2,4(α)-*cis*-Dihydroxymethyl-2,4(β)-*cis*-dimethyl-1,3(α)-*cis*-dicarbamidocyclobutane (15). A mixture of (−)-thymine dimer **3b** (R = H, 20 mg, $[\alpha]_D$ −92° (H_2O)) and sodium borohydride (64 mg) in water (3 ml) was stirred at room temperature for 22 hr. After decomposition of excess hydride with acetone, the solution was adjusted to pH ~5 by the addition of IRC-50 (H^+) resin. Boric acid was removed as methyl ester and the product was purified by chromatography on silica gel and eluted with MeOH–$CHCl_3$ (1:1). The product, a colorless solid (12 mg), was shown to be completely optically inactive in the region 210–700 nm. Recrystallization from methanol gave colorless prisms, mp 210–211°. The ir spectrum had absorption peaks at 3350 (NH, OH), 1650 (ureido carbonyl), 1580, 1530, and 1030 cm^{-1}, which were similar to those of **13**.

Anal. Calcd for $C_{10}H_{20}N_4O_4 \cdot 0.5H_2O$: C, 44.61; H, 7.81; N, 20.82. Found: C, 44.96; H, 7.64; N, 20.74.

Hydrogenolysis of Thymidine Dimers 1 (R = 2-Deoxyribofuranosyl). A mixture of dimer **1** (100 mg) and sodium borohydride (150 mg) in water (15 ml) was stirred at room temperature for 40 hr. After the usual work-up, several products which gave a pink color with the modified Ehrlich reagent were detected on tlc (silica gel; MeOH–$CHCl_3$, 1:1). Three products (mp 149–151, ~150, and 149–154°) were obtained in addition to 2-deoxyribose (chromatography on silica gel, $CHCl_3$–MeOH, 3:2). Acid hydrolysis of the products yielded the monoalcohol **5** and the dicarbinol **6** (tlc on silica gel), spraying with modified Ehrlich reagent. Hydrogenolysis of thymidine dimer **3** (R = 2-deoxyribofuranosyl) gave several products which were positive to the modified Ehrlich reagent.

Free Energies of Hydrolysis of Amides and Peptides in Aqueous Solution at 25°

Alan R. Fersht* and Yolanda Requena

Contribution from the M.R.C. Laboratory of Molecular Biology, Cambridge, England. *Received September 18, 1970*

Abstract: The free energies of hydrolysis of a series of formamides have been determined at 25°. The equilibrium constants (based on the concentration of un-ionized reagents and on an activity of 1.0 for water) for the formation of formamides of hydrazines and primary and secondary alkylamines follow the equation $\log K_{eq} = 0.5 + 0.51 \cdot pK_{a(amine)}$, while the amides of anilines and hydroxylamines are 1.4 kcal, and of ammonia 3 kcal, less stable than predicted. The results are extended to the general case of peptide formation. The "α" effect is discussed and shown to be independent of the stability of the ground-state reagents in the case of hydrazinolysis.

A knowledge of the free energies of hydrolysis of amides and peptides is fundamental to the understanding of biochemical equilibria. However, due to the experimental difficulty of very slow uncatalyzed rates of reaction, a few random equilibrium constants only have been accurately determined. Apart from one study at elevated temperatures,[1] the effect of the structure of the amine moiety on the hydrolysis equilibria of amides has not been systematically investigated.

The few equilibrium constants known do not fall into a simple pattern, except for the generalization that they are virtually independent of the structure of the acyl portion of the amide.[1,2]

Much attention has been paid to the enzymatic catalysis of amide and peptide hydrolysis through the studies on the mechanism of action of the proteolytic enzymes, notably chymotrypsin. However, due to the experimental difficulties of very slow rates and the masking of the water reaction by the acid- and base-catalyzed reactions, there is little known about the solvolysis of amides, apart from some interesting intramolecular acyl transfers.[3]

In a recent paper[4] we have emphasized the use of equilibrium constants for acyl-transfer reactions, derived from free energy of hydrolysis data, to calculate rate constants for otherwise inaccessible reactions. The determination of the amide equilibria also gives the solvolysis rates, provided the ester aminolysis rates are known.

