It is of interest that iodide and perchlorate ions (but not phosphate or sulfate) bind firmly to cyclodextrin, which contains a number of weak hydroxyl group dipoles; the dissociation constant for the cyclodextrin–perchlorate complex is $3.4 \times 10^{-2}$ $M$.[29] It is known that salts bind to polypeptides and amides in the solid state.[6,30] Such binding provides no direct information regarding the occurrence of binding in solution, but it does indicate that amide–ion interactions are of sufficient strength to compete effectively with ion–ion interactions. Finally, it has been shown that dimethylformamide forms a complex with $Al^{3+}$ in an aqueous–organic solvent with a lifetime sufficient to give rise to a distinct nuclear magnetic resonance signal.[31]

(29) F. Cramer, W. Saenger, and H.-Ch. Spatz, *J. Am. Chem. Soc.*, **89**, 14 (1967); H. Schlenk and D. M. Sand, *ibid.*, **83**, 2312 (1961).

(30) J. Bello, D. Haas, and H. R. Bello, *Biochemistry*, **5**, 2539 (1966), and references therein.

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# Hydrogen–Deuterium Exchange in Lactams[1a]

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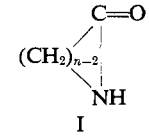
**Abstract:** The kinetics of hydrogen–deuterium exchange have been measured for three *cis* lactams (butyrolactam, valerolactam, and caprolactam), the *cis–trans* lactam (caprylactam), and a *trans* amide (N-methylacetamide) in deuterium oxide solution. Exchange rates and activation energies (12–15 kcal/mole) are comparable for both the *cis* and *trans* configurations. Furthermore, activation energies are of a similar magnitude as those for other model peptides, polypeptides, and proteins.

Recent studies on the hydrogen–deuterium exchange reaction of model peptides and polypeptides[2–9] have attempted to elucidate some of the various factors which affect the observed exchange rates. Even for the simple amide, N-methylacetamide (NMA), the exchange is not instantaneous and depends on the acidity and polarity of the solvent system.[2–4] The acid activation energy in pure $D_2O$ is of the same magnitude as those of other model peptides, polypeptides, and proteins either in the helical or the disordered form.[7,9] Studies with small peptides[6] and polypeptides[7,9] further showed that the parameters for the exchange reaction reflected the inductive effect on the amide group and the local environmental and conformational factors.

A *cis* lactam, butyrolactam, was studied by Klotz and Feidelseit.[5] They found its exchange rate and activation energy to be much higher than that of the *trans* amide, NMA. However, our spectral studies on a series of lactams[10] showed that this lactam is not a good model for the *cis* configuration due to its ring strain. It is therefore interesting to study other *cis* lactams. Such studies will show more conclusively if the rates of exchange for the *cis* and *trans* forms of the amide group are significantly different.

The configuration of the peptide group in the lactams (I) depends on the ring size ($n$).[11,12] The *cis* configuration is the only form present for $n \leq 8$ and the *trans* configuration is the predominant form for $n > 9$. The $n = 9$ lactam is present in both the *cis* and *trans* configurations. The parameters for the exchange reaction of NMA and lactams with ring sizes of 5, 6, 7, and 9 in $D_2O$ as a function of acidity and temperature are reported here.




I

## Experimental Section

**Materials.** NMA of Eastman grade was further purified by vacuum distillation at 59–62° and 1 mm. Butyrolactam ($n = 5$) and valerolactam ($n = 6$) from K and K Laboratories were purified by vacuum distillation at $\lesssim$1 mm, 86–90° and 95–96°, respectively, with precautions to avoid moisture contamination. Caprolactam ($n = 7$) was obtained from Matheson Coleman and Bell and was recrystallized from benzene to give colorless crystals, mp 69–70°. Caprylactam, mp 72–73°, was synthesized in this laboratory.[10]

$D_2O$ of 99.8% purity was purchased from Volk Radiochemical Co. and International Chemical and Nuclear Corp. A solution of 38% DCl in $D_2O$ (99% deuterium) was obtained from Stohler Isotope Chemicals. The anhydrous sodium acetate was Baker analyzed reagent.

