ment occurs through predissociation by intersystem crossing $^3\sigma_0(N\text{–}C) \leftarrow S_1(\pi\pi^*)$ resulting in radical pairs. Subsequently, the radical pairs recombine efficiently in a solvent cage, as has been shown by Shizuka, *et al.*[23–28]

(24) H. Shizuka and I. Tanaka, *Bull. Chem. Soc. Jap.*, **41**, 2343 (1968).
(25) H. Shizuka, *ibid.*, 42, (1969).
(26) H. Shizuka, *ibid.*, **42**, 57 (1969).
(27) H. Shizuka and I. Tanaka, *ibid.*, 42, 909 (1969).
(28) H. Shizuka, T. Morita, Y. Mori, and I. Tanaka, *ibid.*, **42**, 1831 (1969).

Finally, it can be said that the intermediate M (11,12-dihydrocarbazole) is different from the 610-nm transient on the basis of a flash study of diphenylamines. The rate constant k_7 for the process of from the formation of M from the triplet and the decay rate constant k_9 for conversion from M to the starting material have activation energies of 7 and (8 + E_{10}) kcal/mol, respectively, which result in a temperature effect on the quantum yield for carbazole formation. The mechanism for photocyclization of diphenylamine was shown in eq 0–11.

Hydrogen–Deuterium Exchange of a Charged Poly(methacrylamide) and Its Monomeric Analog

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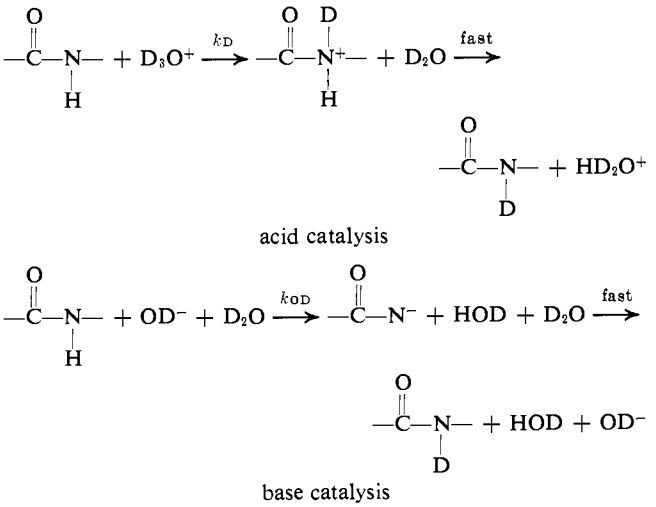
Abstract: The kinetics of hydrogen–deuterium exchange in a charged polymeric amide–poly(ε-aminomethacrylyl-L-lysine)—and its monomeric analog—ε-aminoisobutyryl-L-lysine—were measured in D_2O solutions. The polymer was selected as a model for charge effects on the exchange rates of proteins since its net charge will vary with pH as that of a protein. Both polymer and monomer showed parabolic rate *vs.* pD profiles characteristic of specific acid and base catalysis. The polymer, however, had a drastically reduced rate of minimum exchange, K_{min}, compared with the monomer. The polymer also showed a slightly increased pD of minimum exchange, pD_{min}. The shift in pD_{min} was interpreted as a decrease in K_W in the vicinity of the polymer backbone. Although a decrease in K_W also would decrease k_{min}, the major factor contributing to slow exchange appeared to be steric inhibition inherent with each residue. That interpretation is supported by a possible 3-kcal/mol increase in E_a* for exchange in the polymer over that of the monomer. In addition, internal catalysis by the amino group of the lysine moiety was observed in the polymer. Its effect also showed a drastic reduction over that of small molecules. Interestingly, upon the acquisition of a net positive charge, the rates were *reduced*, despite evidence for some expansion of the molecule. The rate reductions were about twice those predicted by ΔG_{el} from potentiometric titration data. Possibly, the basicities of carboxylate anions and amide groups are affected differently by the proximity of a positive charge.

Hydrogen–deuterium exchange has been shown to be a sensitive method for detecting conformational changes in biological macromolecules.[2–5] Proper interpretation of H → D exchange data in those cases, however, frequently depends on information gained through model systems. To date, considerable data have been gathered on protein analogs. For example, the following facts are known. (i) Labile-side-chain hydrogens (such as –OH, $-NH_3^+$, and COOH) all exchange with half-life times well below 1 min under the usual experimental conditions.[6,7] (ii) H → D exchange of amides usually proceeds much slower (half-life time of several minutes) and shows specific, as well as general, acid and base catalysis.[8–10] (iii) The acid- and base-catalyzed steps shown below probably involve charged amide nitrogen intermediates.[8]



(iv) Exchange rates of an amide hydrogen reflect the local environment of that group.[11,12]

