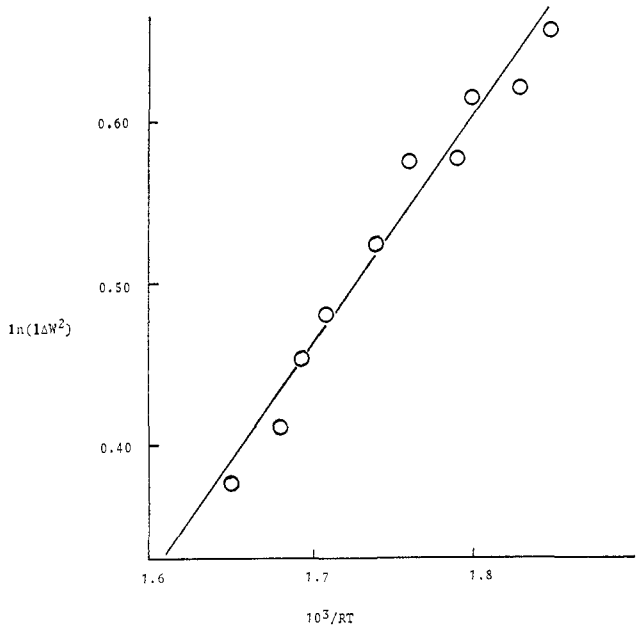

Figure 2. Modified van't Hoff plot for the system BCOT-HMPA-K. ΔW is the line width for the overmodulated spectrum.

From Table II it is clear that the enthalpy of disproportionation increases as the degree of conjugation from the COT ring is extended. This is the expected result since extended conjugation of the COT ring system lowers the electron–electron repulsion effect in the dianion. The entropy term has been shown to be due to ion pairing.[1] Ion pairing decreases as the charge of the anion and dianion is dispersed. Thus, the extension of the conjugation has the expected result of increasing the entropy of disproportionation.

From Table III it is clear that the variance of K_{eq} with counterion is mainly due to the entropy and not the enthalpy term. The case is more dramatic for the COT systems than it is for the PCOT systems. Comparing the system COT-HMPA-K and the system COT-HMPA-Na, we see that the enthalpies are almost identical, but the entropies differ by 20 entropy units. Since the charge on the anions of PCOT is more dispersed than it is for the COT anions, there is less ion pairing and, thus, the entropy term reflects a much smaller dependence upon the cation for the PCOT systems.

Table III. Thermodynamic Parameters for the PCOT and the COT Disproportionation Equilibrium in HMPA at 25°

System	K_{eq}	$\Delta H°$	$\Delta S°$
COT-HMPA-Li	$(5.0 \pm 2) \times 10^{-4}$	-7.8 ± 1	-29 ± 2
COT-HMPA-Na	$(2.3 \pm 1) \times 10^{-3}$	-4.2 ± 0.3	-26 ± 1
COT-HMPA-K	$(2.3 \pm 1) \times 10^{-5}$	-4.6 ± 0.7	-46 ± 1
PCOT-HMPA-Li	$(1.6 \pm 0.5) \times 10^{-4}$	-4.9 ± 0.2	-29 ± 1
PCOT-HMPA-Na	$(4.1 \pm 1) \times 10^{-4}$	-4.6 ± 0.2	-27 ± 1
PCOT-HMPA-K	$(5.6 \pm 1) \times 10^{-5}$	-3.7 ± 0.5	-32 ± 1

Acknowledgments. The authors wish to thank Dr. Alec Grimison for helpful discussion. We are indebted to the Research Corporation for support of this work.

Hydrogen–Deuterium Exchange in Some Polymer Amides

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Abstract: The kinetics of hydrogen–deuterium exchange was followed for two polymers, poly(*N*-ethylacrylamide) and poly(*N*-ethylmethacrylamide), which contain side chain amide groups. Conformational information for these polymers was obtained from viscometry and nuclear magnetic resonance. Infrared spectra showed the presence of hydrogen-bonded and of free amide groups in both polymers. Single first-order exchange processes were observed, and the rate constants, k, obtained from the appearance of OH absorption bands or the disappearance of NH bands, are the same. The polymers exchange more slowly than structurally similar small amides. This behavior is interpretable in terms of steric effects, changes in local dielectric constant in the region of the amide group, and changes in the character of water in the vicinity of the polymers.

The characteristics of many simple chemical reactions may be markedly different in a protein environment than in bulk solution. Some reactions, for example enzyme-catalyzed ones, are markedly accelerated. In contrast, many are strikingly hindered, either thermodynamically or kinetically. Changes in the pK_a of tyrosine residues provide examples of the former, decreases in reactivity of sulfhydryl groups are illustrations of the latter.

The molecular basis for such gross changes in reactivity in a protein environment will be different in details in each case. Nevertheless, some general principles may emerge from a detailed study of a single one of these modified reactions. A fairly comprehensive examination has been made, therefore, of the intrinsic and extrinsic factors that influence one of the simplest of chemical changes, the exchange of H atoms by D atoms.

Studies with small model amides[1,2] show that the exchange rate is very sensitive to H^+ and OH^- as well

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as to general acid and general base catalysis.[3,4] It is also sensitive to the local environment. Substituents in the neighborhood of the amide can produce marked changes in the rate of H–D interchange[5,6] by inductive and other means.

