolysis was carried out at pressures from about 2 to 10 μ and at temperatures ranging from 650 to 1100°K, in a quartz vessel with residence time of about 0.4 sec. Both isomers gave the same primary reaction products, $HNO_2 + C_3H_6$, with HNO_2 decomposing further at the reaction conditions. The first-order rate parameters agreed well with those estimated from low-temperature studies. The activation energies and thermochemistry show that the reverse reaction, the exothermic addition of HNO_2 to an olefin, follows the Markovnikov rule. Addition of the NO_2 group to the CH_3-bearing C atom is favored by 7.2 kcal/mole of activation energy.

Several studies of pyrolysis of nitropropanes were made during the period 1950–1960.[2-6] The most recent of these was made by Smith and Calvert[6] (SC) whose product analyses showed that propylene production from the pyrolysis of 2-nitropropane (2-NP) followed a first-order rate law with $k = 1.11 \times 10^{11-39.3/\theta}$ sec^{-1} ($\theta = 2.303RT$ in kcal/mole). They proposed that HNO_2 was split off from the parent compound with the H coming from the vicinal carbons. Previous workers had proposed, in addition, either C–NO_2 bond breaking, followed by a chain reaction,[2,4] or HNO elimination with the H coming from the geminal position.[5] In all cases, there was secondary oxidation by HNO_2 and its decomposition products ($H_2O + NO + NO_2$).

Some doubt has remained about the nature of the primary process since many of the products of reaction can only be accounted for under the assumption of secondary reaction.[7] The five-center split-out of HNO_2 has not appeared to be a very attractive mechanism in comparison with the simpler C–NO_2 bond break. The HNO elimination appears unlikely on energetic grounds.

Cottrell, Graham, and Reid[3] (CGR) briefly reported the results of some 1-nitropropane pyrolyses. They found that the mechanism probably proceeded with production of propylene and HNO_2, but with an activation energy of about 50 kcal/mole. Their quoted rate constant at 388° was close to 4×10^{-3} sec^{-1}. No correction was made for the appreciable surface reaction observed in the system, and their reported A factor of 10^{13} sec^{-1} should have been a factor of 10 higher to agree with their reported data and activation energy. Gray, Yoffe, and Roselaar[4] (GYR) have also reported on 1-nitropropane pyrolysis, but the results of their experiments were inconclusive. Fréjacques[2] also pyrolyzed the nitropropanes, but his data are probably affected by chain-reaction effects.

The technique of very low pressure pyrolysis[8] (vlpp) is appropriate for determining the primary process in a pyrolytic system, since pyrolyses are carried out at pressures so low that secondary reactions can be either

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