Experimental Section

Materials. Organic reagents were distilled or recrystallized prior to use. Deionized water was used throughout. *N*-Formylmorpholine (bp 92.5° (3.5 mm) (lit.[5] 103–104° (13 mm)); ir (film) ν_{max} 1675 cm^{-1} (lit.[5] 1675 cm^{-1})) and formylhydrazine (mp 57–59° (lit.[5] 57–59°)) were synthesized by the method of Blackburn and Jencks.[5] Formhydroxamic acid (mp 75–77° (lit.[6] 77.5°)) was synthesized by the method of Bernhard, *et al.*[6]

Equilibrium Constants. Solutions were thermostated at 25.0 ± 0.01° either in stoppered test tubes in a constant-temperature bath or in the cuvettes in the Gilford 2400 spectrophotometer.

The equilibria between semicarbazide or thiosemicarbazide and the formyl derivatives were measured by assaying directly for the semicarbazide or thiosemicarbazide, the experimental concentrations being given in Table I.

The following assay was developed. Semicarbazide and thiosemicarbazide were found to give adducts with 1:2 napthaquinone-4-sulfonic acid (NSA) giving visible maxima at 460 nm. (This reagent has been used as a spot test.)

To 5.0 ml of 10^{-3} *M* NSA were added an aliquot of sample and sufficient sodium hydroxide to give a final concentration of 10^{-2} *M* hydroxide ion on addition of water to give a final volume of 10 ml. (Both the formation and decomposition of the adduct are base catalyzed and the concentration of 10^{-2} *M* NaOH was found to be most satisfactory.)

The absorbance at 460 nm was measured after 2.0 min. Under these conditions the semicarbazide adduct has an extinction coefficient of 8.2×10^3 and the thiosemicarbazide 13×10^3. Beer's

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(4) A. R. Fersht and W. P. Jencks, *ibid.*, **92**, 5442 (1970).
(5) G. M. Blackburn and W. P. Jencks, *ibid.*, **90**, 2638 (1968).
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Table I. Experimental Conditions for Direct Determination of Formation Constants of Amides at 25° and Ionic Strength 1.0[a]

Amide of formic acid and amine	Final concn, M HCl	Amine	Amide	pH	$t_{1/2}$[b] equilibrium, min
Thiosemicarbazide[c]	0.2[d]	3.6×10^{-3}	1.64×10^{-2}	0.59	39.1
	0.4[d]	5.0×10^{-3}	1.50×10^{-2}	0.33	22.5
	0.4[e]	5.2×10^{-3}	1.50×10^{-2}	0.32	31.8
Thiosemicarbazide[f]	0.4[d]	5.56×10^{-3}	1.444×10^{-2}	0.35	462
Semicarbazide[c]	0.2[d]	1.2×10^{-2}	7.6×10^{-3}	0.59	141
	0.4[d]	1.54×10^{-2}	4.45×10^{-3}	0.30	90.7
Hydroxylamine[c]	0.1[d]	0.892	7.94×10^{-3}	0.75	360
	0.3[d]	0.698	2.38×10^{-3}	0.39	120
	0.3[g]	0.708	2.64×10^{-3}	0.40	128
Semicarbazide[h]		$<3 \times 10^{-4}$	$>1.97 \times 10^{-2}$	3.66	600

[a] Ionic strength maintained at 1.0 with added KCl. [b] Half-life for attainment of equilibrium. [c] 2 M formic acid initially. [d] No amide initially. Equilibrium for 1-acetylthiosemicarbazide formation. [e] No amine initially. [f] 2 M acetic acid initially. [g] 1.015×10^{-2} M amide initially. [h] 1.0 M formic acid, 1.0 M potassium formate initially; no amide present.

Table II. Experimental Conditions for Determination of Equilibrium Constants between Formhydroxamic Acid (FHA), Hydroxylamine, Amines, and Amides at 25° and Ionic Strength 1.0[a]

