The Thermodynamics of Transfer of Amides from an Apolar to an Aqueous Solution*

Gordon C. Kresheck and Irving M. Klotz

ABSTRACT: The heat of transfer of *N*-methylacetamide from CCl_4 to water (-7.8 kcal/mole) has been calculated from calorimetric measurements of heats of solution. This combined with other thermodynamic data indicates that there is a negative enthalpy change when an amide hydrogen bond in an apolar environment is exposed to aqueous solvent, as may occur in unfolding of proteins. The concentration dependence of the relative apparent molal heat content has been used in addition to obtain an independent evaluation of the equilibrium constant and enthalpy of self-association of *N*-methylacetamide in CCl_4 solution.

The association of amides in aqueous and apolar solutions has been studied in several laboratories (Davies *et al.*, 1955; Davies and Thomas, 1956; Schellman, 1955; Klotz and Franzen, 1962) in an effort to provide an estimate of the stability of the peptide hydrogen bond in proteins. In model amides the reaction, in aqueous solution

$$(\text{N—H}\cdots\text{O=C})_{\text{aq}} \rightleftharpoons (\text{N—H, O=C})_{\text{aq}} \qquad (1)$$

where (NH, O=C) represents nonbonded groups, proceeds far to the right, but $\Delta G°$ is positive for the bond dissociation in apolar solvents

$$(\text{N—H}\cdots\text{O=C})_{\text{apolar}} \rightleftharpoons (\text{N—H, O=C})_{\text{apolar}} \qquad (2)$$

Of greater relevance than eq 2 in regard to proteins is the dissociation

$$(\text{N—H}\cdots\text{O=C})_{\text{apolar}} \rightleftharpoons (\text{N—H, O=C})_{\text{aq}} \qquad (3)$$

since this corresponds to the disruption of a hydrogen bond in the interior of a protein and its exposure, dissociated, to the external aqueous solvent. Recently Klotz and Farnham (1968) have been able to calculate $\Delta G°$ for the dissociation in eq 3 by combining previous data for eq 2 with measurements of the equilibrium constant for the transfer of a model amide from an apolar to an aqueous solvent

$$(\text{N—H, O=C})_{\text{apolar}} \rightleftharpoons (\text{N—H, O=C})_{\text{aq}} \qquad (4)$$

These results indicated that $\Delta G°$ for eq 3 is not greatly different from that for eq 1 and hence led to the conclusion that the intrinsic interaction in the N—H$\cdots$O=C hydrogen bond is not sensitive to the nature of the surrounding solvent.

In view of the interest of these observations in regard to protein stability it seemed worthwhile to broaden the thermodynamic investigations to encompass enthalpy and entropy changes also. For this purpose calorimetric measurements of heats of solution of *N*-methylacetamide in water and in carbon tetrachloride were made. These provided the information needed to compute $\Delta H°$ and $\Delta S°$ for the transfer process of eq 4 and hence for the dissociation reaction 3. In addition, since the experimental measurements also contain implicitly the concentration dependence of the heat content of the amide in CCl_4, it was possible to obtain an independent determination of the thermodynamic parameters of self-association of the amide in this solvent.

Experimental Section

Materials and Methods. Commercial grade (Fisher) *N*-methylacetamide was dried overnight over $CaSO_4$ and distilled at about 0.24 mm, and the fraction boiling at 70° was collected. The *N*,*N*-dimethylacetamide (Eastman) was used without purification. Both amides were free of detectable impurities as tested (by Dr. Charles Spangler) by gas chromatographic analysis. Both were stored over $CaCl_2$. Analyzed reagent grade CCl_4 (Matheson) was used directly, or after passing through a Linde-type 4A activated Molecular Sieve to remove final traces of water, without significant difference in results obtained. Laboratory distilled water was deionized by means of a Barnstead mixed-bed demineralizer.

