

Ultrasonic Relaxation Evaluation of the Thermodynamics of Syn-Anti Glycosidic Isomerization in Adenosine

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Abstract: An ultrasonic approach is proposed which is capable of measuring all thermodynamic and kinetic quantities associated with the syn-anti conformational equilibrium in nucleosides. Application of the method to adenosine indicates that both syn and anti regions are significantly populated at room temperature. Together with high resolution nmr results the proposed method promises to lead to a precise definition of the syn-anti conformational problem in solution.

In 1960 Donohue and Trueblood suggested that there are two broad regions of stability in nucleosides (nucleotides) as one rotates the sugar (sugar phosphate) around the nucleic acid base using the glycosidic C-N bond as a pivot.¹ The two regions were named syn and anti and considerable experimental and theoretical research has been performed trying to pinpoint (a) these regions in various substrates and (b) the reasons for such conformational preferences.²

The principal physical methods employed for delineating preferred conformations have been X-ray crystallography in the solid state,³ MCD⁴ (magnetic circular dichroism), high resolution⁵ and nuclear Overhauser enhancement⁶ (NOE) nmr spectroscopy along with ultrasonic absorption studies⁷ in solution. The early work by Ts'o's group strongly implied predominantly anti conformations (essentially placing the ribose nearer to C-8) for purines in solution.^{5a} The more recent NOE⁶ and MCD⁴ results strongly suggest that in purines the syn conformation (placing the ribose C-5' atom near the C-2 of purine) is not excluded and is especially favored by placing a bulky substituent on C-8⁴ or by a change to nonaqueous solvent.⁴ The most recent NOE data indicate significant syn populations for 2', 3', and 5'-guanosine monophosphates⁹ as well as in adenosine and guanosine.⁶

A few years ago Rhodes and Schimmel performed ultrasonic relaxation studies on adenosine and a number of its derivatives and suggested that the concentration independent relaxation observed between 10 and 200 MHz was due to the syn-anti conformational equilibrium.⁷ Being an isomerization process, this should give rise to a single concentration independent relaxation.

While Rhodes and Schimmel demonstrated the existence of such a relaxation and proposed a numerical

value for the barrier of the process, they had to assume an equilibrium constant of 1 for the syn-anti interconversion. Although this number is probably of the correct order of magnitude based on theory⁹ for a purine nucleoside (with C-2'-endo puckered ribose), it is obviously highly desirable to remove the uncertainty associated with this assumption.

We have developed a set of equations which enable us to measure the barrier (ΔH^\ddagger), the $K_{\text{syn-anti}}$, ΔH , ΔG , ΔS , and ΔV for the syn-anti equilibration process purely from ultrasonic relaxation measurements. We cannot overemphasize the fact that with the assumption that we are indeed looking at the syn-anti equilibrium (and we believe Rhodes and Schimmel's data gave strong evidence of this), *we are proposing a method to determine all kinetic and thermodynamic quantities associated with this event.*

Ultrasonic Absorption Determination of the Thermodynamics of Fast Isomerization Processes

Ultrasonic absorption measurements have been used in the study of a number of chemical phenomena. These include studies on ion pair formation in solution,¹⁰ cation¹¹ and anion¹² desolvation, rotational energy barriers,¹³ vibrational relaxation processes,¹⁴ viscoelastic properties of liquids¹⁵ and molecular association processes.¹⁶

The ultrasonic technique is applicable to a range of time (10^{-6} - 10^{-9} sec) in which a multitude of chemical and physical processes occur. The physical processes include rotational and conformational changes. Since nmr techniques do not measure such fast processes, ultrasonics promises to be a valuable supplementary technique.