	Final concn,[b] M Amine	Amide	Hydroxylamine	FHA	pH	$t_{1/2}$,[c] hr
Methoxyamine	0.776	4.04×10^{-3}[d]	0.138	6.26×10^{-3}	6.42	1
Aniline[f,g]	0.1	5.15×10^{-3}	0.195	4.85×10^{-3}[e]	6.15	38
	0.1	5.85×10^{-4}[d]	0.20	9.4×10^{-3}	6.15	38
Trifluoroethylamine	0.84	9.78×10^{-3}[d]	0.16	4.06×10^{-4}	6.06	31
	0.393	6.7×10^{-3}[d]	0.607	2.56×10^{-3}	7.12	53
Hydrazine	0.195	4.82×10^{-3}[d]	0.605	6.75×10^{-3}	6.28	2
	0.206	4.5×10^{-3}	0.594	5.6×10^{-3}[e]	6.27	2
	0.294	4.34×10^{-3}[d]	0.404	5.72×10^{-3}	6.06	3
	0.304	5.54×10^{-3}	0.396	4.36×10^{-3}[e]	6.09	3
Morpholine	1.1	9.52×10^{-3}	1.0	4.85×10^{-4}[e]	7.88	5.5
	1.1	9.43×10^{-3}	1.0[d]	5.66×10^{-4}	7.88	5.5
Ethylamine	0.442	8.13×10^{-3}	0.508[d]	1.87×10^{-3}	10.75	10
	0.502	8.6×10^{-3}	0.498	1.7×10^{-3}[e]	10.75	10
Dimethylamine[f,h]	0.6	9.3×10^{-3}	0.4	7.1×10^{-4}[e]	7.16	550
	0.6	9.5×10^{-4}	0.4[d]	8.95×10^{-3}	7.16	550
			Hydrazine	Formylhydrazine		
Ammonia[i]	0.95	2.03×10^{-3}[j]	17.2×10^{-3}	2.97×10^{-3}	9.48	10
	0.95	2.31×10^{-3}	12.5×10^{-3}[k]	2.69×10^{-3}	9.48	10

[a] Ionic strength maintained with KCl. [b] Total concentrations of base and conjugate acid. [c] Approximate half-time for attainment of equilibrium (reactions not performed under exact pseudo-first-order conditions). [d] No amide initially. [e] No FHA initially. [f] Reaction not followed to equilibrium. Initial concentration changes used to calculate K_{eq}. [g] Initial changes after 49 hr. Identical K_{eq} estimated after 237 hr ($6.25 \times t_{1/2}$). [h] Followed for 130 hr. Each experiment performed in duplicate with controls for FHA hydrolysis substituting triethylamine hydrochloride for dimethylamine hydrochloride. [i] Hydrazine and formylhydrazine used instead of hydroxylamine and FHA. [j] No formamide initially. [k] No formylhydrazine initially.

law is obeyed up to the highest concentrations routinely used of 2×10^{-4} M (in the 10-ml assay mixture). The "blank" absorbance is about 0.06.

Formhydroxamic acid formation from formic acid and hydroxylamine was determined by the ferric hydroxamate method,[7] 0.5-ml aliquots being added to 2.0 ml of 20% ferric chloride hexahydrate in 1.4 N HCl, and monitoring at 540 nm, after 2.0 min. The experimental concentrations are listed in Table I. This method was also used to study equilibria between hydroxylamine and amide, and amine and formhydroxamic acid. A stock buffer of amine and hydroxylamine was prepared and 25.0-ml samples were added successively to flasks containing, respectively, the amide and the FHA, to give an initial concentration of 10^{-2} M in each. The experimental conditions are given in Table II.

The equilibrium between hydrazine and formamide, and ammonia and formylhydrazine, was monitored by the direct spectrophotometric observation of the formylhydrazine at 230 nm.[5] This method was not as satisfactory as the ferric hydroxamate procedure. The initial and final pH were recorded on a Radiometer 26 meter calibrated at pH 1.10, 4.005, 6.48, and 8.96. The concentrations of un-ionized and ionized amines were calculated from the pK_a and the measured final pH.

The pK_a of dimethylamine was determined from pH values of partially neutralized solutions of 0.35 M total amine concentration, with added KCl. The pK_a of FHA was found by titration of a 0.01 M solution to be 8.51 ± 0.02 at ionic strength 1.0 (added KCl) and 25°. As the concentration of free hydroxylamine in acid solution was accurately required and this had to be obtained from the pH and pK_a, the effect of ionic strength and nature of the cation employed was investigated. The results are given in Table III.

Results

The most rigorous method of determining equilibrium constants is to approach the equilibrium from both sides and measure the equilibrium concentrations of reagents. However, in some cases the attainment of equilibrium is too slow or side reactions become too important to measure equilibrium concentrations.