Calorimetry. The calorimetric data were obtained with an adiabatic solution calorimeter modeled after the one described by Benjamin (1963), except that a nonaqueous cell without epoxy resin was constructed for the determinations in CCl_4. The thermistors and heater in this cell were embedded in stainless steel wells with epoxy resin, and a two-post Latrobe seal was used as a

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* From the Department of Chemistry, Northern Illinois University, DeKalb, Illinois 60115, and Biochemistry Division, Department of Chemistry, Northwestern University, Evanston, Illinois 60201. *Received August 12, 1968.* This work was supported in part by a research grant (GM-13623) from the National Institutes of General Medical Sciences, U. S. Public Health Service.

fuse connector. Measurements were carried out at temperatures close to 25° (±0.05°) and corrections were not made for small deviations from this temperature. The observed enthalpy changes, q_{obsd}, were corrected for the heat change, q_b, associated with opening the sample cells. Integral heats of dilution were measured and extrapolated to infinite dilution by standard techniques. Results are expressed in units of the calorie defined as 4.184 absolute joules.

Evaluation of Thermodynamic Parameters. Heats of transfer corresponding to the process represented by eq 4 are not easily accessible experimentally. On the other hand, heats of solution, ΔH_S, in the apolar and aqueous solvents, respectively, can be readily measured calorimetrically. If these heats are obtained as a function of concentration and extrapolated to zero concentration in each solvent, then

$$\Delta H_{\mathrm{transfer}} = \Delta H_S{}^{\circ}{}_{(\mathrm{aq})} - \Delta H_S{}^{\circ}{}_{(\mathrm{apolar})} \tag{5}$$

From the concentration dependence of heats of solution and dilution, one can also obtain the thermodynamic parameters of aggregate formation. Previous investigations (Mizushima *et al.*, 1950; Davies and Thomas, 1956) have shown that *N*-methylacetamide forms linear aggregates of dimers, trimers, and higher oligomers. For such a self-association with multiple steps one may write

$$\left.\begin{array}{ll} \mathrm{A} + \mathrm{A} = \mathrm{A}_2; & K_2 = \dfrac{(\mathrm{A}_2)}{(\mathrm{A})^2} \\[2ex] \mathrm{A}_2 + \mathrm{A} = \mathrm{A}_3; & K_3 = \dfrac{(\mathrm{A}_3)}{(\mathrm{A}_2)(\mathrm{A})} \\ \quad\vdots & \quad\vdots \\ \mathrm{A}_{n-1} + \mathrm{A} = \mathrm{A}_n; & K_n = \dfrac{(\mathrm{A}_n)}{(\mathrm{A}_{n-1})(\mathrm{A})} \end{array}\right\} \tag{6}$$

The stoichiometric molality, m, may be represented as

$$m = (\mathrm{A}) + 2(\mathrm{A}_2) + \cdots + n(\mathrm{A}_n) \tag{7}$$

If all the equilibrium constants in eq 6 are equal, *i.e.*

$$K_2 = K_3 = \cdots = K_n \equiv K \tag{8}$$

then eq 7 may be expressed in closed form (*e.g.*, Schellman, 1955) when $K(\mathrm{A}) < 1$

$$m = \frac{(\mathrm{A})}{[1 - K(\mathrm{A})]^2} \tag{9}$$

If the enthalpy change, ΔH, is assumed to be the same for each of the steps in eq 6, then the relative apparent molal heat content, ϕ_L, may be expressed as (Schellman, 1955)

$$\phi_L = \frac{(\Delta H)K}{m}\left[\frac{(\mathrm{A})}{1 - K(\mathrm{A})}\right]^2 \tag{10}$$

TABLE I: Heat of Solution, ΔH_S, and Relative Apparent Molal Heat Content, ϕ_L, of *N*-Methylacetamide at 25°.

m	Solvent	q^a (cal)	ΔH_S (cal/mole)	ϕ_L (cal/mole)
0.05393	CCl_4	7.334	3206	3390
0.05261	CCl_4	7.665	3388	3210
0.03377	CCl_4	5.567	3747	2850
0.03214	CCl_4	5.431	4010	2590
0.02473	CCl_4	4.757	4402	2200
0.02023	CCl_4	4.102	4671	1930
0.00699	CCl_4	1.714	5644	960
0.00627	CCl_4	1.527	5638	960
[0.00000]	CCl_4		(6600)	0
0.20187	H_2O	−6.494	−1201	
0.16126	H_2O	−5.254	−1187	
0.08482	H_2O	−2.697	−1198	
	H_2O		−1195 (av)	

[a] $q = q_{\mathrm{obsd}} - q_b$; $q_b^{\mathrm{CCl_4}} = 0.109$ cal, $q_b^{\mathrm{H_2O}} = 0.055$ cal.