**Preparation of Reaction Mixtures.** Buffers of the desired pH were obtained by the addition of 1 $M$ DCl to a solution of 0.02 $M$ sodium acetate in $D_2O$. In order to minimize the time required for solu-

(1) (a) This research was supported by two grants from the Division of Molecular Biology, National Science Foundation, GB-4835 and GB-2049. (b) This paper includes portions of the thesis of C. Y. S. Chen, submitted in partial fulfillment of the requirements for the Ph.D. degree at the University of Iowa, Aug 1968. (c) To whom correspondence should be directed.

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(11) R. Huisgen, H. Brade, H. Walz, and I. Glogger, *Chem. Ber.*, **90**, 1437 (1957).

(12) R. Huisgen and H. Walz, *ibid.*, **89**, 2616 (1956).

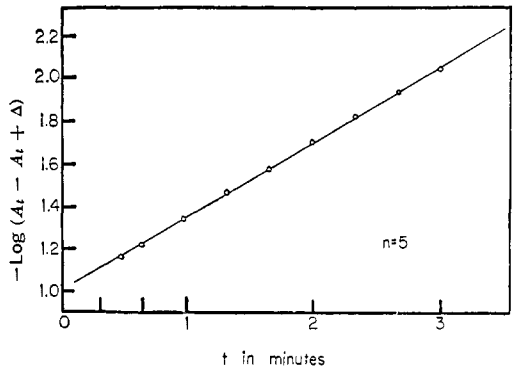


Figure 1. A typical $-\log (A_t - A_{t+\Delta})$ *vs.* $t$ plot (for the $n = 5$ lactam, at pD 4.42 and 6.1 ± 0.4°).

tion, NMA and the lactams, $n = 6$, 7, and 9, were dissolved in small amounts of water to make concentrated stock solutions of 12.5, 9.8, and 7.5, and 4.0 $M$, respectively. These solutions and the pure liquid in the case of the $n = 5$ lactam were then mixed with the buffered $D_2O$ solutions for the kinetic studies.

**pH Measurements.** All pH values were measured with a Radiometer Model 25 pH meter which used a Radiometer Type G202B glass electrode and a Type K-401 reference electrode at 25°. Fisher or Sargent pH 4.01 buffer solution was used to standardize the meter. The meter readings were converted to pD values according to the equation of Glasoe and Long.[13]

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

The addition of sample solution usually caused a slight shift in the pD of each buffer solution, and the magnitude of the shift varied with the pD of the buffer used. Therefore, the pD values of the solutions measured after the exchange reaction were used in the rate *vs.* pD plot and the calculations.

**Temperature Control.** Buffer solution (2.5 ml) in a 1-cm quartz cell in a jacketed cell holder was equilibrated at the desired temperature. During the time course of a typical run, the temperature fluctuation was less than ±1.0°. The actual temperature of the reaction medium was measured to the nearest tenth of a degree with a thermistor sealed in the polyethylene cap of the cell, at 0.5- to 1-min intervals for each run.

**Optical Measurements.** A Cary Model 14R recording spectrophotometer, which was flushed with dry nitrogen, was used for all the measurements. Rates of exchange were followed by the decrease in the absorption of the N–H group (with the 0–0.1 absorbance unit slide wire). The wavelengths for observation were 1.49, 1.50, 1.495, 1.505, and 1.485 $\mu$ for the $n = 5$, 6, 7, and 9 lactams and NMA, respectively. Pure $D_2O$ in a 1-cm cell was used in the reference beam for background compensation.

The stock solution (0.20 ml of the $n = 5$ and 6 lactams and 0.25 ml of NMA and the $n = 7$ and 9 lactams) was added to the buffer solution (2.5 ml in 1-cm cell) at the desired temperature with a 0.25-ml Hamilton syringe (for the $n = 5$, 6, and 7 lactams and NMA) or a 1-ml syringe (for the $n = 9$ lactam). The reaction mixture was kept in the cell holder while mixing thoroughly with a small polyethylene stirrer. In a typical run, the recording was started 15–20 sec after the addition of the sample and was continued until apparent equilibrium was reached. The total change in absorbance was 0.03–0.1.

The $n = 9$ lactam has a surprisingly lower solubility in $D_2O$ than in water. The reaction medium was slightly cloudy for pD >5.4; therefore, a larger error in the determined rate constants is expected.

