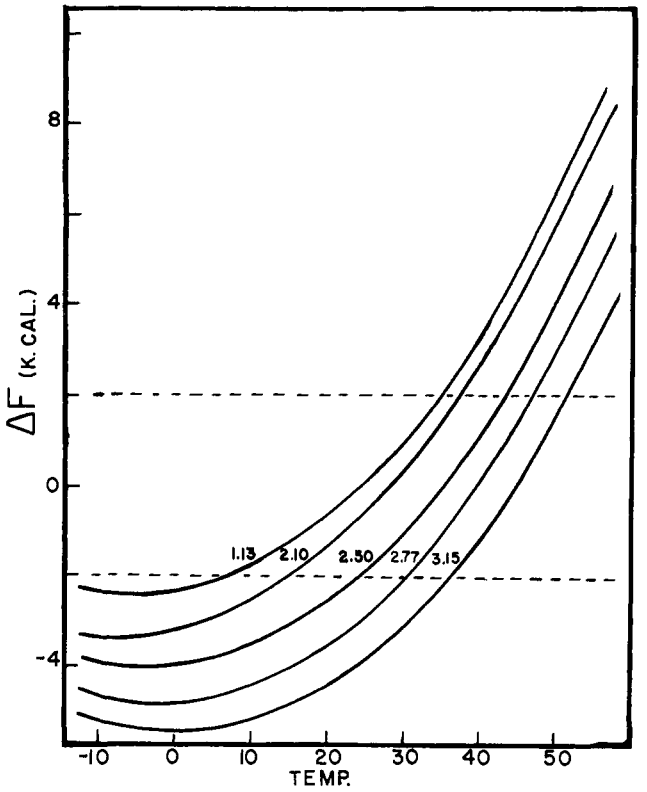

Figure 2. Free energy profiles for the ribonuclease transition assuming an equilibrium between two conformational states. These continuous curves are derived from the experimental data of Figure 1 at each pH (see text).

The best values of the three parameters at each pH were obtained by fitting eq. 1 to the experimental data by least-squares treatment. The average deviation of the experimental values of $\Delta F°$ from the calculated values using the best parameters corresponds to spectrophotometric errors of less than 2×10^{-4} optical density unit. Thus, the data of Figure 1 are completely consistent with the two-state transition as outlined above. The smooth curves in Figure 2 are the free-energy values obtained from eq. 1 at each pH. Accurate values of $\Delta F°$ can be measured directly only within the limits of $+2000$ to -2000 cal. as indicated by the dashed lines. The extension of the curves outside this range is based on the assumption that the E, F, and G parameters are temperature independent. The qualitative features of these stability curves for ribonuclease are seen to be extremely similar to those for chymotrypsinogen.[5]

The ΔC_p values in Table I are nearly independent of pH but strongly dependent on temperature. On a weight basis, these values of ΔC_p are within 10% of the values determined for the serum albumin[7] and the ferrihemoglobin[8] transitions by direct calorimetric measurements, and this provides strong support for the validity of the two-state analysis.

In conclusion, we wish to suggest that the ribonuclease transition is consistent with the assumption of an equilibrium between only two conformational states. The apparent complexity in the thermodynamics, which had previously been interpreted as an indication of intermediate conformational states, is predictable from model compound data and likely results from a structural transition in the solvent phase as the temperature is increased. The thermodynamics associated with this clathrate transition are superimposed upon the thermodynamics of the normal polypeptide unfolding transition and are most clearly manifested in the large positive ΔC_p. It is anticipated that the essential features of the ribonuclease transition will be found to be characteristic of most reversible conformational transitions when examined closely.

(7) P. Bro and J. M. Sturtevant, *J. Am. Chem. Soc.*, 80, 1789 (1958).
(8) W. W. Forrest and J. M. Sturtevant, *ibid.*, 82, 585 (1960).