Combination of eq 9 and 10 allows one to eliminate (A) and to obtain the expression (Gill *et al.*, 1967; Stoesser and Gill, 1967)

$$\phi_L = \Delta H - \left(\frac{\Delta H}{K}\right)^{1/2}\left(\frac{\phi_L}{m}\right)^{1/2} \tag{11}$$

Thus in principle both ΔH and K can be evaluated if ϕ_L is known as a function of the stoichiometric molality.

That $\Delta H_2 = \Delta H_3 = \cdots = \Delta H$ seems a reasonable approximation to actual behavior since the interaction energy between N—H and O═C is not likely to depend very much on the length of chain to which each is attached. On the other hand it is much less likely that $\Delta G_2{}^{\circ} = \Delta G_3{}^{\circ} = \cdots = \Delta G^{\circ}$ since entropic factors would be significantly different for dimer, trimer, etc. Hence, the assumption that $K_2 = K_3 = \cdots = K$ may be unreliable. It is possible, nevertheless, to calculate ΔH and K_2 from the experimental data even if eq 8 is not applicable.

The relative apparent molal heat content, ϕ_L, is related to ΔH (Gill *et al.*, 1967) by the equation

$$\phi_L = \Delta H \frac{(\mathrm{A}_2) + 2(\mathrm{A}_3) + \cdots + n(\mathrm{A}_n)}{m} \tag{12}$$

which may be expressed more concisely as

$$\phi_L = (\Delta H)\alpha \tag{13}$$

where α is the fraction of NH groups which are in the N—H···O═C state. From eq 13 it follows that ΔH can be evaluated from

$$\lim_{\alpha \to 1} \phi_L = \lim_{m \to \infty} \phi_L = \Delta H \tag{14}$$

TABLE II: Heat of Dilution, ΔH_D, and Relative Apparent Molal Heat Content, ϕ_L, of *N*-Methylacetamide in CCl_4 at 25°.

m_i^a	m_f^b	q^c (cal)	ΔH_D (cal/mole)	$-\phi_L$ (cal/mole)
1.61354	0.11245	4.471	887	5400
1.17255	0.07746	4.434	1224	5140
1.02517	0.07000	4.320	1337	4880
0.69339	0.04666	3.745	1719	4850
0.44372	0.02889	3.187	2349	4790
0.35130	0.02504	3.182	2559	4730
0.22407	0.01435	2.053	3126	4670
0.10743	0.00718	1.078	3243	4230
0.08856	0.00678	0.910	3137	4100
0.03330	0.00234	0.221	2098	2540

^{a} m_i = initial molality. ^{b} m_f = final molality. ^{c} $q = q_{obsd} + q_b$; $q_b = 0.068$ cal.

Likewise it follows (Klotz and Franzen, 1962) that

$$\lim_{m\to 0}\frac{\alpha}{1-\alpha}\,\frac{1}{m} = \lim_{m\to 0}\frac{\phi_L}{\Delta H - \phi_L}\,\frac{1}{m} \qquad (15)$$

$$\simeq \lim_{m\to 0}\frac{\alpha}{1-\alpha}\,\frac{1}{(A)} = K_2$$

In practice $m(1-\alpha)$ can be used as an approximation to (A) and should become increasingly reliable as $m \rightarrow 0$.

Results

Heats of solution, ΔH_S, and heats of dilution, ΔH_D, are assembled in Tables I–IV.

TABLE III: Heat of Solution, ΔH_S, and Relative Apparent Molal Heat Content, ϕ_L, of *N,N*-Dimethylacetamide at 25°.

m	Solvent	q^a (cal)	ΔH_S (cal/mole)	ϕ_L (cal/mole)
0.26679	CCl_4	3.513	313	269
0.12013	CCl_4	2.238	454	128
0.07494	CCl_4	1.647	507	75
(0.00000)	CCl_4		(582)	0
0.08634	H_2O	−11.778	−5010	
0.02898	H_2O	− 3.834	−5003	
	H_2O		−5010 (av)	

^{a} $q = q_{obsd} - q_b$; $q_b^{CCl_4} = 0.109$ cal, $q_b^{H_2O} = 0.055$ cal.