(1) An undergraduate research participant from the Department of Chemistry, Kansas State University.
(2) (a) A. Hvidt and S. O. Nielsen, *Advan. Protein Chem.*, **21**, 287 (1966); (b) S. W. Englander in "Poly-α-Amino Acids," Vol. I of "Biological Macromolecules," G. D. Fasman, Ed., Marcel Dekker, New York, N. Y., 1967.
(3) T. F. Emery, *Biochemistry*, **8**, 877 (1969).
(4) D. O. Ulmer and J. H. R. Kägi, *ibid.*, 7, 2710 (1968).
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(7) E. F. Caldin, "Fast Reactions in Solution," Wiley, New York, N. Y., 1964.
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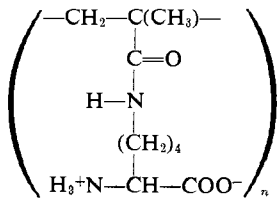
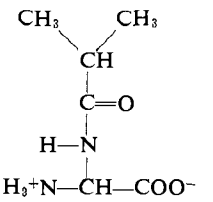
(9) S. O. Nielsen, *Biochim. Biophys. Acta*, 37, 146 (1960).
(10) I. M. Klotz and B. H. Frank, *J. Amer. Chem. Soc.*, 87, 2721 (1965).

Despite the fairly well-understood model behavior, a particularly significant gap related to (iii) needs to be clarified. Proteins are charged molecules, and the net charge varies with pH. Therefore, to be certain that H → D exchange is reflecting a conformational change in regions where the net charge is varying, the effect of the charge itself must be known. Furthermore, it would be valuable to know whether or not such charge effects can be satisfactorily estimated from electrostatic free energies calculated from the more readily obtainable titration data, as first suggested by Hvidt and Nielsen.[2a]

In an attempt to ascertain those effects, we initiated H → D exchange studies on a charged vinyl polymer—poly(ε-aminomethacrylyl-L-lysine)— and its monomeric analog—ε-aminoisobutyryl-L-lysine. We selected that polymer because it would behave as a protein toward pH changes but yet have all its amide groups in nearly identical environments. Thus, the kinetics were simplified and the desired effects more readily observed. Additional criteria for a successful model system required a carboxylate pK_a of 3 or less to give a region of essentially zero net charge over which exchange data could be gathered as the basis for the proper assessment of charge effects. The presence of charged groups at all pH values also virtually assured the solubility in aqueous solutions. Further, the continuous presence of charged groups was expected to minimize any possible conformational changes occurring upon the acquisition of a net charge. Finally, each amide group is separated from the charged α-amino acid moiety by five saturated carbon atoms and should surely be free from any inductive effects.



poly(ε-aminomethacrylyl-L-lysine)
(PMAL)



ε-aminoisobutyryl-L-lysine
(IBL)

Experimental Section

Materials. Isobutyryl chloride was an Eastman White Label product, MA grade L-lysine·HCl was purchased from Mann Research Laboratories, and methacrylyl chloride was obtained from Monomer-Polymer Corp. In addition, Fisher reagent grade isopropyl alcohol and Baker Analyzed cupric carbonate were employed. All those reagents, representing the highest quality available, were used without further purification. D_2O was purchased as 99.8 atom % D from either Bio-Rad Laboratories or from Merck Sharp and Dohme. DCl (37%) and NaOD (50%) were also obtained from Merck Sharp and Dohme.

Preparation. PMAL was prepared by the method of Morawetz and Sammak.[13] Their procedure, in which the monomer, ε-aminomethacrylyl-L-lysine (MAL), is formed by treating methacrylyl chloride with the copper complex of L-lysine, was modified only in that the product was purified by repeated crystallizations from water–isopropyl alcohol mixtures. Isopropyl alcohol was added to a nearly saturated solution of the monomer in water; when an alcohol-to-water ratio of 5:1 was reached, cloudiness appeared and needle-shaped crystals were produced overnight. The purified monomer was polymerized in water with azobisisobutyronitrile under a nitrogen atmosphere; the reaction mixture was maintained at 65° and stirred continuously. Viscosity studies showed the reaction to be complete after 5 days. Exhaustive dialysis of the polymer solution against distilled water was used to remove incompletely reacted material. Subsequent lyophilization (1%) yielded a white, fluffy product, readily soluble in water.

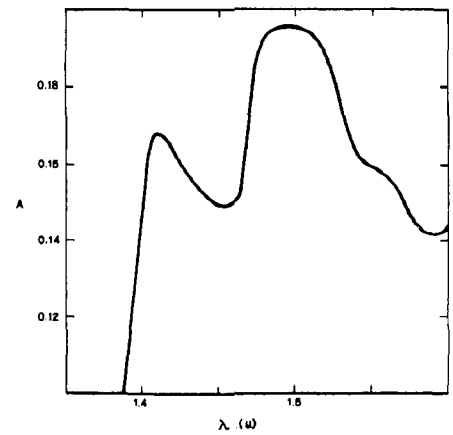


Figure 1. Near-infrared spectrum of 0.28 residue *M* poly(ε-aminomethacrylyl-L-lysine) in D_2O with less than 1% of the amide hydrogens exchanged; 2-cm cells; 25.0°.

The preparation and purification of IBL was analogous with that for the monomer, except isobutyryl chloride was substituted for methacrylyl chloride.

Characterization of the Polymer and Monomers. Potentiometric titrations under a nitrogen atmosphere were used to check purity of the preparations. Basic titrations of MAL and PMAL gave equivalent weights of 214.4 ± 1.4 and 220 ± 5 g, respectively (theoretical is 214.3 g). Similarly, IBL gave 220 + 5 g (theoretical is 216.3). The slight discrepancies in the last two cases could have resulted from incomplete drying. Ionization constants for the carboxylate groups were determined at the same concentrations as for H → D exchange.