Table I. Values of the Enthalpy, Entropy, and Heat Capacity Changes for the Ribonuclease A Transition Assuming a Two-State Reaction

	0°			60°		
pH	$\Delta H°$, kcal.	$\Delta S°$, e.u.	ΔC_p, cal.	$\Delta H°$, kcal.	$\Delta S°$, e.u.	ΔC_p, cal.
1.13	11	32	1230	137	442	3050
2.10	15	44	1175	135	436	2920
2.50	10	23	1180	131	416	2925
2.77	8	12	1210	132	416	3005
3.15	6	1	1180	126	395	2930

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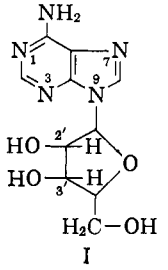
Proton Ionization from Adenosine

Sir:

Adenosine (I) is known to have at least one acidic site ($pK \sim 12.5$) in aqueous solution.[1] This would be



I

expected to be a reactive site and would likely be involved in hydrogen bonding. Zamecnik has recently[2] postulated an intramolecular hydrogen bond between the C-2′ and N-3 positions of adenosine to explain the biosynthesis of proteins. Broom and Robins[3] have shown that in a homogeneous solution of water and 1,2-dimethoxyethane methylation of adenosine by diazomethane occurs at the 2′-position, suggesting this as the acidic site. However, McLaughlin and Ingram[4] and Wolfenden, *et al.*,[5] have shown that a rapid equilibration exists between the 2′- and 3′-aminoacyl isomers in neutral and basic solution, suggesting a close similarity of the 2′- and 3′-positions.

The purpose of this communication is to report a

(1) P. A. Levene, L. W. Bass, and H. S. Simms, *J. Biol. Chem.*, 70, 229 (1926); (b) P. A. Levene, H. S. Simms, L. W. Bass, *ibid.*, 70, 243 (1926).
(2) P. C. Zamecnik, *Biochem. J.*, 85, 257 (1962).
(3) A. D. Broom and R. K. Robins, *J. Am. Chem. Soc.*, 87, 1145 (1965).
(4) C. S. McLaughlin and V. M. Ingram, *Science*, 145, 942 (1964).
(5) R. Wolfenden, D. H. Rammler, and F. Lipmann, *Biochemistry*, 3, 329 (1964).

thermometric titration study of adenine, ribose, adenosine, 2′-deoxyadenosine, 3′-deoxyadenosine, and 2′-O-methyladenosine which conclusively shows the acidity of adenosine to be associated with the presence of both the 2′- and 3′-OH groups and that substitution of H for either the 2′- or 3′-OH group or OCH_3 for the 2′-OH group results in loss of this acidity. In addition pK, $\Delta H°$, and $\Delta S°$ values obtained by the entropy titration method[6] are reported for adenosine ionization.

The chemicals used were the highest purity available from California Biochemical Corp. (Grade A ribose, adenine, adenosine) and Sigma Chemical Co. (Sigma grade deoxyadenosine) or were synthesized (2′-O-methyladenosine, 3′-deoxyadenosine).[7]

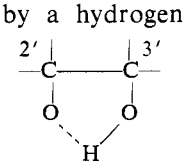
In Figure 1 are plotted thermometric titration data taken from thermograms obtained by titration with 0.6 F NaOH solution of 0.01 F solutions of adenosine, ribose, 2′-deoxyadenosine, sodium adenate ($NaC_5H_4N_5$), 0.002 F 2′-O-methyladenosine, and 0.004 F 3′-deoxyadenosine. The thermograms were obtained using a precision thermometric titration calorimeter.[6,8]

The observed temperature increase (Figure 1) during the titration of 3′-deoxyadenosine, 2′-O-methyladenosine, and 2′-deoxyadenosine is quantitatively accounted for by the heat of dilution of the titrant and heat from stirring. The small differences observed among the individual curves for these substances are quantitatively accounted for by small changes between determinations in the rate of heat input from stirring. The curve for sodium adenate shows a relatively large initial increase due to the reaction of a small amount of the hydrolyzed adenate species (adenine pK = 9.8).

Calculations indicate that under the conditions used in this study proton ionization is detectable provided the pK is less than ~13.5.