As a step in transposing results obtained with small model amides to the interpretation of the behavior of proteins, it would clearly be desirable to examine the behavior of amides in a polymeric matrix that is not a polypeptide. In this way one could see what influence a polymer environment *per se* has on H–D exchange before we attempt to assess the contribution of the characteristic α-helical and β-structure components of proteins.

Experiments have been reported with polyisopropylacrylamide[7] and with polyvinylacetamide.[8] In each polymer the rate constants for H–D exchange are markedly reduced in comparison to those observed with corresponding monomers.

The present investigation is an extension of experiments in the polyacrylamide series. Structural variations have been made in the amide side chain and in the polymeric backbone. The behavior of these modified polymers, compared to that of corresponding monomers, provides additional insight into the molecular basis of attenuation of isotopic exchange rates.

Experimental Section

Materials and Methods. *N*-Ethylacrylamide and methacrylyl chloride were purchased from Borden Chemical Monomer-Polymer Laboratories. The former compound was purified by vacuum distillation at a pressure of 0.40–0.50 mm; the fraction boiling from 59 to 60° was collected and checked for purity by gas-phase chromatography and nmr. Methacrylyl chloride was distilled under N_2; the fraction boiling at 98° was collected and checked for purity by nmr.

Ethylamine (anhydrous) was purchased from Eastman Kodak and Matheson Co., Inc. It was used directly.

Isobutyryl chloride and propionyl chloride were obtained from Eastman Kodak. The isobutyryl chloride was used without further purification. The propionyl chloride was distilled; the fraction boiling at 78° was collected and checked for purity by nmr.

Deuterium oxide (99.8–99.9% D), deuterium chloride (38% in D_2O, warranted to contain 99.5% D in the labeled position), and sodium deuteroxide (40% in D_2O, warranted to contain 99% D in the labeled position) were purchased from Bio-Rad Laboratories.

Cacodylic acid was obtained from J. T. Baker Chemical Co., and dried *in vacuo* at 80° prior to use. Reagent grade sodium acetate was obtained from Allied Chemical, and dried *in vacuo* at 120° prior to use.

Infrared spectra in the fundamental region were scanned with a Beckman Model IR-5 spectrophotometer. A Cary 14R spectrophotometer was used in the overtone ir. The temperatures of solutions in the optical cells were measured with a thermistor probe attached to a telethermometer of the Yellow Springs Instruments Co. The Varian T-60 and A-60 were used for nmr work.

A Spinco Model E ultracentrifuge was used in the determination of polymer molecular weight.

A Radiometer pH meter, Model TTT 1b, and a Corning Model 12 pH meter, in conjunction with Corning combination electrodes, were used for pH measurements. pH readings were changed to pD by means of the conversion equation of Glasoe and Long.[9]

$$pD = pH + 0.40 \qquad (1)$$

Viscosities were measured at 25.0° using a coil viscometer. The flow time of water in the viscometer was 115.8 sec.

The densities of polymer solutions in water were measured at 25.0° using pycnometers, one with a volume of 59.1 ml for poly(*N*-ethylmethacrylamide) and another with a volume of 20.9 ml for poly(*N*-ethylacrylamide).

Preparation of Small Amides. *N*-Ethylmethacrylamide was prepared under nitrogen by treatment of a benzene solution of methacrylyl chloride (115.7 g) with gaseous anhydrous ethylamine (100 g, Eastman Kodak) at about 0°. The mixture was allowed to warm to room temperature and solid ethylamine hydrochloride was removed by filtration. The solvent was stripped off by roto-evaporation. The resulting residue, the amide, was distilled twice; the fraction boiling from 48° (15 μ) to 35° (10 μ) was collected. Purity of the amide was determined by nmr.

N-Ethylisobutyramide was also prepared under nitrogen. A solution of anhydrous ethylamine (100 g, Eastman Kodak) was prepared in benzene at 5° and then cooled to 0°. Isobutyryl chloride (100 g) was added dropwise with stirring. The mixture was allowed to warm to room temperature and filtered to remove ethylamine hydrochloride. The filtrate was dried over anhydrous $MgSO_4$ and roto-evaporated to yield a white crystalline solid wet with a yellow liquid phase. Final purification was obtained by column chromatography on silica gel using purified ethyl acetate as the eluting solvent. The amide was isolated by roto-evaporation and yielded colorless crystals on sublimation. The melting point was 68.5–69.0° (lit.[10] mp 68°). An nmr spectrum obtained in CCl_4 was consistent with its structure. Two triplets observed at δ 1.1 and 1.2 (relative to tetramethylsilane) can be ascribed to the methyl protons of the *N*-alkyl and *C*-alkyl groups, respectively; a septuplet observed at δ 2.4 comes from the α proton, a quintet at δ 3.1 from the methylene of the *N*-ethyl group and a broad band at δ 7.1 from the N–H. The position of the triplet at δ 1.1 was in accord with published data.[11] The infrared spectrum of the amide dissolved in CCl_4 was consistent with its structure.