Chemical homogeneity of the polymer preparation was established using a free-boundary electrophoresis technique. Migrations of 1% PMAL solutions at 1° in pH 9.5 carbonate or pH 7.5 phosphate buffers were followed with Schlieren optics under a current of 20 mA. The ascending peak of the pH 9.5 solution moved relatively rapidly and maintained a single nearly symmetrical band over the 40-min run time. Migration of the pH 7.5 solution was much slower, as would be predicted, but it also showed a virtually symmetrical single band over a 3-hr run. The slight asymmetry on the cathode side of the pH 7.5 peak might be due to a molecular weight distribution skewed toward large values.

Infrared spectra of IBL in KBr were taken on a Perkin-Elmer Model 137 instrument. Based on Stadtler spectral data, prominent bands that could not be assigned to the lysine moiety were found at 3370, 3270, 1650, 1545, 1360, and 1250 cm^{-1}. The first two bands, which form a doublet, could be assigned[14,15] to the associated amide N–H stretching modes. The 1650- and 1545-cm^{-1} bands correspond to amide I and II modes, respectively. Although assignments were less certain for the last two bands, the 1250-cm^{-1} absorption also could have been associated with the amide group, in that it falls within the range of the amide III band. Furthermore, the 1360-cm^{-1} band which did not appear in the spectrum of either lysine or isobutyramide appeared to be associated with N–CH_2; *N*-benzylisobutyramide showed an absorption at 1382 cm^{-1}.

A near-infrared spectrum of PMAL in D_2O at an early time is shown in Figure 1. The band at 1.407 μ (7107 cm^{-1}) was caused by the HOD produced from the exchange of α-amino groups. The band near 1.50 μ resulting from amide >N–H was much broader and shifted from that of simple amides. Its intensity also indicated extensive solvation of the amide moiety. HOD, however, also had an extremely broad band in the same region.[16] Therefore, to ascertain the true shape of the >N–H stretching overtone

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(16) J. D. Worley and I. M. Klotz, *J. Chem. Phys.*, **45**, 2868 (1966).

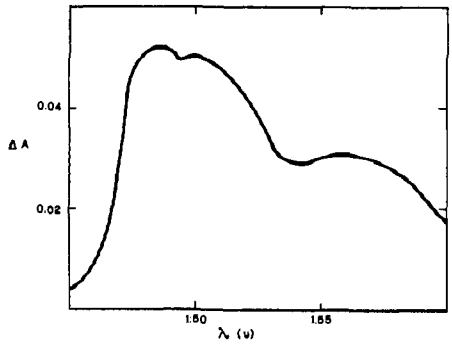


Figure 2. Near-infrared difference spectrum between 0.28 residue M poly(ε-aminomethacrylyl-L-lysine) and HOD in D_2O; 25.0°.

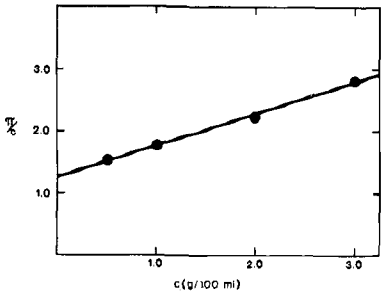


Figure 3. Osmotic pressure of poly(ε-aminomethacrylyl-L-lysine) solutions; 25° (π in centimeters of water).

region, the spectrum of HOD, at the concentration given by the absorbance at 1.410 μ, was subtracted from that of PMAL. The resulting difference spectrum is shown in Figure 2. Surprisingly, the true band showed an even greater broadness in the >N–H region.

One way in which >N–H stretching modes can be broadened and shifted is through formation of hydrogen bonds.[17] Spectral analysis of hydrogen bonding of amide monomers in the near-infrared region[18] has shown that >N–H not bonded to >C=O produces a relatively sharp band with a maximum near 1.47 μ (6710 cm^{-1}). Under conditions where >N–H···O=C< bonding does occur, additional bands are seen at 1.53 and 1.57 μ (6536 and 6370 cm^{-1}).

Analysis of the difference spectrum (Figure 2) indicated that bands at 1.47, 1.53, and 1.57 μ could account for the observed broadness. Those bands, however, were not so clearly defined as were those of poly(*N*-isopropylacrylamide).[11] Possibly this polymer has a variety of hydrogen bonds with considerably different energies.

Viscosity studies were made using Cannon-Fenske type Ostwald viscometers (ASTM size no. 25 and 50) in pure water at several pH values. In general, it was not possible to reliably extrapolate the pure-water results to infinite dilution, because of the extreme nonlinearity of η_{red} *vs.* c plots. Comparisons, however, at a concentration of 3.2×10^{-3} g/ml of PMAL showed $\eta_{red} = 16.6$ at pH 5.20 and 30.3 at pH 3.00 in pure water.

Molecular weight was determined with a Hellfritz Model G1 static osmometer using a Schleicher and Schuell No. O6 membrane filter. Measurements in 0.1 M NaCl at pH 6.0 and 25.00 ± 0.01° gave a number-average molecular weight of 199,000 after correcting for density and extrapolating to zero concentration, as illustrated in Figure 3. Densities were determined with a Weld pycnometer at 25.00 ± 0.01°. These measurements also gave a partial specific volume of 0.74 cm^3/g.