The curve for ribose shows the presence of an acid–base reaction but the data have not yet been treated quantitatively. The curve for adenosine has been analyzed quantitatively,[6] assuming one dissociable proton, to obtain, at zero ionic strength and 25°, pK = 12.35 ± 0.03, $\Delta H°$ = 9.7 ± 0.1 kcal./mole, and $\Delta S°$ = −23.9 ± 0.3 e.u. for the reaction adenosine → adenosine$^-$ + H^+. This pK value is in good agreement with that reported by Levene, *et al.*[1] (12.5).

The curves for sodium adenate and ribose clearly show that the proton in adenosine is ionized from the ribose moiety. Further, the fact that substitution of CH_3 for H on the 2′-hydroxyl or substitution of H for OH in either the 2′- or 3′-position in adenosine causes a loss of acidity indicates that the two adjacent hydroxyl groups are a necessary structural feature for that acidic character to exist. Two possible explanations for this are (1) the combined inductive effect of the vicinal 2′- and 3′-hydroxyl groups and/or (2) the anion is stabilized by a hydrogen-bonded ring; *e.g.*



(6) L. D. Hansen, J. J. Christensen, and R. M. Izatt, *Chem. Commun.* (London), 3, 36, 1965.

(7) The authors greatly appreciate the loan of 50 mg. of 2′-O-methyladenosine and 100 mg. of 3′-deoxyadenosine by Dr. Roland K. Robins for use in this study.

(8) J. J. Christensen, L. D. Hansen, and R. M. Izatt, *Rev. Sci. Instr.*, in press.

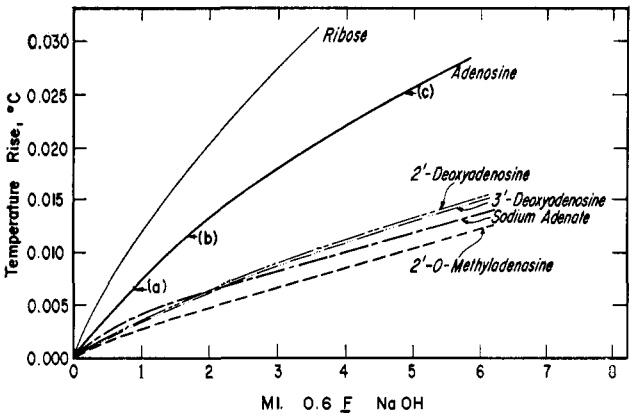


Figure 1. Actual thermometric titration curves for titration of aqueous solutions of the indicated compounds with 0.6 F NaOH. Temperature 25°; initial volume in calorimeter 100 ml; pH of adenosine solution 11.6 (a), 12.0 (b), and 12.5 (c).

It would now be of interest to determine whether 3′-O-methyladenosine is acidic; however, this compound is not yet available for study.

The data presented here suggest that the known acidity[1,9] of the ribose in RNA is a result of the combined effect of the 2′- and 3′-hydroxyl groups. Substitution of H for OH in the 2′-position (DNA) should result in loss of this acidity. Since the known chemical reactivity of the 2′- and 3′-positions in these substances would be expected to parallel the acidity of the OH groups, the different biological functions of DNA and RNA may be closely related to these acidity differences.

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(9) A. M. Michelson, "The Chemistry of Nucleosides and Nucleotides," Academic Press, Inc., New York, N. Y., 1963, p. 340.

(10) (a) To whom inquiries should be directed. (b) National Defense Education Act Predoctoral Fellow.

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Absolute Configurations of Sulfoxides by Asymmetric Oxidation of Sulfides[1]

Sir:

The oxidation of nondissymmetric sulfides by dissymmetric oxidizing agents leads to optically active sulfoxides,[2,3] generally in low optical yields.[4] It has been

(1) We gratefully acknowledge support by the National Science Foundation (GP-3375).

(2) A. Mayr, F. Montanari, and M. Tramontini, *Gazz. chim. ital.*, 90, 739 (1960).

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(4) K. Mislow, M. M. Green, P. Laur, J. T. Melillo, T. Simmons, and A. L. Ternay, Jr., *J. Am. Chem. Soc.*, 87, 1958 (1965).