Microscopic Protonation Equilibria and Solution Conformations of Coenzyme A and Coenzyme A Disulfides

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Microscopic acid dissociation constants have been determined for the protonated N1 nitrogen of the adenine ring and the 3′-phosphate and thiol groups of coenzyme A (CoASH) and the protonated N1 nitrogen and 3′-phosphate groups of symmetrical coenzyme A disulfide (CoASSCoA) and coenzyme A–glutathione mixed disulfide (CoASSG) from chemical shift vs pD data. The microscopic constants for the N1 nitrogen and 3′-phosphate groups in the three compounds are essentially identical. In addition to the expected sensitivity of chemical shifts to titration of proximate acidic groups, resonances for the carbon-bonded protons of the pantoate moieties in all 3 coenzyme A compounds shift upfield upon deprotonation of the distant N1 nitrogen and 3′-phosphate groups, while those of the cysteamine moiety shift upfield upon deprotonation of the N1 nitrogen. The increased shielding of the pantoate and cysteamine protons is interpreted to indicate a decrease in the population of folded conformations in which the pantetheine tail is coiled around the adenine ring. The results also indicate that oxidation of the thiol group has little affect on the acid/base chemistry and the solution structures of the coenzyme A moiety in coenzyme A symmetrical and mixed disulfides.

Introduction

Coenzyme A serves multiple functions in biological systems, foremost being the transfer of acyl groups. Its mode of action in acyl transfer reactions involves the thiol group of its cysteamine moiety accepting an acyl group from a donor and transferring it to an acceptor. The thiol group of coenzyme A (CoASH) is also readily oxidized, for example, by thiol/disulfide interchange reactions, with the formation of symmetrical coenzyme A disulfide (CoASSCoA) and mixed disulfides such as coenzyme A–glutathione mixed disulfide (CoASSG). The latter disulfide has been identified in a number of organisms,[1-4] where it has been suggested to be a storage form of coenzyme A during certain stages of growth.[5]

In this paper, we report the results of an NMR study of the microscopic acid/base chemistry of coenzyme A, its symmetrical disulfide, and its mixed disulfide with glutathione. Our objectives in this work were 2-fold: (i) to obtain protonation constants for use in studies of the solution chemistry of coenzyme A and its derivatives, including studies of the kinetics and equilibria of their thiol/disulfide interchange reactions and (ii) to learn about the solution structures of the coenzyme A moiety in disulfide derivatives of coenzyme A. Coenzyme A itself is reported to exist as a fast exchange system of linear and folded species.[6]

Results and Discussion

Coenzyme A (Figure 1) consists of two major parts, adenosine 3′,5′-diphosphate and pantetheine phosphate, with the two parts joined via a pyrophosphate linkage at the 5′-phosphate. The adenosine 3′,5′-diphosphate part consists of two subunits, an adenine ring and a ribose ring with phosphate groups on the 3′- and 5′-hydroxyls, while the pantetheine tail is made up of pantoic acid, β-alanine, and cysteamine subunits, which are linked via amide bonds. Coenzyme A has multiple sites with Bronsted acid/base properties in aqueous solution, including the 3′-phosphate, pyrophosphate, and thiol groups and the N1, N3, and N7 nitrogens of the adenine ring. The structure in Figure 1 shows coenzyme A as it exists at neutral pH, with all groups deprotonated except the cysteamine thiol group. Over the pH range 2–12, the N1 nitrogen of the adenine ring and the 3′-phosphate and thiol groups undergo protonation/deprotonation reactions.[7] Microscopic acid dissociation constants were determined for these groups from the dependence of the chemical shifts of the carbon-bonded protons and the phosphorus atoms on pD.