N-Ethylpropionamide was prepared under nitrogen at −5 to −2°. Anhydrous ethylamine (Matheson) was dissolved in 300 ml of anhydrous ethyl ether. Propionyl chloride (30 g) dissolved in 170 ml of dry ether was added dropwise with stirring to the amine solution. After addition of the acid chloride was complete, the solution was allowed to warm to room temperature, filtered to remove ethylamine hydrochloride, dried over anhydrous K_2CO_3, and roto-evaporated. The crude amide was taken up in benzene, dried over anhydrous $MgSO_4$, roto-evaporated, and distilled, the fraction boiling from 85.0 to 85.6° (1.2 mm) being collected. An acidic impurity was removed by extraction of a benzene solution with 2% aqueous Na_2CO_3 and salting out of the aqueous layer with NaCl. The organic layer was dried over anhydrous K_2CO_3 and $MgSO_4$, roto-evaporated, and distilled. The fraction boiling at 56.5° (0.5 mm) was collected and its purity determined by thin layer chromatography on silica (Eastman, Chromogram sheet 6061) using ethyl acetate as the developing solvent. An nmr spectrum of the amide in CCl_4 showed two overlapping triplets at δ 1.1 arising from methyl groups, a quartet from the *C*-methylene group at δ 2.1, a quintet from the *N*-methylene group at δ 3.2, and a broad NH band at δ 8.0. The position of the triplets is in agreement with published data[11] as is the ir of the amide in CCl_4.[12]

Preparation of Polymers. Poly(*N*-ethylmethacrylamide) was prepared first in a bulk polymerization of *N*-ethylmethacrylamide. The monomer (0.5 g) and 0.0005 g of benzoyl peroxide were heated to 80–90° under N_2 for 1.25 hr. A glassy solid was obtained which was utilized for solubility determinations only.

A solution polymerization of *N*-ethylmethacrylamide in water was also carried out. To 488 ml of deoxygenated water under N_2 at 38.1° were added 74 ml of deoxygenated aqueous 0.2% $(NH_4)_2S_2O_8$, 14.9 ml of deoxygenated 0.1% $NaHSO_3$, and 30.3 g of *N*-ethylmethacrylamide. Polymerization was allowed to proceed for 43 hr. The polymer was obtained as a white, gummy solid by slow addition of its aqueous solution to acetone. The product was dissolved in, and dialyzed against, glass-distilled water. The yield of polymer was 73% (22.1 g).

Poly(*N*-ethylacrylamide) was prepared first in bulk from 0.8585 g of monomer and 0.0528 g of benzoyl peroxide at 103° under N_2. The polymer was utilized only for solubility determinations.

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(4) I. M. Klotz and B. H. Frank, *J. Amer. Chem. Soc.*, **87**, 2721 (1965).
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Poly(*N*-ethylacrylamide) was also prepared in aqueous medium. To 480 ml of deoxygenated glass-distilled water was added 35.5 g of *N*-ethylacrylamide at 10° under N_2. The resulting suspension was treated with the following quantities of deoxygenated aqueous solutions: 31.2 ml of 0.5% $NaBrO_3$, 3.75 ml of 0.5% $Na_2S_2O_3$, and 5.75 ml of 0.5% H_2SO_4. The suspension was maintained at 10–15° for 40 min after which 6.4 ml of 0.5% $(NH_4)_2S_2O_8$ was added. The polymerization was allowed to proceed for 6 hr after which the solution was heated to 80°. A rubbery mass of polymer precipitated. The polymer was washed with reagent grade acetone, dissolved in glass-distilled water, and dialyzed against glass-distilled water for 7 days. The product was isolated as a fluffy solid after lyophilization from its aqueous solution. Its infrared spectrum, in a KBr pellet, was in accord with that in the literature.[13]

Preparation of Solutions for Kinetics Studies. The polymers synthesized by solution polymerization in water were utilized for kinetics studies. Prior to their use, each polymer was lyophilized from aqueous solution to yield a dry, fluffy amorphous solid that facilitated rapid dispersal and dissolution of this solid in exchange media. The exchange media consisted of buffers in D_2O at preset pD values. Sodium acetate (0.2 *M*) was used as a buffer in solutions of pD values less than 6.2, 0.02 *M* sodium cacodylate served for pD values greater than 6.2. In these buffers the pD remained constant within ±0.02 unit during a typical kinetics experiment.

About 0.4 g of polymer was used in each experiment with poly(*N*-ethylmethacrylamide). The solid was weighed into a polypropylene bottle containing beads of Teflon. Solution of the polymer was achieved after addition of 18.9 ml of D_2O buffer to the bottle and vigorous shaking for 5–6 min on a modified Cole-Palmer supermixer. After dissolution, a polymer concentration of about 0.2 residue molar was obtained. The polymer solution then was transferred rapidly under pressure through a glass wool plug to a cylindrical optical cell with a 5-cm optical path, fitted with a temperature probe. The cell was placed in the nitrogen-purged sample compartment of a Cary 14R spectrophotometer. After each exchange experiment, the polymer was recovered as described by Scarpa, *et al.*[7]

Poly(*N*-ethylacrylamide) was treated similarly. Due to the large viscosities encountered with its solutions, only dilute concentrations of polymer were used. Approximately 0.33 g of the solid was dissolved, as described for poly(*N*-ethylmethacrylamide), in 35.5 ml of D_2O buffer to yield a 0.1 residue molar solution. This solution was transferred to a 10-cm cylindrical optical cell which was fitted with a temperature probe. The cell was placed in the Cary 14R spectrophotometer. After each experiment the polymer was recovered by dialysis of exchange solutions against glass-distilled water followed by lyophilization.

For small amides, exchange media in all cases consisted of buffered D_2O solutions. For values of pD less than 6.2, 0.02 *M* NaOAc solutions were used as buffers; 0.02 *M* sodium cacodylate solutions served for pD values greater than 6.2. The pD of 10-ml aliquots of the buffers was set at an approximate value with stock solutions of DCl and NaOD. Prior to use in kinetics experiments, these buffers were preequilibrated to 25°.