A check for possible hydrolysis of the polymer at low pH was made by letting a 10% solution stand at pH 1.5. There was no detectable change in pH during a 16-hr period.

Rate Measurements. H → D exchange rates were obtained using the near-infrared region of a Cary 14R spectrophotometer as

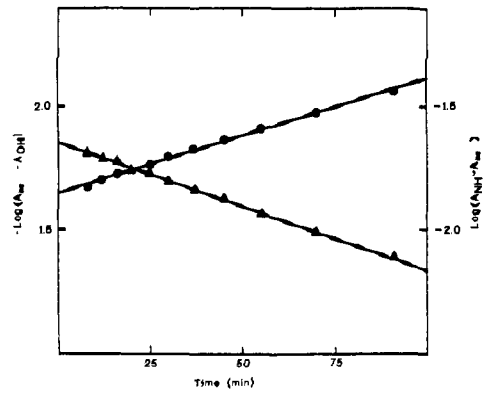
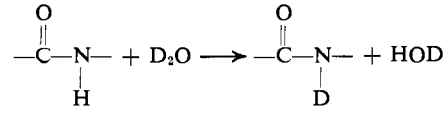


Figure 4. First-order plots of –OH absorbance increase (left ordinate) and >N–H decrease (right ordinate) *vs.* time for poly(ε-aminomethacrylyl-L-lysine) at pD 4.41; 25.0°.

reported previously.[11] Briefly, the sample is dissolved in D_2O and the changes in absorbance with time are followed at 1.41 and 1.50 μ. In general, 0.28 residue M and 0.16 M solutions in 2-cm cells were used for PMAL and IBL, respectively. After the complete exchange reading was taken, the pH was measured with a Beckman Century SS pH meter equipped with a single electrode. Temperatures were controlled by passing water from a Haake Model FE constant-temperature circulating bath through the cell compartment. Temperatures were monitored with a Yellow Springs Instrument Co. Model 42 Tele-thermometer employing a thermistor probe.

Results

Representing the exchange process as



the reaction rate will become pseudo first order in the forward direction for low concentrations of polymer in 99.8% D_2O. Therefore, the rate expression may be formulated as

$$-d(NH)/dt = d(OH)/dt = k(NH) \qquad (1)$$

Accordingly, rate constants were calculated from plots of $\log (A_\infty - A_{OH})$ or $\log (A_{NH} - A_\infty)$ *vs.* time, as shown in Figure 4. The infinity reading, A_∞, was taken after at least eight half-life times. Data from each run were subjected to least-squares analysis. The constants calculated in the two regions almost always agreed to within 15%, generally within 10%, as indicated in Table I. Rates calculated from the 1.53-μ absorbance

Table I. Rates for PMAL Calculated from HOD Production and N–H Diminution at 25°

	λ, μ		
	1.410	1.500	1.530
pD	$k \times 10^3$, min^{-1}		
4.19	16.5	17.1	15.9
5.43	3.87	4.22	3.45
6.68	17.4	16.7	19.0

are generally less accurate because of the smaller net extinction in that region. Therefore, average values of the rates determined at 1.41 and 1.50 μ were used in all subsequent calculations.

(17) G. C. Pimentel and A. L. McClelland, "The Hydrogen Bond," W. H. Freeman, San Francisco, Calif., 1960.

(18) I. M. Klotz and J. S. Franzen, *J. Amer. Chem., Soc.*, **84**, 3461 (1962).

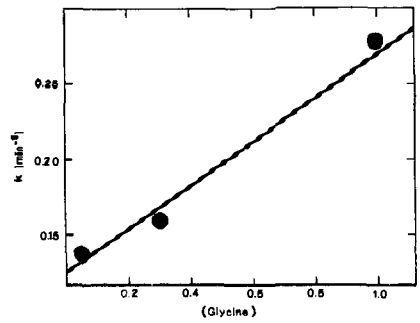


Figure 5. Observed rate constants *vs.* concentration of glycine for 0.16 *M* ε-aminoisobutyryl-L-lysine in 0.01 *M* acetate at pD 5.28; 25.0°.

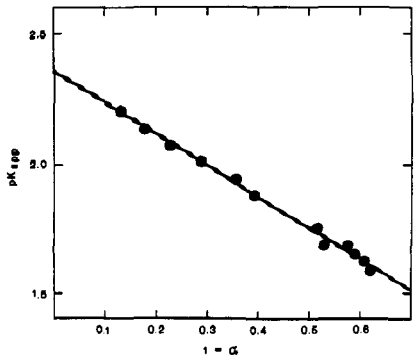


Figure 6. Acid titration data for poly(ε-aminomethacrylyl-L-lysine) in H_2O.

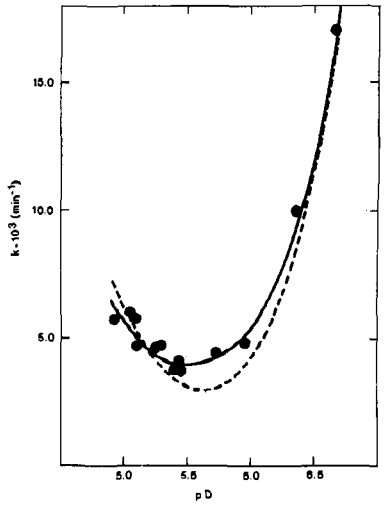


Figure 7. Rate–pD profile for hydrogen–deuterium exchange of poly(ε-aminomethacrylyl-L-lysine) in D_2O; 25.0°. Points are experimental.