We wish to report an extension of the ultrasonic technique which enables measurement of the thermo-

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- (2) The literature on this topic has virtually exploded. A very detailed list of references can be found in E. D. Bergmann and B. Pullman, Ed. (Proceedings of Jerusalem Symposium), Israel Academy of Sciences and Humanities, Jerusalem, Vol. 5, 1973.
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- (15) G. J. Gruber and T. A. Litovitz, *J. Chem. Phys.*, **47**, 2185 (1967).
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dynamics of fast isomerization processes. Such measurements are ordinarily very difficult to obtain for the following reason. Assume some measurable property of the system P which is dependent upon the concentrations of the isomeric species A and B with constants of proportionality p_A and p_B . Then the observable

$$P = p_A A_{\text{eq}} + p_B B_{\text{eq}}$$

Since the total (initial) concentration $C_T = A_{\text{eq}} + B_{\text{eq}}$ and

$$K = B_{\text{eq}}/A_{\text{eq}}$$

$$P = (p_A/(1 + K) + p_B K/(1 + K))C_T$$

This equation has three unknowns unless p_A and p_B can be determined independently. For a process $A + X \rightleftharpoons B$, the quantities can be determined by using judicious amounts of X leading to varying extents of conversion to B . For an isomerization process this conversion cannot be brought about isothermally; hence the problem is without solution. Suppose, however, that the isomerization process has a nonzero ΔV and/or has a moderate value of ΔH and the process kinetically is in the ultrasonic time domain. A solution of the isomerizing solute will show an excess sound absorption compared to the solvent. The experimental quantity μ is given by

$$\mu = (\alpha - \alpha_0)\lambda$$

where α and α_0 are the sound absorption coefficient of solution and solvent, respectively, and λ is the wavelength of the sound. The frequency dependence of μ is given by

$$\mu = 2\mu_{\text{max}}\omega\tau/(1 + \omega^2\tau^2)$$

where μ_{max} is the maximum value of μ , ω the angular frequency, and τ the relaxation time of the system. The quantity μ_{max} is determined by the thermodynamics of the system and the solvent properties¹⁷

$$\mu_{\text{max}} = \frac{\pi}{2\beta RT}(\Delta V_s)^2\Gamma^{-1} \quad (1)$$

and

$$\Delta V_s = \Delta V - \frac{\alpha}{\rho C_p}\Delta H \quad (2)$$

and for an isomerization process

$$\Gamma^{-1} = KC_T/(1 + K)^2$$

Equation 1 has three unknowns ΔH , ΔV , and K . There are two distinct solutions depending on whether or not $\Delta V \equiv 0$.

1. If $\Delta V = 0$ one has two remaining unknowns. Assuming that all thermodynamic functions but K are temperature independent, within a narrow range of temperatures, one can investigate the effect of tempera-

ture on μ_{max} . Define a new quantity G such that $G = \beta RT\mu_{\text{max}}/\pi C_T$, and with $\Delta V = 0$

$$G = \frac{\alpha^2}{\rho^2 C_p^2}(\Delta H)^2 \frac{K}{(1 + K)^2}$$

the temperature dependence of G then is

$$\frac{d \ln G}{d 1/T} = \frac{2d \ln Q}{d 1/T} - \frac{\Delta H 1 - K}{R 1 + K} \quad (3)$$

and $Q = \alpha/\rho C_p$, a quantity calculable as a function of temperature for most common solvents. Measurement of $d \ln G/d 1/T$ at several temperatures then leads to a second independent equation in ΔH and K . Hence, from eq 1 and 3 one can determine these quantities. As a check on the method one can employ the assumption that ΔH should be reasonably constant with temperature. Furthermore, calculation of the quantity $d \ln K_{\text{obs}}/d 1/T$ obtained by plotting $\ln K$ vs. $1/T$ should equal $-\Delta H/R$. Such a ΔH value should be close to $\Delta \bar{H}$, the average of the ΔH values at each temperature. If the ΔH values found by the two approaches are significantly different the assumption $\Delta V \equiv 0$ is probably invalid. For aqueous systems one tests the assumption by making measurements at 4°. At this temperature G is zero for $\Delta V = 0$ (since α vanishes at 4°), and any excess absorption must be due to the ΔV term. Since the absorption of water is large at 4° a small ΔV may, unfortunately, be difficult to detect. In these cases comparison of $\Delta \bar{H}$ and ΔH from the $\log K_{\text{obs}}$ vs. $1/T$ plot is a better criterion for indication of $\Delta V \neq 0$. Even a small ΔV causes inconsistencies in the two ΔH values especially at low temperatures in aqueous medium.