Samples of *N*-ethylisobutyramide were weighed into test tubes 12 cm in length. Two glass beads were placed in each tube to facilitate dissolution of the amide. Enough thermally equilibrated buffer was added to each sample to produce an amide concentration between 0.4 and 0.5 *M* and the tube was agitated for 1 min to effect solution. The solution was transferred to a 1-cm cuvette which was fitted with a temperature probe and the cell placed in the Cary 14R spectrophotometer.

Samples of *N*-ethylpropionamide were also weighed into test tubes 12 cm in length. Enough thermally equilibrated buffer was added to each amide sample to produce a final amide concentration of between 0.44 and 0.47 *M*. The tube was agitated by hand and centrifuged to remove air bubbles and its contents transferred to a 1-cm cuvette. The cuvette was fitted with a temperature probe and placed in the Cary 14R spectrophotometer. The time between addition of the buffer and placement of the solution in the spectrophotometer did not exceed 1.5 min.

The pH of the exchange solutions of the polymers and small amides was determined at room temperature after the reaction had gone to completion.

Results

Characterization of Polymers. Poly(*N*-ethylacrylamide) and poly(*N*-ethylmethacrylamide) are stable water-soluble polymers. Infrared analysis showed that prolonged dialysis of their solutions against water does not lead to hydrolysis of the amide groups.

The intrinsic viscosities of each polymer were measured in water at 25.0°. Poly(*N*-ethylacrylamide) had an $[\eta]$ of 5.6 (dl g^{-1}) and poly(*N*-ethylmethacrylamide) had an $[\eta]$ of 0.73 (dl g^{-1}). The limiting slope of a plot of reduced viscosity *vs.* concentration yielded Huggins constants of 0.45 and 0.56, respectively.

High speed sedimentation equilibrium experiments[14] were performed with each polymer at 20° in 0.1 *N* NaCl. Coupled with values of the partial specific volume, $\bar{v}$, of 0.83 (ml/g) and 0.82 (ml/g) for poly(*N*-ethylacrylamide) and poly(*N*-ethylmethacrylamide), respectively, the equilibrium experiments led to calculated weight-average molecular weights of 880,000 and 143,000, respectively. Plots of $\sigma_w(r)$[14] *vs.* $c(r)$ yielded three different curves of similar shape for three different loading concentrations of each polymer used in the high speed experiments. This behavior is indicative of polydispersity and nonideality.[14]

Solutions of poly(*N*-ethylmethacrylamide) (2%) in water scatter light to an extent visible to the eye; such behavior is indicative of some large aggregates. Ultracentrifuge experiments did not show higher aggregates, presumably because they were sedimented to the bottom of the cell. Indeed, a pile-up of polymer at cell bottom was apparent in the Rayleigh photographic plates.

Space-filling models[15] were constructed for atactic poly(*N*-ethylacrylamide), poly(*N*-isopropylacrylamide), and poly(*N*-ethylmethacrylamide). The polyacrylamides were found to possess flexible backbones which allow these polymers to assume loose, random-coil conformations in solution. In poly(*N*-ethylmethacrylamide), on the other hand, the methyl of the methacrylyl group constrains the freedom of motion of the polymeric backbone in solution. This conclusion from the models is in accord with viscosity, nmr, and other studies of similar systems.[16]

The parameters of the Mark–Houwink equation[17] are unknown for the polymers studied in this paper. If the dependence of intrinsic viscosity on molecular weight is the same for all three polymers, then $[\eta]$ for poly(*N*-ethylacrylamide) is larger than expected, whereas $[\eta]$ for poly(*N*-ethylmethacrylamide) is considerably smaller than anticipated. The low $[\eta]$ for the latter polymer, combined with the high value of its Huggins constant, indicates that poly(*N*-ethylmethacrylamide) macromolecules exist in water in a compact conformation dispersed in a poor solvent.[18] Molecular models indicate that the backbone methyl group constrains the polymer so severely that some of the side chain amide groups are shielded from the polymer–solvent interface and are prevented from interacting with solvent.

The relative freedom of motion of side chain ethyl groups in poly(*N*-ethylacrylamide) and in poly(*N*-ethylmethacrylamide) was inferred from nmr data of each polymer in D_2O, at a concentration of 0.35 residue molar. Assignments of the nmr signals were made with

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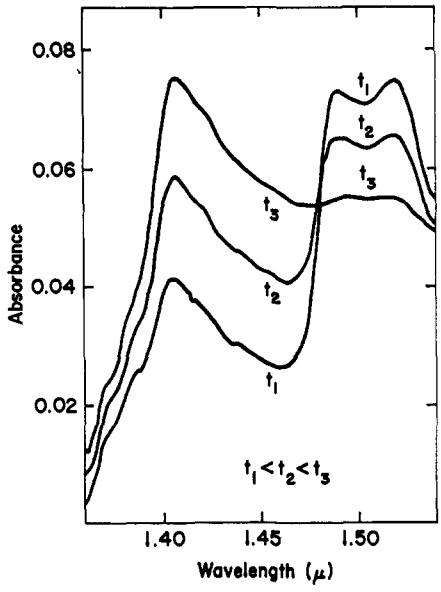


Figure 1. Change of spectra with time during hydrogen-deuterium exchange of poly(*N*-ethylacrylamide) in D_2O at 25°.