TABLE IV: Heat of Dilution, ΔH_D, and Relative Apparent Molal Heat Content, ϕ_L, of *N,N*-Dimethylacetamide in CCl_4 at 25°.

m_i^a	m_f^b	q^c (cal)	ΔH_D (cal/mole)	ϕ_L (cal/mole)
1.38211	0.07552	2.489	590	666
0.53077	0.03541	0.744	451	487

^{a} m_i = initial molality. ^{b} m_f = final molality. ^{c} $q = q_{obsd} + q_b$; $q_b = 0.068$ cal.

From the concentration dependence of ΔH_S for *N*-methylacetamide (Table I) in CCl_4 and in water, respectively, we find, on extrapolation to zero concentration, the following $\Delta H_S°$'s

$$N\text{-methylacetamide (s)} = N\text{-methylacetamide (aq, } m = 0) \qquad \Delta H° = -1.195 \pm 0.01 \text{ kcal/mole} \qquad (16)$$

$$N\text{-methylacetamide (s)} = N\text{-methylacetamide (}CCl_4, m = 0) \qquad \Delta H° = 6.60 \pm 0.1 \text{ kcal/mole} \qquad (17)$$

From these results it follows that

$$N\text{-methylacetamide (}CCl_4) = N\text{-methylacetamide (aq)} \qquad \Delta H° = -7.8 \text{ kcal/mole} \qquad (18)$$

The concentration dependence of ϕ_L for *N*-methylacetamide, as well as dimethylacetamide, has been plotted in Figure 1. It is obvious that the former has a much greater variation with molality, particularly below 0.2 *m*. If ϕ_L is plotted *vs.* $1/m$ and extrapolated to $m \rightarrow \infty$ (see eq 14) a value of −5.1 kcal/mole is obtained for ΔH. With this ΔH eq 15 can be used to evaluate K_2 from calorimetric data alone, and an estimate of 40 ± 5 m^{-1} is obtained.

The data have also been plotted according to eq 11. The points do not fall on a straight line, as would be required if all the *K*'s are equal (eq 8). Nevertheless if one fits a least-squares line through the points, one finds $\Delta H = -6.2$ kcal/mole and $K = 37\ m^{-1}$.

Discussion

The calorimetric data can be combined with previous measurements (Klotz and Franzen, 1962; Klotz and Farnham, 1968) to yield a more detailed thermodynamic description for a variety of processes for the amide group. These can be concisely summarized if we represent the four possible states of the amide group in water and in CCl_4 by I–IV

$$\underset{\text{I}}{(\text{N—H}\cdots\text{O=C})_{CCl_4}} \qquad \underset{\text{II}}{(\text{N—H, O=C})_{CCl_4}}$$

$$\underset{\text{III}}{(\text{N—H, O=C})_{H_2O}} \qquad \underset{\text{IV}}{(\text{N—H}\cdots\text{O=C})_{H_2O}}$$

The thermodynamic parameters for various transitions between these states are assembled in Table V.

TABLE V: Thermodynamic Parameters for Transfer Processes Involving the Bonded and Nonbonded Amide Group in an Aqueous and Apolar Solvent at 25°.[a]

Process	$\Delta G°$ (kcal/mole)	$\Delta H°$ (kcal/mole)	$\Delta S°$ (eu)
I → II	2.4	5.1	9
II → III	-4.1^b	-7.8	-12
III → IV	(3.1)	(0)	(-10)
I → III	-1.7	-2.7^c	-3

[a] Quantities in brackets are based on the results of Klotz and Franzen (1962). [b] Klotz and Farnham (1968). [c] The effect of the nonpolar substituents on this quantity may be estimated by combining the $\Delta H°$ data for process II → III for *N*-methylacetamide and *N,N*-dimethylacetamide. By assuming that each $\Delta H°$ reflects approximately the sum of the contributions of the methyl groups and hydrogen bonding groups of each molecule, the individual contribution is -0.85 kcal/mol CH_3 and -3.05 kcal/mol H-bond, since *N*-methylacetamide and *N,N*-dimethylacetamide contain two and three methyl groups and two and one hydrogen bonding groups, respectively. Therefore, -1.7 kcal/mole should be removed from -2.7 kcal/mole to give -1.0 kcal/mole for the hydrogen-bond contribution to process I → III.