## Results

**Calculations of Observed Rate Constants.** The exchange reaction can be represented as

$$\underset{\underset{\text{O}}{\|}}{\text{—C—NH—}} + \text{D}_2\text{O} \xrightarrow{k'} \underset{\underset{\text{O}}{\|}}{\text{—C—ND—}} + \text{HOD} \qquad (1)$$

Under the experimental conditions, the exchange reaction can be regarded as pseudo first order, since in all cases $D_2O$ was present in large excess to the amide or lactam (greater than 50:1). The total amount of water present was in most cases negligible except in the reaction medium for the $n = 9$ lactam, where about 2 moles/l. was present compared to 50 moles/l. of $D_2O$. Therefore, the rate expression for (1) is

$$-\frac{\text{d[CONH]}}{\text{d}t} = \frac{\text{d[HOD]}}{\text{d}t} = k'[\text{CONH}] \qquad (2)$$

where $t$ is the time in minutes, [CONH] represents the concentration of the amide or lactam, [HOD] is the concentration of HOD, and $k'$ is the pseudo-first-order rate constant.

Absorbance data were used to compute rate constants by applying the procedure of Guggenheim.[14] Here

$$A_t = A_0 e^{-k't} \quad A_{t+\Delta} = A_0 e^{-k'(t+\Delta)} \qquad (3)$$

$$\ln (A_t - A_{t+\Delta}) = -k't + \ln A_0(1 - e^{-k'\Delta}) = -k't + C \qquad (4)$$

where $A_t$ is the absorbance at time $t$, $A_{t+\Delta}$ is the absorbance at time $t + \Delta$, $\Delta$ is a constant interval of greater than two to three half-lives, $k'$ is the observed rate constant, and $C$ is a constant. A plot of $-\log (A_t - A_{t+\Delta})$ *vs.* $t$ has a slope of $k'/2.303$.

A typical $-\log (A_t - A_{t+\Delta})$ *vs.* $t$ plot is shown in Figure 1. The experimental scatter was generally small so that the slope could easily be determined. A typical plot of $k'$ *vs.* pD is shown for the $n = 7$ lactam in Figure 2.

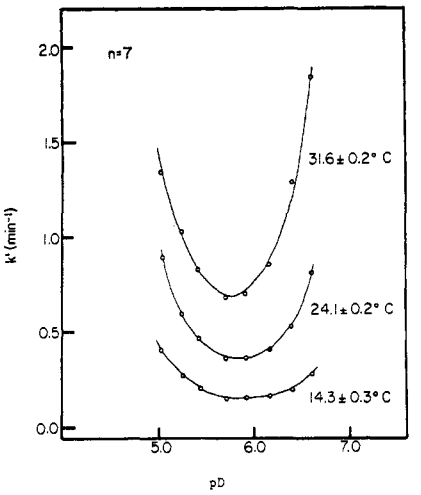


Figure 2. The typical observed rates *vs.* pD plots for the hydrogen-deuterium exchange reaction at different temperatures (for the $n = 7$ lactam).

**Acid and Base Catalytic Constants.** Both acid and base catalyses of hydrogen-deuterium exchange have been observed for the *cis* lactams ($n = 5$, 6, and 7) and the *cis–trans* lactam ($n = 9$) as well as NMA.

The catalysis of $D^+$ and $OD^-$ may be written

$$k' = k_0 + k_D[D^+] + k_{OD}[OD^-] \qquad (5)$$

$$= k_0 + k_D[D^+] + k_{OD}(K_w/[D^+]) \qquad (6)$$

where $k_0$ is the rate constant in $\text{min}^{-1}$ in the absence of catalysts, $k_D$ is the acid catalytic constant in $M^{-1}\ \text{min}^{-1}$, $k_{OD}$ is the base catalytic constant in $M^{-1}\ \text{min}^{-1}$, and

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

(14) E. A. Guggenheim, *Phil. Mag.*, **2**, 538 (1926).