An alternative method is to measure the rate constants for the forward and backward reactions so as to determine the equilibrium constant from their ratio. However, as pointed out by Krupka, *et al.*,[8] this technique leads to incorrect results if the forward and reverse

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(8) R. M. Krupka, H. Kaplan, and K. J. Laidler, *Trans. Faraday Soc.*, **62**, 2754 (1966).

Table III. Effect of Salts on the pK_a of Hydroxylamine at 25°

H_2NOH, M	H_2NOH–HCl, M	K^+, M	Na^+, M	Cl^-, M	Ionic strength	pK_a[a]
0.1	0.1	0.9		1.0	1.0	6.13
0.1	0.1	0.1	0.8	1.0	1.0	6.06
0.5	0.5	0.5		1.0	1.0	6.10
0.1	0.1	1.5		1.6	1.6	6.16
0.1	0.1	0.1	1.4	1.6	1.6	6.02
0.02	0.02	0.01	1.99	2.0	2.0	5.97
0.004	0.004	0.004		0.004	0.004	6.01
0.010	0.010	0.010		0.010	0.010	6.02

[a] The pH of half-neutralization, ±0.02 pH unit.

rate constants are determined under conditions of differing kinetic order.

In the particular case of the equilibria between amides and esters or acids, the rate expressions generally contain kinetic terms of complex order in amine concentration.[9,10] Also, changes of the rate-determining step occur from formation to breakdown of the tetrahedral intermediate with changing pH. This may be exemplified from the classic study of Blackburn and Jencks[5] on the aminolysis of methyl formate. The reaction of hydrazine with methyl formate has both first- and second-order terms in hydrazine concentration of 5.0 min^{-1} M^{-1} and 700 min^{-1} M^{-2}, respectively. Also, the reaction undergoes a change in the rate-determining step from tetrahedral intermediate formation to breakdown at about pH 9, leading to a 50-fold change in observed rate constant. It is clear that the determination of the equilibrium constant between hydrazine and methyl formate and methanol and formylhydrazine by the method of ratios of initial rates will be in great error unless each is determined under identical conditions of amine concentration and pH.

Following the procedure of Jencks, who has emphasized the importance of such measurements,[2,11] we have usually followed reactions from both sides using reaction mixtures identical in the buffer components which are in large excess over the changing reagents. For such systems it may be demonstrated easily that the ratio of initial *changes* in concentration at any time t is identical with those of final changes in concentrations.

$$A + B + X \rightleftharpoons A + B + Y;\ A,B \gg X,Y \qquad (1)$$

$$\left(\frac{\Delta X}{\Delta Y}\right)_t = \left(\frac{\Delta X}{\Delta Y}\right)_{t=\infty} \qquad (2)$$

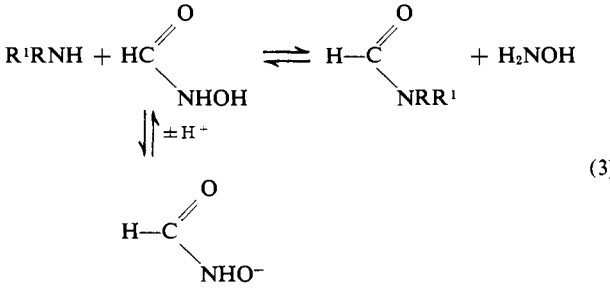
It is not necessary to determine initial rates or rate constants but just concentration changes to determine the equilibrium constant.

The major difficulty in determining the equilibrium constants is the slow rate of attainment of equilibrium, for example, the study of Morawetz and Otaki.[1] In some cases, however, enzymatic catalysis has been used to attain equilibrium, *e.g.*, for ammonia with glutamic acid catalyzed by gluataminase,[12] *N*-benzoyl-L-tyrosine with glycinamide[13] and glycanilide,[14] *N*-acetyl-L-tyrosine and hydroxylamine[2] catalyzed by chymotrypsin, and other examples reviewed by Jencks.[2] The most thoroughly investigated equilibria are those involving hydroxamic acids,[2] due to their ease of assaying by the ferric chloride method.[4]

Earlier studies[2,15,16] have shown that the kinetically reactive species in the formation of amides from amines and acid are the undissociated forms of the reagents (although amines do react very slowly with carboxylate ions[1]). Also the reactions are subject to acid catalysis.[2]

With these observations in mind, and also the well-known high reactivity of formates, the equilibria of formic acid with reactive α-effect nucleophiles of low pK_a were measured directly in acid solution. In acid solution (0.2–0.4 *N* HCl) the apparent equilibrium constants for amide formation from semicarbazide and thiosemicarbazide with formic acid are close to unity, allowing the reagent concentrations to be directly and accurately measured by the convenient assay for hydrazines given in the Experimental Section.