Exchange rates of the polymer did not show a significant concentration dependence. For example, PMAL at a concentration of 0.40 residue *M* gave a *k* of 4.68×10^{-3} min^{-1} at pD 5.303, compared with a calculated value of 4.30×10^{-3} min^{-1} for 0.28 residue *M*. Similarly, IBL at pD 5.50 gave $k = 0.138$ and 0.142 min^{-1} for concentrations of 0.16 and 0.068 *M*, respectively. Solubility limitations (approximately 0.23 *M* at 25°) unfortunately prevented a satisfactory demonstration of concentration dependence with the monomer. To decide whether some dependence of exchange rate on concentration could result from catalysts by the α-amino acid group, glycine was added to the reaction mixture. The results are shown in Figure 5. The slope gave a catalytic constant of 0.14 M^{-1} min^{-1}.

Before the H → D exchange data on the polymer could be properly presented, it was necessary to know the charge *vs.* pH relationship. Traditionally, charge effects in the ionization of polyacids have been evaluated by eq 2 for pK_{app}, the apparent dissociation constant[19]

$$pK_{app} = pH - \log(\alpha/1 - \alpha) = pK_0 + 0.43\Delta G_{el} \quad (2)$$

where ΔG_{el} is the electrostatic free energy and a function of α, pK_0 is the intrinsic ionization constant, and α is the degree of dissociation of the acidic group. In general, this equation has been shown to be valid for many polyacids and bases over the range $0.1 \leqq \alpha \leqq 0.9$. Alternately, assuming ΔG_{el} to be proportional to α, eq 2 may be written for our application as

$$pK_{app} = pK_0 - w'(1 - \alpha) \quad (3)$$

or

$$pK_{app} = pK_0 - wZ \quad (4)$$

where w' and w are electrostatic interaction factors and the net charge $Z = 930(1 - \alpha)$. Figure 6 shows a plot of pK_{app} *vs.* $1 - \alpha$ for PMAL as obtained from acid titration data. From the intercept at $1 - \alpha = 0$ of this and other plots an average of 2.42 ± 0.06 was obtained for pK_0. This is virtually identical with the 2.34 obtained for MAL. In addition, the slope corresponded to a w of 0.0013, which agreed reasonably well with values for other polyacids and bases.[20,21] Similar data in D_2O gave a pK_0 of 2.72 and a w of 0.0010. The acid titration data in D_2O were also used to prepare a plot of pD *vs.* $1 - \alpha$, from which Z could be calculated for each of the H → D exchange experiments. For example, at pD 4.98 and 3.64, $1 - \alpha$ was 0.01 and 0.1, respectively.

Turning to H → D exchange behavior of PMAL, Figure 7 shows the variation of the observed rate constants over a pD range of 4.9–6.7. The parabolic rate *vs.* pD profile, similar to that found for other monomeric and polymeric amides, was characteristic of specific acid and base catalysis. Accordingly, the dashed line in Figure 7 was computed from a nonlinear least-squares[22] fit to the equation

$$k = k_D(D^+) + k_{OD}(OD^-)$$

expressed as

$$k = k_D(D^+) + k_{OD}K_{D_2O}/(D^+) \quad (5)$$

where k_L and k_{OCL} are the specific acid and base catalytic constants, respectively, and K_{D_2O} is the autoionization constant of D_2O (1.35×10^{-15} at 25°).[23] With this equation the agreement was poor, as shown by a standard deviation of $\pm 9.27 \times 10^{-4}$ min^{-1}, about twice the expected 10% experimental error. To the contrary, the solid line in Figure 7 was computed with the same

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(20) A. M. Kotlair and H. Morawetz, *J. Amer. Chem. Soc.*, 77, 3692 (1955).
(21) A. Katchalsky, K. Rosenheck, and B. Altman, *J. Polym. Sci.*, 23, 955 (1957).
(22) D. D. Mueller, Ph.D. Dissertation, The University of Oklahoma, 1966.
(23) R. G. Bates, R. A. Robinson, and A. K. Covington, *J. Phys. Chem.*, 70, 3820 (1966).

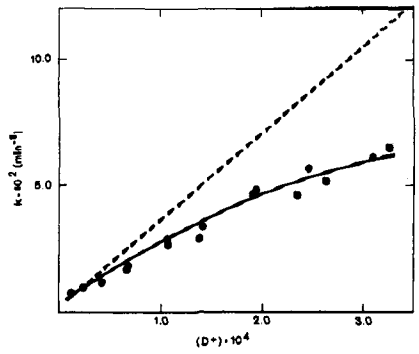

Figure 8. Observed rate constants *vs.* concentraton of deuterium ion for poly(ε-aminomethacrylyl-L-lysine) in D_2O; 25.0°. Points are experimental.