Table I. Exchange Rate Parameters for the Lactams ($n$ = 5, 6, 7, and 9) and NMA

| Compound | Temp, °C | $pD_{min}$ | $k_{min}$, $min^{-1}$ | $10^{-4}k_D$, $M^{-1}$ $min^{-1}$ | $10^{-8}k_{OD}$, $M^{-1}$ $min^{-1}$ | $k_0$, $min^{-1}$ |
|---|---|---|---|---|---|---|
| Lactam | | | | | | |
| $n$ = 5 (I) | 9 ± 2 | 5.09 | 0.380 | 2.27 ± 0.06 | 9.66 ± 0.23 | 0.012 ± 0.009 |
| | 12.9 ± 0.5 | 5.02 | 0.622 | 2.81 ± 0.02 | 16.5 ± 0.1 | 0.085 ± 0.004 |
| | 21.3 ± 0.2 | 4.99 | 1.420 | 6.17 | 41.7 | 0.158 |
| (II) | 6.1 ± 0.4 | 5.13 | 0.310 | 1.98 ± 0.06 | 7.08 ± 0.22 | 0.017 ± 0.009 |
| | 21.6 ± 0.2 | 5.02 | 1.474 | 6.61 ± 0.13 | 39.1 ± 0.7 | 0.213 ± 0.023 |
| $n$ = 6 | 14.6 ± 0.4 | 5.39 | 0.208 | 0.827 ± 0.022 | 0.887 ± 0.024 | 0.141 ± 0.002 |
| | 24.9 ± 0.2 | 5.29 | 0.450 | 1.60 ± 0.04 | 2.78 ± 0.06 | 0.283 ± 0.004 |
| | 33.7 ± 0.2 | 5.25 | 0.985 | 2.97 ± 0.04 | 6.06 ± 0.04 | 0.651 ± 0.002 |
| $n$ = 7 | 14.3 ± 0.3 | 5.92 | 0.141 | 3.71 ± 0.13 | 0.347 ± 0.012 | 0.053 ± 0.003 |
| | 24.1 ± 0.2 | 5.85 | 0.350 | 7.82 ± 0.18 | 1.01 ± 0.03 | 0.129 ± 0.005 |
| | 31.6 ± 0.2 | 5.77 | 0.685 | 12.7 ± 0.4 | 2.39 ± 0.07 | 0.253 ± 0.013 |
| $n$ = 9 | 20.7 ± 0.3 | 5.62 | 0.095 | 0.552 ± 0.033 | 0.203 ± 0.011 | 0.069 ± 0.002 |
| | 32.0 ± 0.5 | 5.40 | 0.310 | 0.979 ± 0.029 | 1.01 ± 0.04 | 0.232 ± 0.002 |
| | 45.5 ± 1.0 | 5.39 | 1.117 | 3.88 ± 0.28 | 4.16 ± 0.27 | 0.801 ± 0.023 |
| NMA | 12.6 ± 1.0 | 5.47 | 0.130 | 1.82 ± 0.02 | 1.36 ± 0.02 | 0.007 ± 0.002 |
| | 22.5 ± 0.6 | 5.42 | 0.345 | 4.39 ± 0.02 | 4.12 ± 0.02 | 0.012 ± 0.002 |
| | 32.9 ± 0.2 | 5.32 | 0.872 | 9.59 ± 0.40 | 14.3 ± 0.6 | |

$K_w$ is the self-dissociation constant for $D_2O$. Following the analysis of Leichtling and Klotz,[7] $k_D$, $k_{OD}$, and $k_0$ can be determined according to eq 7–9 which are derived from eq 6

$$k' - k_{min} = k_D[D^+_{min}]\frac{(n-1)^2}{n} \quad (7)$$

$$[D^+_{min}]^2 = \frac{k_{OD}}{k_D}K_w \text{ or } k_{OD} = \frac{[D^+_{min}]^2}{K_w}k_D \quad (8)$$

$$k_{min} = k_0 + 2k_D[D^+_{min}] \quad (9)$$

where $k_{min}$ is the rate constant at the minimum ($pD_{min}$) of the $k'$ *vs.* pD plot, and $n$ is a variable defined as $n = [D^+]/[D^+_{min}]$.