Representative chemical shift titration curves for carbon-bonded protons of the adenine, ribose, pantoate, and cysteamine subunits are shown in Figures 2 and 3.[8] The chemical shifts of carbon-bonded protons near sites of protonation or deprotonation are expected to change due to through-bond electronic effects.[9] For example, the chemical shifts of protons 2 (Figure 2) and 8 of the adenine ring change over the pD range 2–6 as the protonated N1 nitrogen is titrated[7] and then remain essentially constant up to pD 13. Likewise, the chemical shifts of protons f (Figure 2) and g of the cysteamine moiety change over the pD range 8–12 as the thiol group is titrated. The results in Figure 2 show, however, that the chemical shift of the f protons is also sensitive to titration of the protonated N1 nitrogen of the adenine moiety, even though they are separated from N1 by 25 bonds. Likewise, it is found that the chemical shifts of protons of the pantoate moiety also change as quite distant acidic groups are titrated. For example, the data in Figure 3 show that the chemical shift of pantoate methyl group b changes by a total of ~0.111 ppm over the pD range 2–6 as the protonated N1 nitrogen and the 3′-phosphate group are titrated, even though the methyl protons are separated by 17 and 14 bonds, respectively, from the protonation sites. The sensitivity of the pantoate chemical shifts to titration of distant groups

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(6) Lee, C.-H.; Sarma, R. H. *J. Am. Chem. Soc.* **1975**, *97*, 1225–1236.

(7) The N1 nitrogen is assumed to be the protonation site of the adenine ring of CoASH on the basis of ^{15}N NMR results which show that N1 is the protonation site of adenine and several of its derivatives: Markowski, V.; Sullivan, G. R.; Roberts, J. D. *J. Am. Chem. Soc.* **1977**, *99*, 714–718 (b) Gonnella, N. C.; Nakanishi, H.; Holtwick, J. B.; Horowitz, D. S.; Kanamori, K.; Leonard, N. J.; Roberts, J. D. *J. Am. Chem. Soc.* **1983**, *105*, 2050–2055. (c) Schindler, M. *J. Am. Chem. Soc.* **1988**, *110*, 6623–6630.
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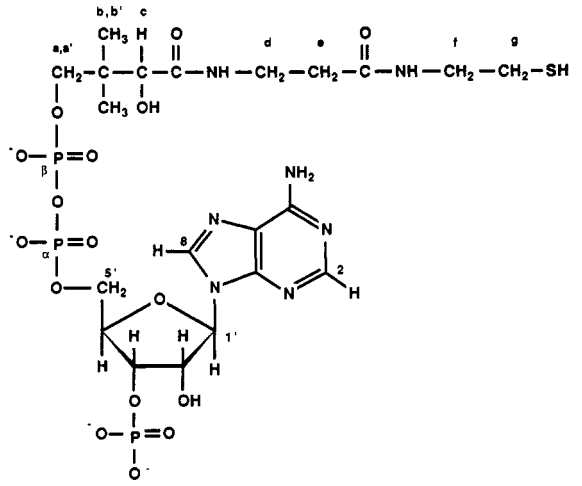

Figure 1. The structure of coenzyme A.