Having directly determined the equilibrium constant for formhydroxamic acid formation from hydroxylamine and formic acid (as done for acetohydroxamic acid[2]), the equilibrium constants between formhydroxamic acid and other amides were measured by setting up the following equilibria (eq 3). Control



(3)

experiments show that the setting up of such equilibria is fast with respect to the hydrolysis of the amides. The FHA ionizes with a pK_a of 8.51 at 25° and ionic strength 1.0. This was found to be convenient since at high pH the apparent equilibrium constant for the FHA formation is enhanced since the kinetically inert hydroxamate ion prevents the equilibrium from being displaced in favor of the amides of the more basic amines. The free

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Table IV. Hydrolysis Equilibria for Formamides at 25°, Ionic Strength 1.0[a]

Amide	pK_a[b]	K_{eq}[c] $\frac{[>NC(=O)H]}{[>NH][HCO_2H]}$	$K'_{pH\ 7.0}$[d]	ΔG_{hydr}, pH 7.0, kcal
N-Formyl-				
Thiosemicarbazide	1.88	51.3 ± 2.5	0.0234	−2.22
Semicarbazide	3.86	(5.15 ± 0.25) × 10^2	0.235	−0.86
Trifluoroethylamine	5.84	(3.5 ± 0.25) × 10^3	1.60	+0.28
Hydrazine	8.20	(1.07 ± 0.06) × 10^5	3.09	+0.67
Morpholine	8.87	(2.24 ± 0.1) × 10^5	1.38	+0.19
Ethylamine	10.97	(1.7 ± 0.1) × 10^6	0.084	−1.48
Dimethylamine	11.06	(6.1 ± 0.7) × 10^6	0.243	−0.86
Methoxyamine	4.80	106 ± 10	0.048	−1.80
Aniline	4.85	118 ± 18	0.054	−1.73
Hydroxylamine	6.10	934 ± 37	0.38	−0.84
Ammonia	9.45	(2.42 ± 0.2) × 10^3	0.004	−3.54
N-Acetyl-				
Thiosemicarbazide	1.88	57.2	0.256	−0.81

[a] Maintained with KCl. [b] At 25° and ionic strength 1.0.[a] [c] An activity of 1.0 for water assumed. [d] Apparent equilibrium constant at pH 7.0 based on total sum of ionic and nonionic species. A pK_a of 3.66 is used for formic acid.

energies of hydrolysis of the amides were thus measured from the equilibria with FHA and the free energy of hydrolysis of the FHA. The results are given in Table IV.

The value of K_{eq} for FHA formation is about twice as large as measured for other acylhydroxamic acids under similar conditions.[2] However, the equilibrium constants for *N*-acetylthiosemicarbazide and *N*-formylthiosemicarbazide measured under identical conditions here are nearly identical. There is fair agreement between the values determined in this study for formamide and *N*-ethylformamide and those for propionamide and *N*-methylpropionamide determined in ref 1. However, there is a vast discrepancy between dimethylformamide and dimethylpropionamide. The values quoted are generally accurate to better than ±10% for this study. The dimethylpropionamide equilibrium constant as with the other propionamide measurements was determined by ratios of rate constants for forward and reverse reaction[1] under conditions which were not identical in amine concentration or pH. These values, for the reasons pointed out earlier, must be considered unreliable.