From eq 7, a graph of $k'$ *vs.* $(n-1)^2/n$ will give a straight line with a slope of $k_D[D^+_{min}]$. Since $[D^+_{min}]$ is known from the $k'$ *vs.* pD plot, $k_D$ can be determined. The $k_{OD}$ is then calculated from eq 8 using a value of $1.54 \times 10^{-15}$ for $K_w$,[15] and the $k_0$ is calculated from eq 9. This method was found to be better than solving eq 6 directly using various observed rate constants and pD values. A typical $k'$ *vs.* $(n-1)^2/n$ plot is shown in Figure 3. Catalytic constants and their experimental

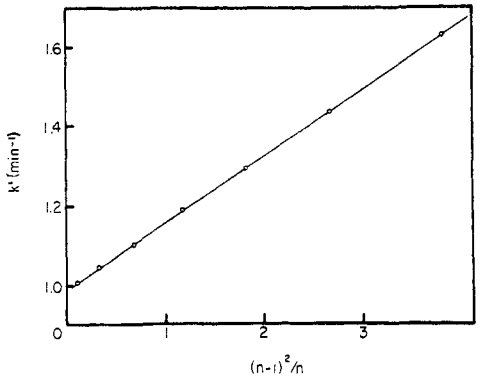


Figure 3. A typical $k'$ *vs.* $(n-1)^2/n$ plot for determining $k_D$ (for the $n$ = 6 lactam, at 33.7 ± 0.2°).

scatters obtained for the various lactams and NMA are listed in Table I.

(15) R. W. Kingerley and V. K. LaMer, *J. Am. Chem. Soc.*, **63**, 3256 (1941).

**Activation Parameters.** Using standard procedures,[16] the activation energy for exchange, $E_a^*$, was calculated from the temperature variation of the acid catalytic constant; the free energy of activation, $\Delta F_a^*$, the heat of activation, $\Delta H_a^*$, and the entropy of activation, $\Delta S_a^*$, were then calculated from $E_a^*$ and $k_D$. The results are shown in Table II.

Table II. Parameters for the Activation at 25°

| Compound | $E_a^*$, kcal/mole | $10^{-4}k_D$, $M^{-1}$ $min^{-1}$ | $\Delta F_a^*$, kcal/mole | $\Delta H_a^*$, kcal/mole | $\Delta S_a^*$, cal/mole °C |
|---|---|---|---|---|---|
| Lactam | | | | | |
| $n$ = 5 | 14.7 | 8.65 | 13.3 | 14.1 | 2.6 |
| $n$ = 6 | 11.9 | 1.66 | 14.1 | 11.3 | −9.4 |
| $n$ = 7 | 12.4 | 8.24 | 13.1 | 11.8 | −4.6 |
| $n$ = 9 | 14.8 | 6.49 | 14.7 | 14.2 | −1.6 |
| NMA | 14.0 | 5.32 | 13.4 | 13.4 | 0 |

## Discussion

The observations on the exchange reactions of all the lactams studied and NMA are consistent with the proposed mechanisms.[2] The first-order catalytic effect

$$\text{—CONH— + } OD^- \overset{k_{OD}}{\rightleftharpoons} \text{—CON}^-\text{— + HOD} \quad (10)$$

$$\text{—CONH— + } D_3O^+ \overset{k_D}{\rightleftharpoons} \text{—CO}^+\text{NHD— + } D_2O \quad (11$$

of $OD^-$ and $D^+$ was observed previously for NMA,[3,4] the $n$ = 5 lactam and N-ethylacetamide,[5] small peptides,[6] and polypeptides[7–9] either in the helical or disordered form. The results on NMA obtained from the present study agree reasonably well with those reported for $pD_{min}$, $k_{min}$, $k_D$, and $E_a^*$,[3–5] whereas, in the case of the $n$ = 5 lactam, only the $k_{min}$ and the $k_D$ at 11° of Klotz, *et al.*, fit our data. Their values for $k_D$ at 19.5° and for $E_a^*$ are almost twice as high as found in the present study. This is not too surprising if one examines closely their $k'$ *vs.* pD plot at 19.5°. Actually there are only two points to define the slope of the lower pD branch of the curve and for one the rate is so fast that a considerable error in its value is likely. Such an error will affect both the position of the minimum and

(16) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," 2nd ed, John Wiley and Sons, Inc., New York, N. Y., 1961, pp 22–24, 91, 98–100.

the slope of the curve which gives the $k_D$ value and the activation energy. We studied the $n = 5$ lactam at five temperatures and for eight different buffer pH's. The $k'$ *vs.* pD plots obtained have well-defined slopes and are reasonably symmetrical with respect to the minimum (Figure 4). The two experiments on this lactam gave a value of 15 kcal/mole for $E_a$* with an experimental scatter of $\pm 1$ kcal/mole. This agrees with an estimate of the error in the measurements.