For the transfer process II → III, corresponding to eq 4, $\Delta H°$ (-7.8 kcal/mole) is the compelling factor being substantially more negative than $\Delta G°$ (-4.1). The transfer of *N*-methylacetamide from CCl_4 to H_2O is thus accompanied by a negative $\Delta S°$ (-12 eu) as might have been expected if the amide is hydrogen bonded to the H_2O molecules in the aqueous solvent.

With this $\Delta H°$, the enthalpy change for the transfer I → III is readily obtained. It is an exothermic process as well as an exergonic one. Thus this process which corresponds to the disruption of a peptide hydrogen bond in the interior of a protein molecule and its exposure to an aqueous solvent is energetically favored, at least in the model amide.

The equilibrium constant K_2 and the corresponding $\Delta G°$ obtained calorimetrically are not in good agreement with the overtone infrared results (Klotz and Franzen, 1962), but they are in better agreement with the results of Davies and Thomas (1956).

For both *N*-methylacetamide and *N,N*-dimethylacetamide in CCl_4 endothermic heats of dilution are observed, being much larger for the former solute. The entire ϕ_L for *N*-methylacetamide has been assigned to the N—H ··· O=C bonds in solution in the calculation of the equilibrium for aggregate formation but it is recognized that this cannot be strictly valid since nonzero values of ϕ_L are found for dimethylacetamide. Endothermic heats of dilution have also been reported for dilute solutions of pyridine and of quinoline in CCl_4 (Marcom and Travers, 1966; Gray *et al.*, 1968). These have been ascribed to solute–solute interactions. Therefore, it would appear that both *N*-methylacetamide and *N,N*-dimethylacetamide undergo self-interaction in dilute CCl_4 solution. The large difference between the magnitude of the heat effect noted when comparing these compounds undoubtedly reflects different mechanisms of interaction. The aggregation of *N*-methylacetamide in apolar solvents has been shown by infrared (Davies and Thomas, 1956; Klotz and Franzen, 1962) and nuclear magnetic resonance (LaPlanche *et al.*, 1965) studies to involve peptide-like hydrogen-bond formation, whereas *N,N*-dimethylacetamide cannot aggregate in this manner, but probably undergoes some form of dipole–dipole association (Phillips, 1965).

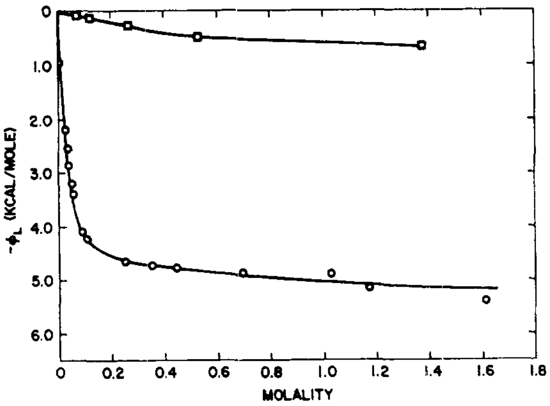

FIGURE 1: Relative apparent molal heat content, ϕ_L (kilocalories per mole), of *N*-methylacetamide (circles) and *N,N*-dimethylacetamide (squares) in CCl_4 at 25° as a function of molality.

Calorimetric measurements thus provide a fuller picture of amide group interactions in different solvent environments.

Acknowledgments

The assistance of Machinists R. Bjelk and E. White, Faraday Laboratories, Northern Illinois University, during the construction of the calorimeter is appreciated. Also, Dr. L. Stein, Chemistry Division, Argonne National Laboratory, provided helpful suggestions concerning the construction of the nonaqueous cell.