program but using the expression

$$k = k_0 + k_D(D^+) + k_{OD}K_{D_2O}/(D^+) \quad (6)$$

where k_0 reflects any direct exchange processes occurring in the reaction. This equation fitted to a standard deviation of $\pm 4.44 \times 10^{-4}$ min^{-1}, which was near the expected experimental error (Table I). Calculations on the acid side were limited to pD 4.9[$(1 - \alpha) \cong 0.01$] to minimize any charge effects. The calculated constants for both the monomer and polymer are given in Table II. Exchange data on the monomer,

Table II. Hydrogen–Deuterium Exchange Parameters at 25°

Compound	IBL	PMAL
k_0, min^{-1}		$(2.0 \pm 0.1) \times 10^{-3}$
k_D, M^{-1} min^{-1}	17,200 ± 700	343 ± 21
$k_{OD}k_{D_2O}$, M min^{-1}	$(3.27 \pm 0.14) \times 10^{-7}$	$(3.16 \pm 0.08) \times 10^{-9}$
pD_{min}	5.36 ± 0.03	5.52 ± 0.03
k_{min}, min^{-1}	0.15	4.12×10^{-3}

IBL, which showed a similar rate–pD profile, were adequately fitted, using eq 5. Based on the catalytic constant for glycine a k_0 term of 0.02 min^{-1} would be predicted. Unfortunately, that was only slightly beyond the experimental error and could not be detected.

From the catalytic constants, the pD at which the minimum exchange rate occurred (pD_{min}) could be calculated, using the relationship of eq 7,[11] provided that k_0 is independent of deuterium ion concentration (see Discussion section). As shown in Table II the calcu-

$$(D^+_{min})^2 = k_{OD}K_{D_2O}/k_D \quad (7)$$

lated pD_{min} for the polymer appeared slightly higher than that of the monomer within the 1σ errors given.

Figure 8 shows the extension of the exchange data into the acid region of the profile. Clearly, the rates did not coincide with those predicted from the fit between pD 4.9 and 6.7 (dashed line). Further, the discrepancy increased with increasing deuterium ion concentration.

Slowing the exchange in pH regions where the polymer carries a net positive charge would be expected if the reaction involved a protonated intermediate, as has been proposed for acid catalysis. Also, if all the reduction was due to electrostatic effects, presumably the discrepancy could be accounted for by a relationship analogous to eq 2. Consequently, the solid line in Figure 8 was calculated from the expression

$$k = k_0 + k_DO^{-wz}(D^+) + k_{OD}10^{wz}K_{D_2O}/(D^+) \quad (8)$$

where w is treated as an adjustable parameter and k_0, k_D, and $k_{OD}K_{D_2O}$ are maintained as calculated above pD 4.9. The value of w giving the best agreement between observed and calculated rates was $(2.4 \pm 0.5) \cdot 10^{-3}$. Thus, eq 8 satisfactorily accounted for the charge effects, but w was approximately twice that obtained from titration data.

Activation energies determined from 16, 20, and 25° data at pD 4.8 for PMAL gave an E_a^* of 19 kcal/mol. Similarly, from data for IBL at 5, 10, 15, and 25° and pD 4.4, an E_a^* of 16 kcal/mol was found. With the polymer, the difference between O–H and N–H rates at the lower temperatures was such to indicate a possible error of ±3kcal/mol.

Discussion

(A) Internal Catalysis. The necessity for a k_0 term in the fit of PMAL exchange data is unusual. No previous quantitative studies on hydrogen exchange of model compounds have revealed a significant direct-exchange process in pure aqueous solution.[9,10,12] In our study, the k_0 term seemed to reflect a direct exchange (catalysis) by the α-amino group of the lysine moiety. Figure 7 shows that near pD_{min} the spontaneous rate represents approximately half the observed rate constant, but at higher or lower pD values k_0 contributes a smaller portion to the total rate constant. This is precisely what would be expected from catalysis by the α-amino acid group, because in the pD range of 5–7 the α-amino group is fully protonated while the carboxylate anion undergoes little if any protonation ($pK_0 = 2.72$). Furthermore, amino groups are known to exchange instantaneously by the technique employed and are in the $>ND_3^+$ form throughout the experiment.

It also seems apparent that most if not all of the catalysis resulted from $>ND_3^+$ rather than from the carboxylate group. For example, Klotz and Frank[10] have shown that acetate ion had no effect on the exchange of *N*-methylacetamide in D_2O, but that several amines proved to be effective catalysts. The lack of any notable concentration dependence on the observed rates for the polymer further indicated that most of this catalysis was internal and not a result of polymer–polymer interactions. This observation is consistent with that of Morawetz and Song,[24] who found that catalysis of solvation of nitrophenyl side-chain esters in a copolymer with histamine occurred internally as well.

It is of interest to compare internal catalysis in a polymer with that between monomeric compounds. If a uniform density of residues in the polymer is assumed, an effective concentration of 6.4 residue M can be calculated. That concentration of amino groups would imply an internal catalytic constant for the protonated form of 3.4×10^{-4} M^{-1} min^{-1}. Taking the rate-enhancement data for glycine ($k_{cat} = 0.14$ M^{-1} min^{-1}) as representative of the α-amino group, it can be seen that its catalytic effect was greatly reduced in the polymeric environment. At least part of the reduction might have been caused by hindrance of the approach of $>ND_3^+$ to the amide group. Molecular models have shown that it is possible for the α-amino group to approach the amide hydrogen of the same residue as well

(24) H. Morawetz and W. R. Song, *J. Amer. Chem. Soc.*, **88**, 5714 (1966).

as adjacent residues only with considerable strain. Across-the-chain catalysis, or catalysis by widely separated residues, therefore, might be more likely. A more nearly complete answer to these possibilities, however, will have to await further experimentation.