The other lactams studied have $k_{min}$ values similar to the corresponding value for NMA except the $n = 9$ lactam which has a much slower exchange rate. The analysis of Leichtling and Klotz[7] indicates that $k_{min}$ might be affected by the nature of the solvent [$k_{min} = k_0 + b(K_w)^{1/2}$, where $b$ is a function of $k_D$ and $k_{OD}$] and other environmental and conformational effects. A smaller value for $K_w$ is necessary to give a smaller $k_{min}$ if the effect comes from the solvent. Since $K_w$ is lower for $D_2O$ than for $H_2O$, even though more $H_2O$ (or HOD) molecules are present in this reaction system than in other lactams studied, this cannot cause the decrease in $k_{min}$. It is not certain whether the coexistence of the *trans* configuration or the conformation of this lactam has caused a decrease in the accessibility of the amide group. The slightly higher $E_a$* for this $n = 9$ lactam than for the $n = 6$ and 7 seems to agree with such an effect.

Similar values for $pD_{min}$ were observed for NMA and the $n = 6$ and 9 lactams. The values for the $n = 5$ lactam are lower, and those for the $n = 7$ lactam seem to be higher. In all cases, the $pD_{min}$ decreases slightly with increases in temperature. Since the $pK_w$ of water decreases with temperature increases, this temperature dependence of $pD_{min}$ agrees with the derived relation between $pD_{min}$ and $pK_w$[7]

$$pD_{min} = {}^1/_2 pK_w - {}^1/_2 \log (k_{OD}/k_D) \qquad (12)$$

with eq 10 and 11 as the rate-controlling steps. In the $n = 5$ lactam, the catalysis by $D_3O^+$ might be sterically less favorable than the catalysis by $OD^-$, and thus a lower $pD_{min}$ will result. A higher $E_a$* is also expected. These were actually observed (Tables I and II).

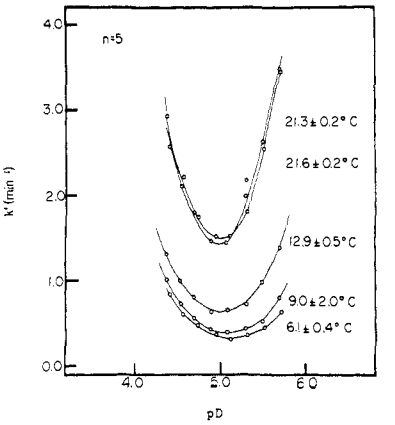


Figure 4. The observed rates of exchange *vs.* pD plots for the $n = 5$ lactam.

The acid catalytic constant, $k_D$, has quite comparable values for the $n = 5$ and 7 lactams and NMA, with slightly lower values for the $n = 6$ lactam. The $k_D$ for the $n = 9$ lactam is much smaller, which might be due to some conformational effect. The $k_{OD}$ values are in the following order: $n = 5 \gg$ NMA $> n = 6$, $7 > n = 9$. A detailed analysis in relation to the conformation is difficult.

In summary, the results of present investigation show that exchange rates of the *trans* amide, NMA, and unstrained *cis* lactams are comparable. The activation energies, 12–15 kcal/mole, are the same for all the compounds studied and are of similar magnitude as those reported for other model peptides, proteins, and polypeptides. The slight differences between individual compounds can be accounted for by the estimated error of this study (5–10%). Some variation in rate constants can be explained by the conformation of the compounds. A slight variation of $pD_{min}$ with temperature was observed which seems to be related to the trend of the $pK_w$ changes. It is interesting to note that the entropy of activation changes sign for the $n = 5$ lactam; however, it is not easy to interpret it in terms of the exact changes involved in the process of exchange.