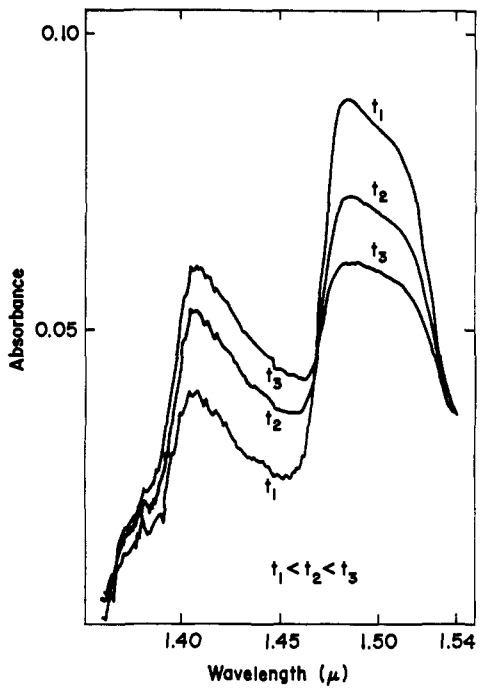


Figure 2. Change of spectra with time during hydrogen-deuterium exchange of poly(*N*-ethylmethacrylamide) in D_2O at 25°.

reference to results for the structurally analogous small amides, *N*-ethylpropionamide and *N*-ethylisobutyramide, in CCl_4. The methylene components of the side chain ethyls in the polymers were clearly separated from other proton signals. Poly(*N*-ethylmethacrylamide) had a broad methylene band with some splitting whereas poly(*N*-ethylacrylamide) showed a sharply resolved quadruplet centered near 160 cps. The increased broadening in poly(*N*-ethylmethacrylamide) implies that the methylene is restrained from averaging its environment through bond rotation.[19,20] Evidently the alkyl amide group attached to the polymethacrylamide is more wedged in between segments of the backbone than is true in the polyacrylamide lacking the backbone methyl. Thus the nmr data are in accord with expectations from model building.

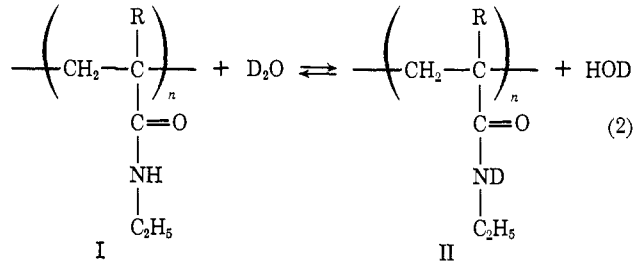
Hydrogen Bonding and Infrared Spectra. Poly(*N*-ethylacrylamide) and poly(*N*-ethylmethacrylamide) possess two bands in the overtone region, 1.48 μ (6800 cm^{-1}) to 1.54 μ (6500 cm^{-1}). These bands were assigned to free and hydrogen-bonded (N-H···O=C) amide groups, respectively, by comparison with related small amides[21,22] and polymers.[7] For poly(*N*-ethylacrylamide) the free NH band appears at 1.490 μ (6700 cm^{-1}), the bonded at 1.519 μ (6500 cm^{-1}); for poly(*N*-ethylmethacrylamide) the free NH band is at 1.485 μ (6740 cm^{-1}), the bonded at 1.509 μ (6630 cm^{-1}). During the course of an isotope exchange, the NH bands decrease in intensity while a band due to an OH stretching overtone increases in intensity at 1.407 μ (7110 cm^{-1}). Spectra of the near-infrared region, showing both the OH and NH bands, are illustrated in Figures 1 and 2 for typical isotope exchanges at 25°.

Studies of the fundamental infrared absorptions of the polymers were made in KBr pellets. Poly(*N*-ethylmethacrylamide) shows a broad band centered at 3.00 μ (3330 cm^{-1}) arising from NH stretch, an "amide I" band at 6.13 μ (1630 cm^{-1}), an "amide II" band at 6.57 μ (1520 cm^{-1}), as well as peaks at 3.40 (2940 cm^{-1}), 3.45 (2900 cm^{-1}), 6.90 (1450 cm^{-1}), and 7.25 μ (1380 cm^{-1}) attributable to CH_2 and CH_3 groups.[23] Poly(*N*-ethylacrylamide) has a broad band centered at 3.02 μ (3310 cm^{-1}) arising from an NH stretch, an "amide I" band at 6.05 μ (1650 cm^{-1}), an "amide II" band at 6.43 μ (1550 cm^{-1}), and absorbance peaks at 3.38 (2960 cm^{-1}), 3.40 (2940 cm^{-1}), 6.88 (1455 cm^{-1}), and 7.21 μ (1390 cm^{-1}) arising from CH_2 and CH_3 groups.[23]

The infrared spectra of the small amides were examined in CCl_4 solution. A 0.42 *M* solution of *N*-ethylisobutyramide shows bands at 2.90 (3450 cm^{-1}) and 3.00 μ (3330 cm^{-1}) attributable to free and bonded NH groups,[24] respectively. *N*-Ethylpropionamide has analogous bands[24] at 2.90 (3450 cm^{-1}) and 3.02 μ (3310 cm^{-1}).

Space-filling models of the polymers indicate that some intramolecular hydrogen bonds could form, but that intermolecular hydrogen bonding is very unlikely because of steric interferences from the side chains.