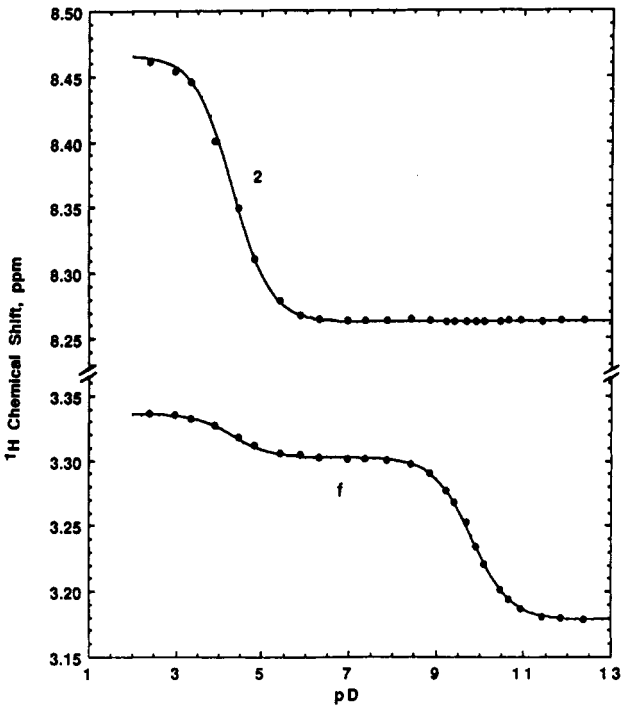

Figure 2. Chemical shifts of proton 2 of the adenine ring and proton f of the cysteamine moiety of CoASH (assignments given in Figure 1) as a function of pD. The smooth curves through the experimental points were simulated using the pK values and limiting chemical shifts obtained from the nonlinear least-squares fits of the data.

is due to through space effects, as discussed below in terms of the solution conformation of CoASH.[6]

Acid dissociation constants for the protonated N1 nitrogen (pK_{N1}), 3′-phosphate (pK_{PO_4}) and thiol (pK_{SH}) groups of CoASH were determined by fitting chemical shift vs pD data to monoprotic or diprotic acid models. For example, pK_{N1} was determined by fitting chemical shift data for protons 2 and 8 of the adenine ring to a monoprotic acid model. The values obtained for pK_{N1} are listed in Table I. The limiting chemical shifts of protons 2 and 8, i.e., their chemical shifts when N1 is in the protonated and deprotonated forms, were also obtained from the fits of the chemical shift data. The smooth curve through the experimental data for proton 2 in Figure 2 was calculated using the values obtained for pK_{N1} and the limiting chemical shifts by fitting the data. Adenine N1 titration shifts for protons 2 and 8, i.e., the change in chemical shift of protons 2 and 8 upon titration of the protonated adenine

Table I. Microscopic Acid Dissociation Constants for Coenzyme A, Coenzyme A Disulfide, and Coenzyme A–Glutathione Mixed Disulfide[a,b]

acid dissocn constant	proton											
	a	a′	b	b′	c	f	g	2	8	1′	5′	average[c]
						CoASH						
pK_{N1}	4.25 (±0.12)	4.20 (±0.10)	4.35 (±0.09)	4.32 (±0.09)	4.46 (±0.16)			4.34 (±0.03)	4.24 (±0.04)	4.34 (±0.03)	4.24 (±0.07)	4.32 (±0.09)
pK_{PO_4}	6.32 (±0.24)	6.28 (±0.21)	6.31 (±0.26)	6.30 (±0.22)	6.37 (±0.26)					6.17 (±0.14)	6.48 (±0.25)	6.31 (±0.08)
pK_{SH}						9.83 (±0.01)	9.82 (±0.01)					
						CoASSCoA						
pK_{N1}	4.39 (±0.09)	4.35 (±0.06)	4.41 (±0.13)	4.41 (±0.13)				4.31 (±0.03)	4.37 (±0.03)	4.34 (±0.04)	4.45 (±0.12)	4.38 (±0.05)
pK_{PO_4}	6.33 (±0.14)	6.27 (±0.06)	6.17 (±0.24)	6.17 (±0.24)							6.49 (±0.26)	6.27 (±0.11)
						CoASSG						
pK_{N1}	4.40 (±0.06)	4.14 (±0.08)	4.33 (±0.02)	4.30 (±0.05)	4.37 (±0.04)			4.42 (±0.03)	4.45 (±0.01)	4.42 (±0.01)	4.33 (±0.06)	4.34 (±0.09)
pK_{PO_4}	6.70 (±0.14)	6.52 (±0.17)	6.52 (±0.07)	6.61 (±0.09)	6.67 (±0.09)					6.55 (±0.08)	6.62 (±0.13)	6.59 (±0.06)
p$K_{Gly\text{-}COOH}$												3.65 (±0.03)
p$K_{Glu\text{-}COOH}$												2.22 (±0.05)
p$K_{Glu\text{-}NH_3}$												9.33 (±0.01)

[a] Determined from nonlinear least-squares curve fits of chemical shift vs pD data for the various carbon-bonded protons; proton assignments are given in Figure 1. [b] Uncertainities are the standard error of the estimate from the nonlinear least-squares fit. [c] Uncertainities are the standard deviation.