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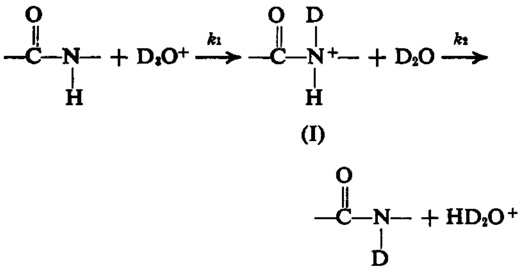
Local Environment Effects on Hydrogen–Deuterium Exchange*

Irving M. Klotz and Delbert D. Mueller†

ABSTRACT: The rate–pH profile of hydrogen–deuterium exchange of an amide NH in a polymer is shifted toward higher pH in the presence of an anionic detergent, sodium lauryl sulfate, which is bound by the polymer. This shift can be accounted for in terms of a change in local environment of the amide group which influences the acid–base character of the CONH moiety. This extrinsic effect is analogous to that produced intrinsically by electron-withdrawing substituents attached to an amide group. H–D exchange rates may be modified by perturbations of the activated state, as well as of the ground state, of the kinetic transition.

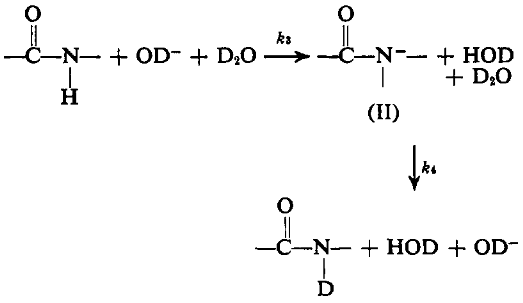
Hydrogen–deuterium exchange is a kinetic process. Thus, as in all rate processes, H–D interchange depends upon a transition from a ground state to an activated state (Figure 1). In studies with polypeptides and proteins it has generally been assumed that any attenuation in exchange rate is to be ascribed to a conformation of the macromolecule which lowers the energy of the ground state (Hvidt and Nielsen, 1966). Originally this state of lowered energy was assumed to be a helical structure. More recently masked conformations have been presumed to be inaccessible for less-defined steric reasons (Hvidt and Nielsen, 1966).

It is also obvious (Figure 1) that a change in the relative energy of the activated state, due to modifications in the environment in which an amide group may be situated, could likewise affect the kinetics of the isotopic exchange (Klotz, 1968).



$$-\overset{O}{\overset{\|}{C}}-\underset{H}{N}- + D_3O^+ \xrightarrow{k_1} \underset{(I)}{-\overset{O}{\overset{\|}{C}}-\underset{H}{\overset{D}{N^+}}-} + D_2O \xrightarrow{k_2} -\overset{O}{\overset{\|}{C}}-\underset{D}{N}- + HD_2O^+ \quad (1)$$

Structural and environmental features that might affect the energy of the activated state reveal themselves when we examine the mechanism of the exchange reaction (Berger *et al.*, 1959; Klotz, 1968). In acid solution the steps are given by eq 1. In view of the weak basicity of the amide NH group one expects k_1 to be rate controlling in this acid-catalyzed reaction. In basic solution we may write



$$-\overset{O}{\overset{\|}{C}}-\underset{H}{N}- + OD^- + D_2O \xrightarrow{k_3} \underset{(II)}{-\overset{O}{\overset{\|}{C}}-N^-}- + HOD + D_2O \xrightarrow{k_4} -\overset{O}{\overset{\|}{C}}-\underset{D}{N}- + HOD + OD^- \quad (2)$$

In view of the weak acidity of the amide NH group, k_3 should be rate limiting in the base-catalyzed reaction.

In either pathway a charged intermediate is formed, a cation (I) in the former, and an anion (II) in the latter. It seems very likely, therefore, that the activated state would also be a charged species.

Recent experiments with a synthetic polymer (Scarpa *et al.*, 1967), polyisopropylacrylamide, have shown that slow hydrogen–deuterium exchange may be observed for amide NH groups attached to a flexible, swollen polymer that is not in a compact conformation. These

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* From the Biochemistry Division, Department of Chemistry, Northwestern University, Evanston, Illinois. *Received August 19, 1968.* This investigation was supported in part by a grant (GM-09280) from the National Institutes of General Medical Sciences, U. S. Public Health Service. It was also assisted by support made available by a U. S. Public Health Service training grant (5T1-GM-626) from the National Institute of General Medical Sciences.

† Present address: Department of Biochemistry, Kansas State University, Manhattan, Kansas 66502.