Calculation of Rate Constants. The isotope exchange process may be represented schematically as



(2)

Since D_2O is present in great excess (~55 *M*), only the

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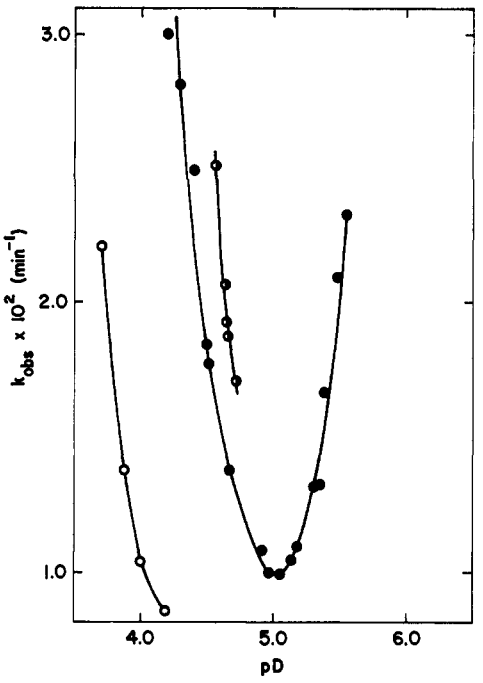


Figure 3. Rate constant-pD profile for H-D exchange of poly(*N*-ethylacrylamide) in D_2O at 15° (○), 25° (●), and 30° (◑).

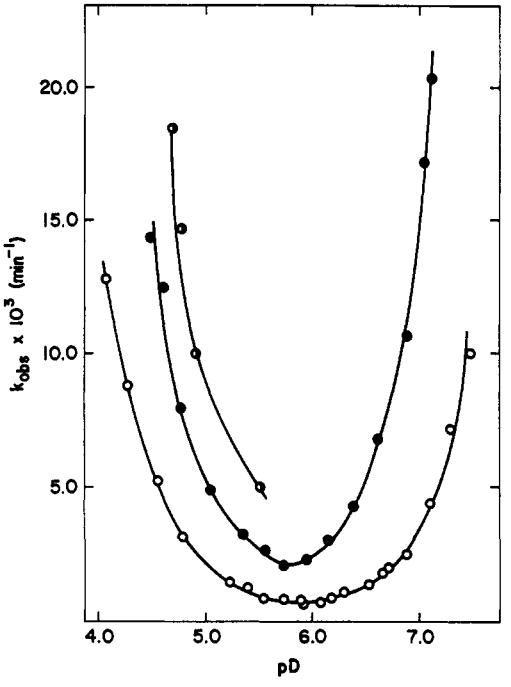


Figure 4. Rate constant-pD profile for H-D exchange of poly(*N*-ethylmethacrylamide) in D_2O at 15° (○), 25° (●), and 30° (◑).

forward reaction is significant. Hence, the H-D exchange rate appears as a pseudo-first-order reaction.

$$-d[NH]/dt = d[OH]/dt = k_{obsd}[NH] \quad (3)$$

In a typical experiment, spectra were scanned from 1.54 to 1.28 μ, and the absorbances at 1.407 μ (for OH), and the respective wavelengths for free and bonded NH groups were monitored as a function of time (Figures 1 and 2). To compensate for light scattering in the polymer solutions, absorbances are related to that at a fixed reference point, 1.310 μ, in a region where the spectra are flat.

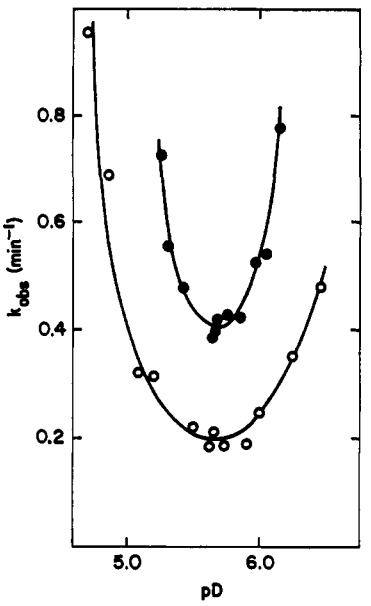


Figure 5. Rate constant-pD profile for H-D exchange of *N*-ethylpropionamide (●) and of *N*-ethylisobutyramide (○), in D_2O at 25°.

Rate constants were usually calculated as described by Scarpa, *et al.*[7] A Guggenheim analysis[25] was used for some of the data for poly(*N*-ethylmethacrylamide) at 15° where exchange was very slow. Typical calculated rate constants are assembled in Table I.

Table I. Rate Constants (at 25°) Calculated from Different Absorption Bands

	k_{obsd} (min^{-1}), calcd from		
pD	OH band	Free NH band	Bonded NH band
	Poly(*N*-ethylacrylamide)		
4.399	1.90×10^{-2}	2.13×10^{-2}	1.95×10^{-2}
4.487	1.75×10^{-2}	1.55×10^{-2}	1.47×10^{-2}
5.107	1.03×10^{-2}	1.28×10^{-2}	1.37×10^{-2}
5.291	1.30×10^{-2}	1.38×10^{-2}	1.39×10^{-2}
	Poly(*N*-ethylmethacrylamide)		
4.488	1.49×10^{-2}	1.41×10^{-2}	1.42×10^{-2}
5.023	4.84×10^{-3}	4.97×10^{-3}	4.87×10^{-3}
4.774	7.84×10^{-3}	7.91×10^{-3}	7.96×10^{-3}

The observed rate constants for both polymers are plotted as a function of pD in Figures 3 and 4. The parabolic dependence of rate on pD is similar to that observed with small amides[4-6,26-28] and polymers.[5,7,8,29]

Isotope exchange of the small amides, *N*-ethylpropionamide and *N*-ethylisobutyramide, was studied at 26.0 ± 1.0°. First-order rates were observed. The rate constant-pD profiles for these amides are shown in Figure 5. Values of k and pD at the minimum are assembled in Table II.