Table II. Titration Shifts for the Carbon-Bonded Protons of Coenzyme A, Coenzyme A Disulfide, and Coenzyme A–Glutathione Mixed Disulfide[a-c]

titration shift	proton a	a′	b	b′	c	d	e	f	g	2	8	1′	5′
						CoASH							
$\Delta\delta_{N1}$	0.055	0.062	0.079	0.079	0.039	*0.034*	*0.035*	*0.031*	*0.021*	0.204	0.162	0.068	0.042
$\Delta\delta_{PO_4}$	0.027	0.030	0.032	0.036	0.019	*0.007*	*0.007*					0.015	0.015
$\Delta\delta_{SH}$	*0.003*	*0.007*	*0.003*	*0.009*	*0.009*	*0.019*	*0.030*	0.124	0.097				
						CoASSCoA							
$\Delta\delta_{N1}$	0.039	0.070	0.053	0.073	*0.019*	*0.017*	*0.022*	*0.019*	*0.022*	0.236	0.150	0.052	0.051
$\Delta\delta_{PO_4}$	0.024	0.028	0.030	0.032			*0.006*	*−0.011*	*−0.013*				0.017
						CoASSG							
$\Delta\delta_{N1}$	0.044	0.055	0.068	0.073	0.032	*d*	*0.033*	*d*	*0.037*	0.203	0.160	0.074	0.034
$\Delta\delta_{PO_4}$	0.021	0.029	0.027	0.031	0.015	*d*	*0.006*	*d*	*−0.008*			0.013	0.016

[a] Changes in chemical shift upon deprotonation of the N1, 3′-phosphate and SH groups. [b] Titration shifts in normal type were obtained from the nonlinear least-squares fits of the data; those in italics were estimated from the chemical shift vs pD titration data. [c] Proton assignments are given in Figure 1. [d] Obscured by overlap.

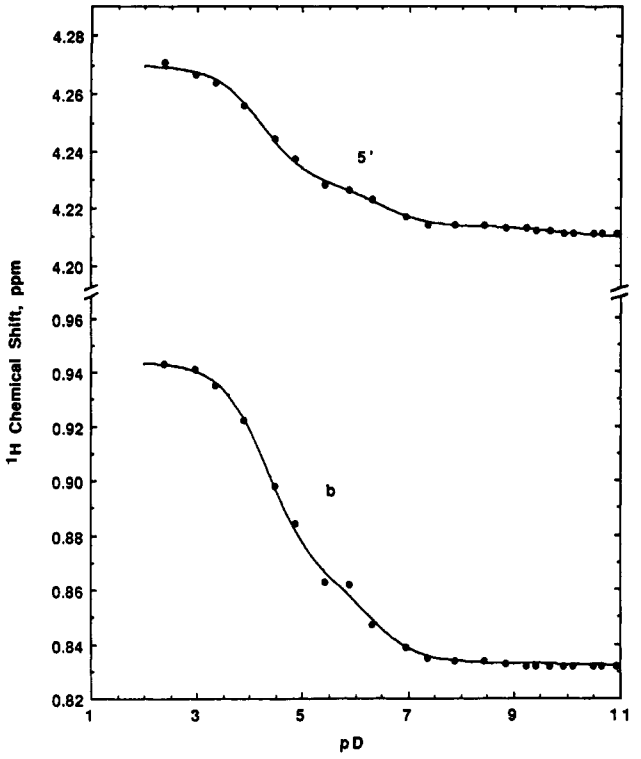

Figure 3. Chemical shifts of the 5′ proton of the ribose ring and methyl group b of the pantoate moiety of CoASH as a function of pD. The smooth curves through the experimental points were simulated using the pK values and limiting chemical shifts obtained from the nonlinear least-squares fits of the data.