(B) Effect of Charge. In Figure 8 it was shown that the acid-catalyzed portion of the exchange profile did not remain linear above 5×10^{-5} (D^+), as would be predicted from eq 6. Indeed, the deviation became greater as the net positive charge on the polymer increased. To appreciate the magnitude of those charge effects in pure D_2O, consider that k already had been reduced by a factor of 2 at pD 3.5, where $1 - \alpha$ was only 0.12.

According to the accepted mechanism, the acid-catalyzed step of amide hydrogen exchange involves a protonated intermediate. Thus, under conditions where the polymer carries a net positive charge, it would be reasonable to assume that electrostatic interactions would raise the energy of the transition state. In addition, the effective concentration of deuterium ions in the vicinity of the polyelectrolyte would be lower because of a preference for anionic counterions. Both effects correlate well with the observed behavior.

Furthermore, it was shown that the charge effect could be accounted for by eq 8. Using this expression it can be shown that

$$(D^+)_{min}^2 = (k_{OD}K_{D_2O}/k_D)10^{2wZ}$$

Thus, a net charge Z produces a shift in pD_{min} of

$$\Delta pD_{min} = -wZ$$

Another relationship derivable from eq 8 is

$$k_{min} = k_0 + 2(k_D k_{OD} K_{D_2O})^{1/2}$$

which shows that k_{min} is unaffected by a net charge. It is convenient, therefore, to view rate reductions in the acid-catalyzed region, which result from a net positive charge, as a decrease in pD_{min} without a change in k_{min}.[25]

More quantitatively, the value of w necessary to account for the exchange data was about twice that found from titration studies. This might mean that ΔG_{el} was no longer proportional to $1 - \alpha$ below 0.1, or that the ionization constants for the carboxylate anion and amide group responded differently toward a net positive charge. The second possibility can be readily understood on the basis of the Brønsted relation $k = bK^{-\beta}$ ($0 < \beta < 1$), where K is the acidity constant and k the rate constant for the protonation of the group. Thus, different values of w could result from differences in β, or b, the Brønsted constants for the two groups.

(C) Slow Exchange in the Polymer. The data in Table II show that exchange in the polymer, when corrected for k_0, was slower by a factor of approximately 70 than that of the monomer. Slowness occurred, despite residues being extensively solvated, as indicated by the nearly identical pK_a for the monomer and polymer and the near-infrared spectrum (Figure 2). Furthermore, viscosity studies indicated considerable expansion of PMAL over the pH range of 5–3. Interestingly, the rates remained slow in spite of such swelling. Apparently, compactness is not an important factor in slowing exchange of vinyl polymers in good solvents. The residues seemingly must also be in nearly identical environments, because exchange rates are strictly first order.

Additional evidence to support the last point can be gleaned from percentage-exchange calculations. Bigeleisen[27] has reported that simple primary amides and ammonia have fractionation factors very close to unity in D_2O. Assuming similar ratios for secondary amides and amines in polymers, exchanges close to 100% can be anticipated. Based on an extinction coefficient of 53.5 cm²/mol for HOD in D_2O at 1.410 μ and 25°, exchanges of 90–100% were found near pD_{min} after correcting for the water remaining in the lyophilized sample.[28] Such percentage of exchange clearly indicated that most, if not all, the amide groups behaved identically toward exchange.

Rapid conformational changes along the vinyl backbone possibly could place all the side chains in similar environments on the time scale of hydrogen exchange. In other words, there is not an inside or outside of the polymer molecule of sufficient duration to be reflected in exchange rates. Recent evidence to support that contention can be derived from the nmr studies of Morawetz and coworkers,[29] who found that the potential barriers to rotation about $-CH_2-C(=O)-$ carbon–carbon bonds were not affected by polymerization.

The position of pD_{min} also gives some information on slowness of exchange in the polymeric state. For example, eq 7 shows that any change in the ratio k_{OD}/k_D or in K_{D_2O} would be reflected in pD_{min}. Because pD_{min} is apparently significantly higher for PMAL than for IBL, it can be inferred that either k_{DO}/k_D or K_{D_2O} is decreased in the polymeric environment. For the catalytic constants to change, the basicity of the amide nitrogen must change—for example, through inductive effects.[30] In the polymer studied here, however, the amide groups were separated by three saturated carbon atoms, which should have minimized any inductive effects. Besides, inductive effects between amide groups would be expected to lower, not raise, pD_{min}. Therefore, the basicity of the amide group probably was not altered by polymerization, and the ratio k_{OD}/k_D presumably was the same as in the monomer.