(25) E. A. Guggenheim, *Phil. Mag.*, 2, 538 (1926).
(26) I. M. Klotz and P. L. Feidelseit, *J. Amer. Chem. Soc.*, **88**, 5103 (1966).
(27) C. Y. S. Chen and C. A. Swenson, *J. Amer. Chem. Soc.*, **91**, 234 (1969).
(28) S. W. Englander and A. Paulsen, *Biopolymers*, 7, 379 (1969).
(29) Y. Kakuda, N. Perry, and D. D. Mueller, *J. Amer. Chem. Soc.*, **93**, 5992 (1971).

Table II. Comparison of Rate Constants for Polymeric and Small Molecule Amides at 25°

Small amides	Polymers	k_{min}, min^{-1}	pD_{min}
N-Ethylpropionamide		0.40	5.66
N-Isopropylpropionamide[a]		0.18	5.60
N-Ethylisobutyramide		0.18	5.70
	Poly(*N*-ethylacrylamide)	0.0098	5.00
	Poly(*N*-isopropylacrylamide)[a]	0.0032	5.00
	Poly(*N*-ethylmethacrylamide)	0.0022	5.75

[a] Taken from J. S. Scarpa, D. D. Mueller, and I. M. Klotz, *J. Amer. Chem. Soc.*, **89**, 6024 (1967).

Activation energies, in the acid range, for the polymers were found to be 20 and 26 kcal/mol for poly(*N*-ethylmethacrylamide) and poly(*N*-ethylacrylamide), respectively.

Discussion

If we examine k_{min} and pD_{min} (Table II) for the monomeric models we find their properties very similar to those observed in earlier studies.[4,26,30] In general, the pD at the minimum exchange rate is near 5.6 and varies only slightly with substitution of alkyl groups on the amides. On the other hand, k_{min} drops with increasing size of alkyl substituent. This is apparent in a comparison of ethyl and isopropyl substituents (Table II) on propionamide. Furthermore, *N*-methylpropionamide has been shown previously[30] to have a k_{min} of 0.44 and thus falls in place in this series of propionamides.

These studies with monomeric models illustrate once again that in small model amides, local environment around the -CONH- group has pronounced effects on the H-D exchange rate.[2,3,5,6,26,30]

Turning to the polymers, we note first that a single (pseudo) first-order rate equation fits the observations of H-D exchange. Thus, either all of the amide N-H groups are in indistinguishable environments, or any differences in local environment due to N-H···O=C hydrogen bonding or to alternative conformations of the polymer are averaged out by rapid rearrangement among states, within the time of isotopic exchange. The latter explanation seems more likely since the unstructured conformation of the polymer would not provide identical environments in the locale of each residue.

The polyacrylamides show the same parabolic k-pD dependence as is observed with the small model amides and as has been found previously with other polymides[7,8,29] and polypeptides.[5] Thus the exchange must be catalyzed by D^+ and OD^-, and the observed rate constants k_{obsd} may be expressed as

$$k_{obsd} = k_O + k_D(D^+) + k_{OD}(OD^-) \quad (4)$$

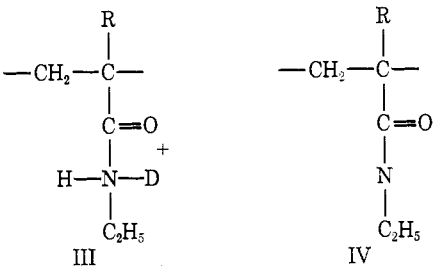
As has been shown previously,[5] the values of k_{min} and of pD_{min} at the minimum of the k-pD profile are given by

$$k_{min} = 2(k_D k_{OD} K_W)^{1/2} + k_O \quad (5)$$

$$pD_{min} = \frac{1}{2} pK_W - \frac{1}{2} \log (k_{OD}/k_D) \quad (6)$$

where K_w is the dissociation constant of water.

Since the kinetics for the polymers fit the same analytical expressions as for the small model amides, it seems likely that the mechanistic pathways for the exchange process are the same. The rate-determining step is generally accepted to be[31] that leading to the formation of the deuterated amide cation (III) in the acid-catalyzed reaction and of the deprotonated anionic species (IV) in the base-catalyzed exchange.



As in any rate process, H-D interchange depends on a transition from a ground state to an activated state. In studies with proteins and polypeptides it has generally been assumed[1] that any attenuation in exchange rate is to be ascribed to a conformation of the macromolecule that lowers the energy of the ground state. For polyvinylacetamide it has also been suggested[8] that the lowering in rate is due entirely to inaccessibility of amide groups on the polymer to solvent. In some conformations of the polymer the amide group is presumed to be totally blocked from exposure to solvent, in others, statistically less frequent, totally exposed and able to exchange with a rate similar to that of a corresponding monomeric model amide.

Studies with small model amides have shown[4-6,26] that exchange rates are very sensitive to the local environment, in the covalent neighborhood, and in the surrounding bulk solution. Thus, substituents of the amide group[5,6] can produce marked changes in H-D interchange by inductive and other means. In the bulk solution, H^+, OH^-, and general acids and bases[1,2] can produce catalytic accentuations of the rates. In essence these effects are manifested through changes in concentration of the activated species in the exchange pathway.

