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Activation Energy of Deuterium–Hydrogen Exchange in Amides

Sir:

When it was found that deuterium exchange rates with protein N–H groups fall roughly into two classes, rapid and slow,[1,2] it seemed reasonable to assign the fast group to unfolded portions of the polypeptide chain and the slow group to helical sections.[3] On this basis one could ascribe the measured heat of activation of 20 kcal./mole for the slow exchange to the need to open three adjacent hydrogen bonds in order to unfold a helical segment,[3] each hydrogen bond presumably requiring 6–8 kcal. It was assumed in this connection that unfolded sections of the polypeptide chain exchanged hydrogens with a greatly reduced heat of activation.

As far as we can find no actual measurements have been made of the activation energy for deuterium–hydrogen exchange in a simple, solvated N–H group. In the course of studies of catalytic effects on such exchanges,[4] we have examined, therefore, the effect of temperature on the kinetics of deuterium exchange in a model amide, N-methylacetamide, $CH_3CONHCH_3$, when it is entirely in the monomeric state.

Exchange rates were followed for solutions of 1 M N-methylacetamide in D_2O by observation of changes in absorbance at 1.43 μ in the near-infrared region of the spectrum. It was found immediately at room temperature that the rate of exchange was acid- and base-catalyzed, in agreement with the report of Nielsen.[5] Experiments were then extended to lower and higher temperatures, over the pD range 4.5–6.5.

In the range pD 4.5–5.5, the exchange was acid-catalyzed at each of three temperatures, 12, 24, and 32°. The activation energy (Fig. 1), calculated from the temperature coefficient of the rate constant at pD 5.0, is 17 kcal./mole. The rate reaches a minimum at approximately pD 5.5. Above pD 5.5 it is base-catalyzed, and rate constants were measured over the range pD 5.5–6.5. Data at pD 6.3 (Fig. 1) were used to calculate the activation energy, and a value of 23 kcal./mole was obtained. Essentially the same energies would be obtained for other pD's in the acid or basic range, respectively. The observed rates at pD values more than 0.5 unit below or above the pD of the minimum rate were greater than 90% acid- or base-catalyzed, respectively.

It seems clear, therefore, that at least in this model of a peptide group, $CH_3CONHCH_3$, a substantial activation energy (near 20 kcal./mole) is essential for exchange with deuterium even when the N—H is not hydrogen bonded to a C=O. If additional energy

(1) H. Lenormant and E. R. Blout, *Nature*, **172**, 770 (1953).
(2) A. Hvidt, G. Johansen, K. Linderstrom-Lang, and F. Vaslow, *Compt. rend. trav. lab. Carlsberg, Ser. chim.*, **29**, 129 (1954).
(3) K. Linderstrom-Lang, Special Publication No. 2, The Chemical Society London, 1955, p. 1.
(4) I. M. Klotz and B. H. Frank, *Science*, **138**, 830 (1962).
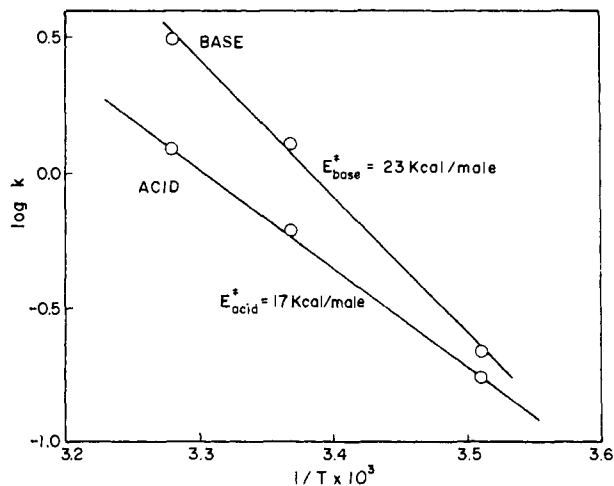(5) S. O. Nielsen, *Biochim. Biophys. Acta*, **37**, 146 (1960).

Fig. 1.—Logarithm of the observed rate constant (in min.$^{-1}$) for the deuterium exchange of D_2O with the N–H hydrogen of $CH_3CONHCH_3$, as a function of temperature. Acid constants at pH 5.0; base constants at pH 6.3.

is needed to break hydrogen bonds in a helix before exchange occurs, then the observed activation energy for the over-all exchange process ought to be substantially greater than 20 kcal./mole.

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X-Ray Structure Determination of Cubane[1]

Sir:

We wish to present the results of a high-precision X-ray analysis as absolute confirmation of the structural assignment of the recently synthesized hydrocarbon cubane.[2]

The space group of cubane[3] is $R\bar{3}$ with the cell constants $a = 5.340 \pm 0.002$ Å. and $\alpha = 72.26 \pm 0.05°$. There is one molecule per unit cell.

Three-dimensional X-ray data were collected with Cu Kα radiation on a GE XRD-5 instrument with a scintillation counter employed as a detector. The intensities were collected using the moving counter–moving crystal technique. The 97 independent structure amplitudes were obtained by averaging the three reflections related by the crystal's threefold axis.

A body diagonal carbon and the atom related to it by the center of symmetry are at special positions on a threefold axis at x, x, x and $\bar{x}, \bar{x}, \bar{x}$. The hydrogens attached to these carbons also lie on this special position. The other carbons and hydrogens, six of each, occupy the general positions of the space group. The coordinates of the independent atoms and the temperature factors for the atoms are given in Tables I and II.