Ultrasonic Relaxation Evidence for a Two-state Glycosyl Conformational Equilibrium in Aqueous Solution of Adenosine 3′,5′-Cyclic Monophosphate

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Summary A concentration-independent relaxation found in both aqueous and 7M urea solutions of the title compound can be assigned unequivocally to a two-state (*syn*⇌*anti*) glycosyl conformational equilibrium.

ALTHOUGH the very important second hormonal messenger, adenosine 3′,5′-cyclic monophosphate[1] (cyclic AMP) exists in both the *syn*[2] and *anti*[3] glycosyl conformations in the solid state,[3] its solution glycosyl conformation is still unresolved. High resolution ^{1}H n.m.r. studies suggested an *anti* conformation.[4] Lanthanide probes in successive studies suggested *anti*[5], *syn*[6], and finally a *syn–anti* rapidly equilibrating mixture[7] for the glycosyl solution conformation. Theoretical calculations on cyclic AMP indicate similar stabilities to the *syn* and *anti* regions separated by a low rotational barrier (*ca.* 6 kcal mol^{-1}).[8]

Recently we reported an application of ultrasonic relaxation to determine both kinetic and thermodynamic quantities of *syn–anti* glycosyl isomerization in adenosine.[9] We here report our findings on cyclic AMP which clearly indicate that in aqueous solution (pH 8·0) two glycosyl conformations of this compound coexist and rapidly equilibrate.

TABLE[a]

Concentration/M of cyclic AMP	(μ_{max}/C_T)/l mol^{-1}
0·050[b]	$1·2 \times 10^{-2}$
0·10[b]	$1·1 \times 10^{-2}$
0·20[b]	$0·98 \times 10^{-2}$
0·15[c]	$1·6 \times 10^{-2}$
0·20[c]	$1·6 \times 10^{-2}$

[a] Relaxation frequency, f_r, 37 ± 2 MHz throughout. [b] [Urea] = 0. [c] [Urea] = 7·0M.

Ultrasonic absorption measurements were carried out in the 10–300 MHz frequency range. Two experimental

techniques were employed: the conventional pulse at 25–300 MHz (described elsewhere[10]), and a swept-frequency interferometer at 10–35 MHz (similar to Eggers' design[11]). Sound absorption measurements on cyclic AMP solutions were compared with analagous solutions containing aqueous NaCl or NaCl in water–urea. All experiments were performed at 25 °C and the pH was adjusted to 8·0 to ensure that the anionic form of the phosphate was present.

The ultrasonic absorption spectrum of cyclic AMP consists of two relaxations in the range 10–300 MHz. The high frequency effect, not observed in adenosine,[9] is due to sodium ion binding to the phosphate and is similar to the one observed in NaH_2PO_4 solutions. Addition of divalent cations (Mg^{2+} or Ca^{2+}) causes dramatic changes in the absorption of this relaxation in cyclic AMP. This supports the assignment of ion binding as the cause of absorption. The low-frequency relaxation is quite similar to the process found in adenosine.[3] A comparable relaxation is also observed in NaH_2PO_4 solutions and it is necessary to demonstrate that the process is not due to ion pair interconversion. If this were the cause of the excess of absorption, the effect would increase dramatically in the presence of excess of Na^+ ion. The magnitude of the low-frequency relaxation remains virtually unchanged in a solution of 0·1M cyclic AMP and 1·0M excess of Na^+ (added as NaCl). The position of the absorption is independent of concentration (f_r is constant over a four-fold concentration range). We therefore conclude that the low-frequency relaxation process is due to an isomerization process within the anion itself. N.m.r. results indicate that the ribose phosphate ring has a rigid conformation in cyclic AMP.[12] The only remaining isomerization is the *syn–anti* interconversion.

For an isomerization process the maximum excess of sound absorption, μ_{max}, is a linear function of the total concentration, C_T.[9] The data in the Table indicate that there is a trend, believed to be outside experimental error, for μ_{max}/C_T to increase with decreasing concentration. This indicates that the *syn–anti* process is coupled to a concentration-dependent equilibrium. We can rule out ion pair formation as the secondary equilibrium since the addition of excess of sodium ion causes no significant effect on the magnitude or relaxation frequency of the slower relaxation. If base stacking, known to occur in aqueous solutions of all such systems,[13] is responsible for the effect, disrupting stacks should free nucleotides for *syn*⇌*anti* isomerization. Concentrated urea is a known denaturant of nucleotides. In 7M urea μ_{max}/C_T and f_r are virtually independent of concentration, while μ_{max}/C_T increases by 50% over the corresponding value found in aqueous solutions. These data strongly suggest that urea has eliminated intermolecular interactions which hinder the *syn–anti* rotation.

This interdependence of stacking interactions and glycosyl conformational equilibration should be considered in all studies on solution conformations of nucleosides and nucleotides.

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