It seems likely that the effect of the polymer environment on the stability of the charged intermediates III and IV is an important factor in determining the exchange rate. It has already been shown[32] that the addition of an anion strongly bound by polyisopropylacrylamide shifts the k-pD profile in the direction predicted if the cationic intermediate (III) is stabilized by the presence of this bound anion. To a first approximation all of the polyamides listed in Table II show essentially equal reductions in rate (about 60-(±20) fold) if each is compared with its corresponding monomer. Even

(30) P. L. Feidelseit, Ph.D. Dissertation, Northwestern University, 1967.

(31) A. Berger, A. Loewenstein, and S. Meiboom, *J. Amer. Chem. Soc.*, **81**, 62 (1959).

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poly(ε-aminomethacrylyl-L-lysine), with charged zwitterionic side chains projecting into the solvent water, shows a k_{min} of 0.0041 (compare with values in Table II), approximately 40-fold reduced with respect to its monomer. It seems unlikely that all of these different polymers spend the same fraction of time, about 98%, in conformations blocked from exposure to solvent. Furthermore, all of these polymers have intrinsic viscosities corresponding to unstructured, relatively swollen configurations. These should be relatively "porous" to solvent.

On the other hand, in the polymer matrix every [-CONH-] group is surrounded by an apolar environment built up by the backbone of the polymer, the immediate substituents of the amide group, and neighboring residues pendant from the backbone chain. In this apolar environment charged intermediates such as III and IV would be unfavored. Thus rate constants for their formation would be diminished. Furthermore, in the local neighborhood of the polymeric matrix, the local concentration of residues would be several molar, even in a relatively open conformation. In essence, then, the local solution would correspond to a relatively concentrated organic–aqueous solution. In such an environment K_w of water is decreased.[33] In addition, in the neighborhood of the polymer there will be interactions between the apolar substituents and H_2O molecules, and the structure of water would be different than in bulk solvent. This will surely affect the solvation of charged intermediates III and IV, as well as of the alkyl groups.

It seems reasonable to conclude, therefore, that the placement of an amide group in a polymeric matrix is accompanied by substantial changes in its local environment and that these can have marked effects on stability of the transition state, as well as of the ground state, in the mechanistic pathway for hydrogen–deuterium exchange.

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(33) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 3rd ed, Reinhold, New York, N. Y., p 756.

Mechanism of Cis–Trans Isomerization about Carbon–Carbon Double Bonds Catalyzed by Silver(I)[1]

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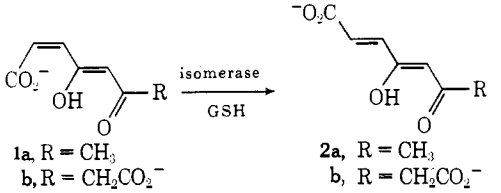
Abstract: Cis–trans isomerizations of maleylacetone, maleylacetoacetate, and similar compounds are catalyzed by enzymes and by silver ion. The possible relations between the two are discussed. Silver ion catalyzed isomerization of maleylacetone proceeds *via* a nonradical path and without vinyl proton exchange. The methyl ester (**6**) and the enol methyl ether (**7**) of maleylacetone were synthesized and the reactions of silver with these substrates are compared with that for silver and maleylacetone. The methyl ester undergoes rapid silver ion catalyzed isomerization while isomerization of the enol methyl ether is not catalyzed by silver. Solvent isotope effects, pH–rate profiles, and the lack of general acid–base catalysis suggest that a π complex between silver and the monoanion (**4**) of maleylacetone is formed. The π complex loses a proton in a preequilibrium step and could then undergo intramolecular nucleophilic attack to form a furanone intermediate, which is able to undergo facile rotation about the carbon–carbon bond that is isomerizing.

Cis–trans isomerizations about carbon–carbon double bonds are important reactions in certain biological systems.[2] Several of these reactions, catalyzed by enzymes, occur during the metabolism of aromatic amino acids and related compounds. Photocatalyzed cis–trans isomerizations are basic to visual processes, but a dark, enzyme-catalyzed trans–cis isomerization is generally required to regenerate the light-sensitive pigment.[3] Although several systems have now been characterized, the mechanism of enzyme action for any of these remains to be elucidated.

One system in which we have been interested is isomerization of maleylacetone (**1a**) or maleylacetoacetate (**1b**) to fumarylacetone (**2a**) or fumarylacetoacetate (**2b**), respectively (eq 1), catalyzed by an enzyme found in animal liver or in *Vibrio 01* bacteria.[4] Glutathione (GSH) is required as a coenzyme for



1a, R = CH_3; b, R = $CH_2CO_2^-$ — 2a, R = CH_3; b, R = $CH_2CO_2^-$ (1)

(1) (a) Research performed under the auspices of the U. S. Atomic Energy Commission. (b) For a preliminary report of this work, see R. A. Johnson and S. Seltzer, *J. Amer. Chem. Soc.*, **94**, 4755 (1972).

(2) For a recent review, see S. Seltzer, *Enzymes, 3rd Ed.*, **6**, 381 (1972).

(3) (a) R. Hubbard, *J. Gen. Physiol.*, **39**, 935 (1956); (b) G. Wald and R. Hubbard, *Enzymes, 2nd Ed.*, **3B**, 369 (1960).

(4) S. Seltzer, *J. Biol. Chem.*, **248**, 215 (1973).