Data in Table II show that k_D for PMAL was $^1/_{50}$th that for IBL, but that the product $k_{OD}K_{D_2O}$ for the polymer was $^1/_{100}$th that for the monomer. Thus, for k_{OD}/k_D to remain constant, K_{D_2O} would have to differ by a factor of 2 between the two systems. A reduction in K_{D_2O} no doubt resulted from the more apolar nature of the polymeric environment in that K_W is lowered by about 10^3 in 50:50 (w/w) mixtures of dioxane and water.[31]

Equation 9 gives the relationship between k_{min} and the catalytic constants. From this expression, it can be seen that a decrease in K_{D_2O} by a factor of 2 does not

(25) It is possible to arrive at a similar conclusion using Brønsted relations, as was shown in an earlier paper.[26]

(26) I. M. Klotz and D. D. Mueller, *Biochemistry*, 8, 12 (1969).

(27) J. Bigeleisen, *Science*, 147, 463 (1963).

(28) It is possible to estimate the water of hydration from the HOD absorbance at zero time. Generally, 10% of the sample was water.

(29) H. Morawetz, *Accounts Chem. Res.*, 3, 354 (1970).

(30) B. H. Leichtling and I. M. Klotz, *Biochemistry*, 5, 4026 (1966).

(31) H. S. Harned and B. B. Owen, "Physical Chemistry of Electrolyte Solutions," 3rd ed, Reinhold, New York, N. Y. 1958.

account for all the lowering of exchange rates. Consequently, activation energies for the monomer and polymer were determined and found to be 16 and 19 kcal/mol, respectively. If this difference of 3 kcal/mol is real, it is more than enough to account for the remaining reductions.

One possibility for an increase in activation energy would be a substantial decrease in effective dielectric constant in the vicinity of the polymeric residues. Model compound studies in 50:50 dioxane–D_2O (dielectric constant of 40), however, have shown only a threefold decrease in the true first-order rate of pD_{min} over that for pure deuterium oxide.[30] Thus, slow exchange in this charged polymer apparently results mostly from steric inhibition of the individual amide groups along the polymer backbone.

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Vibrational Spectra of Liquid Crystals. III. Raman Spectra of Crystal, Cholesteric, and Isotropic Cholesterol Esters, 2800–3100-cm^{-1} Region

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Abstract: The Raman spectra of a series of cholesteric liquid crystals have been recorded in an attempt to elucidate the nature of intermolecular interactions in these phases. While most Raman bands are unaffected by the crystal-liquid crystal and liquid crystal-isotropic liquid phase transitions, certain C–H stretching bands change both frequency and intensity. These bands are assigned using arguments based on model steroids. It is shown that the results are indicative of regions of local order in the cholesteric phase which are similar to those in the crystal.

The nature of the ordering forces in liquid crystals is of current interest. Several techniques, including vibrational spectroscopy, have been employed as a probe of these forces. In this paper we discuss intermolecular effects on the vibrations of cholesteric liquid crystals. Despite several recent reports of vibrational spectra of liquid crystals[1–6] only one spectrum of a cholesteric phase has appeared, that being a brief discussion of the infrared spectrum of cholesteryl propionate.[6]

In previous papers, we have discussed both internal[1] and external[5] modes of vibration of nematic liquid crystals, showing that detailed information about the nematic state and the crystal–nematic transition can be obtained from the spectra. In an attempt to better understand the intermolecular interactions in cholesteric mesophases, the Raman spectra of a number of cholesterol esters have been recorded in crystal, cholesteric, isotropic, and solution phases. The implications of the only significant changes—those occurring in the C–H stretching region—are discussed herein.

The results shed new light on the cholesteric phase by viewing it in terms of the intermolecular interactions of the C–H bonds. Further, the spectra are unique among those recorded for liquid crystals to date in that they show significant differences between liquid-crystal and isotropic phases. In all the previous work, the spectra of these two phases were identical.

Experimental Section

All the cholesterol derivatives used in this study except cholesterol and cholesteryl laurate were purchased from the Vari-Light Corp., Cincinnati, Ohio. The latter two were supplied by Eastman Kodak. Pregnenolone and 19-norprogesterone were National Biochemical Corp., Cleveland, Ohio, products. Ethylestrenol and 19-nortestosterone were obtained from Hoffmann-La Roche. All the chemicals were used without further purification. Reagent grade carbon tetrachloride was used for solution spectra of cholesterol and its derivatives.

The Raman spectra were recorded using a Spex Model 1401 double spectrometer, equipped for photon counting. A Spectra-Physics Model 125 He–Ne laser (632.8 nm) delivered 60 mW at the sample. Spectra were scanned at 5 cm^{-1}/min, with a spectral slit width of approximately 7 cm^{-1}. Samples were illuminated in 1-mm capillaries using the transverse–transverse configuration. The capillary was kept in a heating block which has been described elsewhere.[5] The sequence in which the spectra were recorded was crystal, isotropic liquid, and liquid crystal.

The spectrum of each compound in each phase was run at least three times and the $\Delta\nu$ values of the Raman bands were determined as the average of these measurements. The spectra were reproducible and the uncertainty in the $\Delta\nu$ measurements was estimated as ± 2 cm^{-1}. Although the signal-to-noise ratio was rather poor, all the intensity changes discussed in this paper are for spectra which showed these changes in a highly reproducible fashion. All the compounds whose spectra were studied here have a very large number C–H bonds, and therefore very many C–H stretching bands are expected to appear in the spectra. This consideration precludes the resolution of the observed spectral profile in the C–H

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