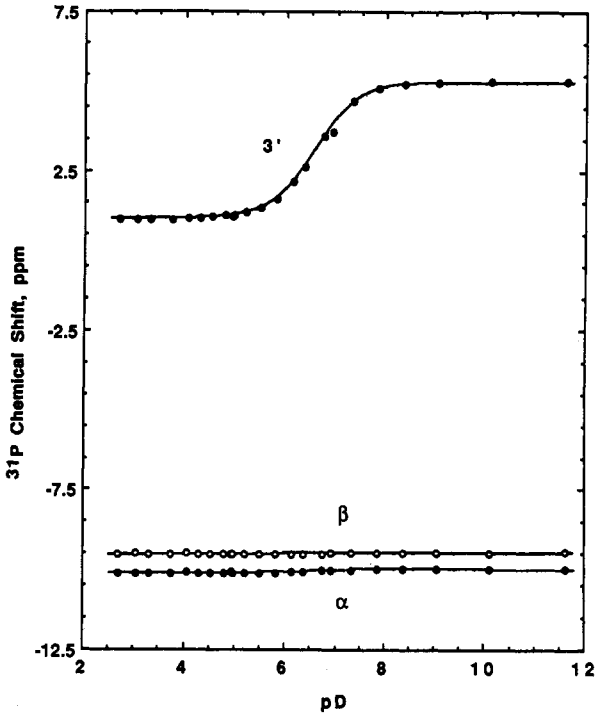

Figure 4. Chemical shifts of the phosphorus atoms (vs H_3PO_4) of the 3′-phosphate and pyrophosphate groups as a function of pD. The smooth curve through the 3′-phosphate chemical shift data was simulated using the values obtained for pK_{PO_4} and the limiting chemical shifts from the nonlinear least-squares fit of the data to a monoprotic acid model.

N1 nitrogen ($\Delta\delta_{N1}$), calculated from the limiting chemical shifts are reported in Table II.

The pD 8–12 regions of the chemical shift titration curves for protons f and g of the cysteamine moiety were fitted to a monoprotic acid model, from which pK_{SH} (Table I) and the limiting chemical shifts of protons f and g, i.e., the chemical shifts when the thiol group is in the thiol and thiolate forms, were obtained.[10] The titration shifts calculated from the limiting chemical shifts are given in Table II. Also reported in Table II are the adenine N1 titration shifts for protons f and g. The smooth curve through the experimental data for proton f in Figure 2 is a simulated curve, calculated using pK values and limiting chemical shifts obtained from computer fits of the data.

The chemical shift titration curves in Figure 3 are more complicated, both showing two overlapping titration steps; the first corresponds to titration of the protonated N1 nitrogen of the adenine moiety and the second to titration of the 3′-phosphate group. Similar chemical shift titration curves were observed for protons a, a′, b′, c, and 1′. The titration curves for the protons of the pantoate moiety and for 1′ and 5′ were all fitted to a diprotic acid model, from which pK_{N1}, pK_{PO_4} (Table I) and the chemical shifts of each protonated form of CoASH were obtained. The titration shifts calculated from the limiting chemical shifts are reported in Table II. The smooth curves through the data in Figure 3 were simulated using the parameters obtained by fitting the chemical shift data.

Chemical shifts vs pD data for the 3′-phosphate resonance and the resonances for the α and β phosphorus

(10) Previous estimates of pK values for CoASH are: (a) pK_{N1} = 4.0 (incorrectly assigned to adenine NH_{3+}), pK_{PO_4} = 6.4 and pK_{SH} = 9.6: Beinert, H.; Von Korff, R. W.; Green, D. E.; Buyske, D. A.; Handschumacher, R. E.; Higgins, H.; Strong, F. M. *J. Biol. Chem.* **1953**, *200*, 385–400. (b) pK_{SH} = 10.35: Pitman, I. H.; Morris, I. J. *Aust. J. Chem.* **1980**, *33*, 1625–1630. (c) pK_{SH} = 9.77: Shiu, M. H.; Braun, R. D. *J. Electrochem. Soc.* **1981**, *128*, 2103–2106.