Discussion

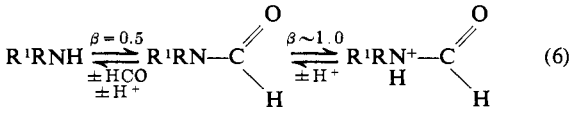
With the exception of aniline, ammonia, methoxyamine, and hydroxylamine the formation constants at 25° and ionic strength 1.0 for the formamides satisfactorily obey the equation (with pK_a values statistically corrected)

$$\log K_{eq} = 0.50 + 0.51pK_{a(amine)} \quad (4)$$

where

$$K_{eq} = \frac{[HCON<]}{[>NH][HCO_2H]} \quad (5)$$

The β value for the transfer of an amine to a constant acyl donor with the subsequent loss of a proton is 0.51. As the β value for proton transfer should be close to unity, this compares well with the β value of 1.56 for the transfer of tertiary amines to a constant acyl donor[17] (eq 6). This may be compared with the analogous transfer of alcohols and phenols to a constant acetyl



(6)

donor, *i.e.*, the formation constants for esters, which obey the equation[11a]

$$\log K_{eq} = -12.1 + 0.70pK_{a(alcohol)} \quad (7)$$

For the general case of amides of hydrazines and primary and secondary amines, an amide is (17.5 − 0.26·pK_a) kcal more stable than an ester of an alcohol of the same pK_a as the amine moiety of that amide. For the anilines and hydroxylamines the energy difference is (16.1 − 0.26pK_a) kcal. The stability of the amide of ammonia is a striking 3 kcal less than a primary or secondary amine. Jencks and coworkers[18] have determined the relative values of the equilibrium constants for acetanilide formation and obtained an absolute β value for acetyl transfer between anilines of 0.61. A value of 0.51 may be calculated from the data of Davis[19] for the formation of formanilides at 100° in 2:1 pyridine–water. In Figure 1 a line parallel to that already shown may be drawn passing through the point for aniline. In general, the equilibrium for the transfer of an acyl group to an amine is 50–60% more sensitive to the pK_a of the amine than is proton transfer to that amine.

The Free Energies of Hydrolysis of Peptides. As the free energies of hydrolysis of amides and esters are somewhat insensitive[20] to the nature of the acyl moiety, eq 4 and 7 should be generally applicable. However, formic acid is a unique carboxylic acid in that it does not contain a hydrophobic group attached to the carboxyl, but just a hydrogen atom. This is reflected in the high reactivity of formates compared with acetates

(17) A. R. Fersht and W. P. Jencks, *J. Amer. Chem. Soc.*, 92, 5432 (1970).

(18) W. P. Jencks, B. Schaffhausen, K. Tornheim, and H. White, personal communication.

(19) O. C. M. Davis, *Z. Phys. Chem.*, 78, 353 (1912); A. C. M. Davis and F. W. Rixon, *J. Chem. Soc.*, 107, 728 (1915).

(20) It is expected that these equilibrium constants be independent of the nature of the acyl portion, as they are just the measure of the relative affinities of an RO– or R′RN– to that of an –OH for a carbonyl group.[11b] However, a *charged* substituent will have a hydrolysis constant dependent on the pK_a of the carboxylic acid; for example, if RO– is O–, that is, $RCO_2H + {}^-OH \rightleftharpoons RCO_2^- + H_2O$, $\log K_{hydr} = pK_{aRCO_2H} - pK_{aH_2O}$. It is of interest that the pK_a of ^-OH ionizing to give O^{2-} may be estimated by extrapolation from eq 5 to be 33 for addition to acetic acid, and 34.4 for addition to formic acid.

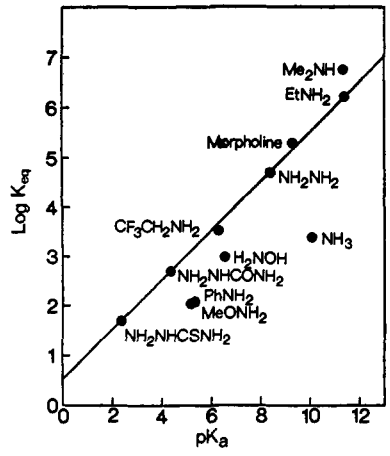


Figure 1. Plot of the formation constants of formamides against the pK_a of the amine constituent. Data are plotted for 25°, ionic strength 1.0, and for the reaction in the form $>NH + HCO_2H \rightleftharpoons HCON< + H_2O$, using an activity of 1 for water.