atoms of the pyrophosphate group are presented in Figure 4. The chemical shift of the 3′-phosphate resonance changes by 4.26 ppm over the pD range 4–8, while the resonances for the α and β phosphorus atoms of the pyrophosphate group shift by ~0.1 and <0.1 ppm, respectively, which confirms that 3′-phosphate is protonated at low pD. A value of 6.17 was obtained for pK_{PO_4} from a fit of the 3′-phosphate chemical shift data to a monoprotic acid model. It is interesting to note that, in contrast to the resonances for the ribose and pantetheine protons, the ^{31}P resonances are not sensitive to titration of the adenine N1 nitrogen or the cysteamine thiol groups.

The chemical shift titration curves for the carbon-bonded protons of coenzyme A disulfide and the coenzyme A part of coenzyme A–glutathione mixed disulfide are similar to those of CoASH, except for the thiol group titration shifts in the CoASH titration curves. Values obtained for pK_{N1} and pK_{PO_4} and the N1 and 3′-phosphate titration shifts by fitting chemical shift titration curves for CoASSCoA and the CoA part of CoASSG are reported in Tables I and II. Also reported in Table I are acid dissociation constants determined for the two carboxylic acid groups and the ammonium group of the glutathione part of CoASSG by fitting chemical shift data for glutathione protons.[11]

The acid dissociation constants in Table I are for specific groups, as distinct from the macroscopic constants which are obtained from pH titration data. Thus, the average pK_{N1} values for CoASH, CoASSCoA, and CoASSG in Table I indicate that the acidity of the protonated N1 nitrogens of the adenine moieties of these three compounds is identical. The same is true of the acidity of the monoprotonated 3′-phosphate groups of CoASH and CoASSCoA, while that of CoASSG is less acidic by ~0.3 pK units. However, because the acidities of the N1, 3′-phosphate, and thiol groups of CoASH are quite different, the microscopic group constants for CoASH in Table I are essentially equal to its macroscopic constants, i.e., pK_1 = 4.32, pK_2 = 6.31, and pK_3 = 9.83.[12] However, for CoASSCoA, the two protonated N1 nitrogens titrate simultaneously, as do the two 3′-phosphate groups, and thus its microscopic group constants in Table I differ from its macroscopic constants. In terms of macroscopic acid dissociation constants, the acid/base chemistry of CoASSCoA is described by four dissociation constants, which are calculated from the average values for the microscopic constants in Table I to be pK_1 = 4.07, pK_2 = 4.67, pK_3 = 5.98, and pK_4 = 6.58.[13] The microscopic group constants are useful for describing the protonation states of specific groups, while the distribution of CoASSCoA among its mono-, di-, tri-, and tetraprotonated forms can be described in terms of the macroscopic constants, as illustrated by the fractional deprotonation and species distribution diagrams in Figure 4. For CoASSG, the macroscopic constants will also differ from the microscopic constants due to overlap of the pH regions for titration of the carboxylic acid groups of the glutathione moiety and N1 of the coenzyme A moiety.

The titration shifts in Table II are due to both through-bond electronic effects and through-space effects. For example, the chemical shifts of the carbon-bonded protons of the pantoate subunits of CoASH, CoASSCoA, and CoASSG change upon titration of the protonated N1 nitrogen and 3′-phosphate groups, even though they are separated from the N1 nitrogen by 15–17 bonds and from the 3′-phosphate group by 10–12 bonds. The through-space titration shifts provide information about the solution structures of CoASH, CoASSCoA, and CoASSG.

Lee and Sarma have established from vicinal coupling constants that, in general, the two major parts of CoASH, i.e., the adenosine 3′,5′-phosphate and pantetheine phosphate moieties, have conformations similar to those of the free subunits.[6] They also concluded from the downfield shift, i.e., the deshielding of the resonances for the pantetheine protons of CoASH relative to those of free pantetheine phosphate, that CoASH exists as a fast exchanging system of linear and folded species, with the pantetheine tail coiled around the adenine ring in the folded species. In this folded structure, the majority of the pantetheine protons are in regions deshielded by adenine ring current effects. It was proposed that interchange between the linear and folded species occurs via rotation about the P–O–P linkage.[6]

The data in Table II show that all the carbon-bonded protons of the pantetheine tail of CoASH experience an upfield shift, i.e., there is less deshielding, upon titration of the protonated adenine N1 nitrogen, which suggests a decrease in the population of folded conformations upon titration of the protonated N1 nitrogen. However, the absence of any change in the chemical shifts of the f ang g protons upon titration of the 3′-phosphate group suggests that the protonation state of the 3′-phosphate group has no affect on the folding of the pantetheine part around the adenine moiety. The titration shifts of the pantetheine protons in Table II suggest that the CoA moieties of CoASSCoA also exist in a linear ⇌ folded equilibrium with the pantetheine tail coiled around the adenine ring in the folded conformation and that the two halves of CoASSCoA behave independently. The results indicate the same is true for the coenzyme A part of CoASSG, which suggests that oxidation of the thiol group to form symmetrical and mixed disulfides has little affect on the solution structure of the coenzyme A moiety.

Experimental Section

Coenzyme A, coenzyme A disulfide, and coenzyme A–glutathione mixed disulfide were obtained from Sigma Chemical Co. Solutions were prepared in degassed D_2O containing 0.15 M NaCl. CoASH, CoASSCoA, and CoASSG concentrations were 0.010, 0.005, and 0.001 M, respectively. 2-Methyl-2-propanol was added for an internal ^1H chemical shift reference. The pD was adjusted with DCl or NaOD, and 0.75-mL NMR samples were removed at the appropriate pD values. ^1H and ^{31}P NMR spectra were measured with a Varian VXR-500S spectrometer at a probe temperature of 25 °C. pD measurements were made using combination microelectrodes which were calibrated with pH 4.00, 7.00, and 10.00 aqueous buffers (Fisher Scientific Co.). The exact pH values of the buffers were determined by comparison with primary standard buffers prepared according to NBS procedures. pH meter readings were converted to pD values for D_2O solutions with the equation pD = pH meter reading + 0.40.

Acid dissociation constants were determined by fitting NMR chemical shift titration data to monoprotic and diprotic acid models by nonlinear least-squares methods. For a monoprotic acid system, the observed chemical shift is given by eq 1

$$\delta_{obs} = f_{HA}\delta_{HA} + f_A\delta_A \quad (1)$$

(11) Microscopic pK values for the COOH groups of the glycine glutamyl residues and the ammonium group of glutathione in H_2O are 3.22, 2.19, and 9.17, respectively. Rabenstein, D. L. In *Glutathione: Chemical, Biochemical and Medical Aspects*; Dolphin, D.; Poulson, R.; Avramovic, O., Eds.; Wiley-Interscience: New York, 1989; Part A, pp 147–186.

(12) The macroscopic constants for CoASH are related to its microscopic group constants as follows: $K_1 = K_{N1} + K_{PO_4} + K_{SH}$, $K_2 = (K_{N1}K_{PO_4} + K_{N1}K_{SH} + K_{PO_4}K_{SH})/K_1$ and $K_3 = (K_{N1}K_{PO_4}K_{SH})/K_1K_2$. Noszal, B. In *Biocoordination Chemistry*; Burgur, K., Ed.; E. Horwood: New York, 1990; pp 18–55.