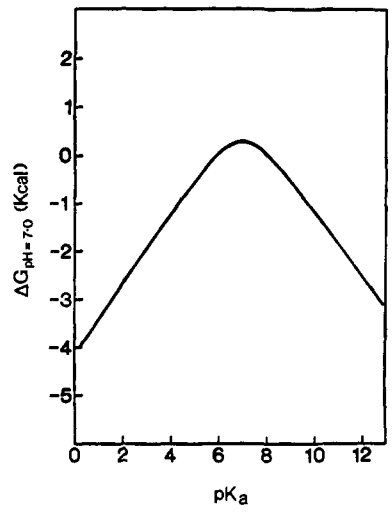


Figure 2. Plot of the free energy of hydrolysis of hypothetical peptides of a constant acyl portion (pK_a 3.66) against the pK_a (statistically corrected) of the amine constituent. Calculations are for pH 7.0 based on the total concentration of all species, ionic and nonionic, and a water activity of 1.0. Varying the pK_a of the acyl portion causes only vertical displacements in the plot (see eq 10).

which is due to the $\Delta S^{\ddagger}$ term in the kinetic equation, and not the $\Delta H^{\ddagger}$, and is presumably due to solvation effects.[21]

Accordingly, the formamides appear to have formation constants 2–3 times greater than for amides of other carboxylic acids including amino acids. For example, formhydroxamic acid has a formation constant 2.57 times greater than that for *N*-acetyltyrosinehydroxamic acid,[22] the constant for *N*-acetyl-L-phenylanine-L-tyrosine ethyl ester is 2.5 times smaller than that predicted from eq 4, and that for *N*-benzoyltyrosineglycinamide[13] is some 2–3 times smaller than predicted. The equilibrium constants for peptide formation may be estimated from those for formamide formation using an attenuation factor of 2.5. Equation 4 may be modified to give eq 8, which predicts the variation of peptide formation constant for varying amine constituent of the peptide bond, concentrations being expressed as in eq 5 and pK_a values statistically corrected. Alternatively, if the

$$\log K_{eq} = 0.1 + 0.51pK_{a(amino)} \quad (8)$$

statistical correction is ignored

$$\log K_{eq} = 0.3 + 0.51pK_{a(amino)} \quad (9)$$

The calculation of the free energy of hydrolysis at any pH must allow for the ionization of both carboxylic acid and amine and is a function of the respective pK_a values. For example, the free energies of hydrolysis at pH 7 and 25° follow eq 10 (no statistical correction) for a peptide of an (amino) acid carboxyl group $pK_a = pK_{HA}$, and an amino (acid) of $pK_a = pK_N$. The amides

$$\Delta G_{pH\ 7.0} = -0.41 + 0.66pK_{a_N} - 1.36pK_{a_{HA}} + 1.36[\log \{1 + 10^{(pK_{a_{HA}}-7)} + 10^{(7-pK_{a_N})} + 10^{(pK_{a_{HA}}-pK_{a_N})}\}] \quad (10)$$

of the anilines and hydroxylamines are, of course, 0.7–0.8 kcal, and those of ammonia 3.1 kcal, less stable than predicted by the equations. Carpenter[23] has given an extensive analysis of the pH dependence of such equilibria and the general relationships between different types of compounds.

Equation 10 is plotted in Figure 2. The free energy of hydrolysis of peptides of an acyl portion of pK_a 3.66 is plotted against the pK_a of the amino portion. The thermodynamically most stable peptides at pH 7.0 are seen to be those of an amino constituent of pK_a 7. Acyl-blocked peptides generally have a pK_a between 7.5 and 8.5, so that at physiological pH naturally occurring peptides are among the thermodynamically most stable amides. Also, at this pH, amines with a pK_a in the region 7–8 are kinetically the most reactive toward the carbonyl group.

Reactivity and Stability. Certain nucleophiles which have an electronegative atom next to the nucleophilic center, such as the hydroxylamines, hydrazines, and hypochlorite ion, are hyperreactive toward the carbonyl group.[24] This is termed the *α effect*.[25] It has been suggested[10] that in general the factors which stabilize the transition state stabilize also the product. Indeed this has been shown to occur for some *oxyanion* nucleophiles with esters[11b] and also for peroxides with saturated carbon.[26] However, it is clear from Figure 1 that the thermodynamic stability of the acylated amine bears no relationship to the kinetic reactivity of the amine toward the carbonyl group. Indeed, the hyperreactive hydroxylamine forms a much less stable product than a non-α-effect equivalent amine. Similarly, aniline and ammonia have normal reactivity toward *p*-nitrophenyl acetate[24] in terms of the Brønsted relationship.