(13) The macroscopic constants are related to the microscopic constants as follows: $K_1 = 2K_{N1} + 2K_{PO_4}$; $K_2 = (4K_{N1}K_{PO_4} + K_{N1}^2 + K_{PO_4}^2)/K_1$; $K_3 = (2K_{N1}^2K_{PO_4} + 2K_{N1}K_{PO_4}^2)/K_1K_2$; and $K_4 = (K_{N1}^2K_{PO_4}^2)/K_1K_2K_3$.

where f_{HA} and f_A represent the mole fractions and δ_{HA} and δ_A the chemical shifts of the acid in its protonated and deprotonated states. The model equation for a monoprotic acid is obtained by expressing f_{HA} and f_A in terms of the acid dissociation constant, K.

$$\delta_{obs} = \frac{[H^+]\delta_{HA} + K\delta_A}{[H^+] + K} \quad (2)$$

The following model equation for a diprotic acid is derived in the same way.

$$\delta_{obs} = \frac{[H^+]^2\delta_{H_2A} + [H^+]K_1\delta_{HA} + K_1K_2\delta_A}{[H^+]^2 + [H^+]K_1 + K_1K_2} \quad (3)$$

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Registry No. CoA, 85-61-0; CoASSCoA, 31664-36-5; CoASSG, 6477-52-7.

The Problem of Regioselectivity in Nucleophilic Additions to Pyridinium and Related Cations. Role of Generalized Anomeric Effect

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A MNDO and AM1 study of the isomerization of pyrans, thiopyrans, dihydropyridines, and their methoxy derivatives was carried out so as to clarify the nature of the effects causing the observed thermodynamic regioselectivity in nucleophilic addition to pyridinium, pyrylium and thiopyrylium cations. The results show that, in contrast to 2*H*-thiopyrans, 2*H*-pyrans and 1,2-dihydropyridines are significantly stabilized by a generalized anomeric effect when the group bonded to the 2 position is OMe. It is pointed out that in the case of reversible additions, a clear distinction should be made between the kinetic and the thermodynamic regioselectivity because, in contrast with current usage, the latter cannot be interpreted in terms of frontier orbital theory but by the presence or absence of a generalized anomeric effect. It is also pointed out that predictions of kinetic regioselectivity based on frontier orbital theory are not supported by literature data. The kinetic regioselectivity seems to be governed by the relative electron density at the carbon under attack, independent of the hard or soft character of the nucleophile.

There has been considerable interest in the regioselectivity of nucleophilic attacks on pyridinium ions, due, in part at least, to the biological importance of the reduction of NAD.[1] The first attempt to rationalize the literature data on these systems was made by Kosower,[2] who related the position of attack by a nucleophile to the possible intermediacy of a charge-transfer complex (CTC) prior to the attack. In particular, nucleophiles with a low ionization potential were presumed to proceed via a CTC which subsequently would evolve into the 4*H* adduct, while nucleophiles not forming the CTC would directly attack the 2 position.

In a more recent and theoretically-rooted approach by Klopman,[3] the regioselectivity was related to the hard/soft character of the nucleophiles. According to this view, since the total charge density in a pyridinium ion is larger at C2 than at C4, whereas the coefficient of the LUMO is larger at C4 than at C2, hard nucleophiles would attack C2 (charge control), while soft nucleophiles would attack C4 (frontier orbital control). Both of the models implicitly assume that the experimental distribution of the products is due to irreversible reactions or that, in the case of reversible reactions, the lower-energy product is formed from the lower-energy transition state. However, this assumption is critical because in many cases it has been reported that the kinetically favored product differs from the product of thermodynamic control (vide infra). In fact, these experimental findings have been overlooked and the Klopman approach to the problem of regioselectivity has gained wide acceptance,[4] though it is mainly based on equilibrium data rather than kinetic data, as required by the theory.

Scheme I

1a-7a 1b-7b

1, X = O, Y = H
2, X = O, Y = OMe
3, X = S, Y = H
4, X = S, Y = OMe
5, X = NMe, Y = H
6, X = NH, Y = H
7, X = NH, Y = OMe

In order to understand the causes of the thermodynamic regioselectivity in nucleophilic additions to pyridinium and to the related pyrylium and thiopyrylium cations, we have undertaken a MNDO[5] and AM1[6] study of the equilibria reported in Scheme I. The results of this investigation are reported herein.

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