A pointer toward this conclusion is in the study of the aminolysis of methyl formate[5] where it is pointed out that although hydrazine has an enhanced rate of attack on methyl formate, the tetrahedral intermediate so formed reverts to reagents at a correspondingly faster rate, so that the adduct formation has an equilibrium constant similar to that for a non-α-effect amine of sim-

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ilar pK_a. It is also shown in the following paper[27] that the initial attack of methoxide ion on formylhydrazine to give the tetrahedral intermediate has a rate constant expected for that of the attack on an amide of a non-α-effect amine of similar pK_a.

The present ideas on the nature of the α effect are discussed in ref 10 and 28. The combination of our study and that of Blackburn and Jencks[5] requires, at least for the case of hydrazine and probably by analogy for hydroxylamine, that any explanation of the α effect based on either the increased stability of the resultant acylhydrazine or decreased stability of the nucleophile is highly suspect. The α effect for hydrazinolysis appears to be due to the increased stability of the transition state for tetrahedral intermediate formation.

The decreased thermodynamic stability of formamide deserves some comment. The geometries of NH_3 and NH_4^+ are highly compatible with that of water which could lead to high solvation energies,[29] but in formamide these interactions are lost. The nucleophilic reactivity of ammonia is not decreased with respect to primary and secondary amines by a factor equivalent to the decreased stability of the resultant amide so that any special solvation effects are not manifested in the transition states of ammonolysis reactions. The obvious structural differences of anilides and hydroxamic acids in relation to the other amides perhaps preclude direct comparison of their free energies of hydrolysis due to possible bond energy changes, but the similarity of formamide makes a solvation energy explanation attractive.

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Acyl-Transfer Reactions of Amides and Esters with Alcohols and Thiols. A Reference System for the Serine and Cysteine Proteinases. Concerning the N Protonation of Amides and Amide–Imidate Equilibria

Alan R. Fersht

Contribution from the M.R.C. Laboratory of Molecular Biology, Cambridge, England. *Received September 18, 1970*

Abstract: The absolute magnitudes of the rate constants for the alcoholysis and thiolysis of some amides and esters have been obtained. Amides show the reactivity order: acylhydroxylamines $\gg$ acylhydrazines $\sim$ acyl primary amines $>$ acyl secondary amines. Within a single class of amides the reactivity increases with increasing pK_a of the amine moiety. Reactivity also increases with decreasing pK_a of the nucleophilic alcohol or thiol. In contrast, the reaction of alcohols with esters increases with increasing nucleophile pK_a and decreasing leaving group pK_a. The requirement for concurrent acid catalysis can lead to an alkoxide ion having a lower nucleophilicity than an apparently un-ionized alcohol. A method is given for the calculation of pK_a's for N protonation of amides. Comparisons are drawn between the enzymatic and nonenzymatic reactions and some speculated mechanisms are shown to be inconsistent. The resonance energy of amides is calculated to be 17–18 kcal/mol.

Despite the large amount of work done on the hydrolysis of peptides and amides by chymotrypsin and papain there are no systematic studies of the intermolecular alcoholysis of amides with which to provide a reference framework for the enzymatic studies. Structure–reactivity relationships have provided in past studies probably the most generally informative knowledge about the nature of chemical reactions. Some such studies have been performed with chymotrypsin, but without controlled chemical studies for comparison.

The absence of information on the alcoholysis of amides is due to the excessively slow rates of the uncatalyzed reactions. In a recent paper[1] we have emphasized the use of free energies of hydrolysis to obtain rate constants for experimentally inaccessible acyl-transfer reactions. Fortunately, the reverse reaction of amide alcoholysis, that is, ester aminolysis, is well studied.[2–5] The rate constants for this, in conjunction with the free energies of hydrolysis of amides which we have just measured,[6] and of esters, measured by Jencks and coworkers,[7–9] provide the rate constants for the alcoholysis reactions. The same is true for the thiolysis reactions. Combined with the fine studies from the laboratories of Schmir[10–13] and Jencks[14] on intramolecular

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