2. If $\Delta V \neq 0$ we suggest the following procedure. Under these conditions eq 3 becomes

$$\frac{d \ln G}{d 1/T} = \frac{2|\Delta H|}{|\Delta V - (\alpha\Delta H/\rho C_p)|} \frac{\partial Q}{\partial 1/T} - \frac{\Delta H 1 - K}{R 1 + K} \quad (4)$$

then define

$$W = \frac{d \ln G}{d 1/T} - \frac{2|\Delta H|}{|\Delta V - (\alpha\Delta H/\rho C_p)|} \frac{\partial Q}{\partial 1/T}$$

and

$$W = -\frac{\Delta H 1 - K}{R 1 + K}$$

or

$$K = (\Delta H - WR)/(\Delta H + WR) \quad (5)$$

Substitution of (5) into (1) leads to a fourth degree equation in ΔH . Small (0.1 cc/mol) incremental ΔV values are now tried with the total range of ΔV estimated from the assumptions $\Delta H = 0$ and Γ^{-1}_{max} (equal to $C_T/4$). With these values ΔV is calculated. To avoid possible errors the range of ΔV can be expanded by taking $\Gamma^{-1} = C_T/10$, which is about as small a concentration factor as can be measured experimentally. Assuming $\Delta V = 0$, a limit can also be placed on ΔH . For each ΔV one calculates four ΔH values from eq 1 (employing a digital computer) at each temperature. Of the four ΔH roots, two will be erroneous usually (imaginary or clearly outside the range of ΔH values allowed). The other two roots are $r_1 \cong -r_2$ (of course $r_1 = -r_2$ when $\Delta V = 0$). Assuming, as previously, that ΔH and ΔV are nearly temperature

(17) The symbols represent the following: Γ^{-1} a concentration function such that $\Gamma = \sum \nu_i^2/c_i$; with ν_i the stoichiometric coefficient of species i and c_i the concentration of species i ; ΔV_s the adiabatic volume change, ΔV the isothermal volume change, and ΔH the enthalpy change for the reaction; α the thermal expansivity of the solution, ρ its density, and C_p its heat capacity; C_T the total solute concentration. β is the adiabatic compressibility of solvent. Since our most concentrated solution is 0.0284 M in adenosine, we have assumed all solution properties to equal the solvent properties, i.e., α, β, ρ, C_p .

independent in the short range which can be studied, the solution lies in finding a ΔV which produces nearly temperature independent ΔH (parenthetically, usually only a narrow range of ΔV values will produce such a ΔH). From such a set of ΔV and ΔH values one can calculate K from eq 5 at each temperature and check for internal consistency of the data by showing that ΔH from the $\log K$ vs. $(1/T)$ plot agrees with the $\Delta \bar{H}$ calculated above.

Experimental Section

Ultrasonic absorption instrumentation has been described previously.¹² Adenosine was obtained from Aldrich Chemical Co. and used as received. In repeating measurements of Rhodes and Schimmel we generally used somewhat more dilute solutions since the solutions used by these authors seem to be supersaturated.¹⁸ Investigations covered the frequency range 10–300 MHz. Only one concentration was studied at 10°, this being $1.75 \times 10^{-2} M$. This represents a saturated solution. The results of our determination as well as literature data are given in Table I.

Results and Estimation of Errors

Using the data in Table I and properties of water

Table I. Results of Ultrasonic Absorption Measurements^c

$T, ^\circ\text{C}$	$\mu_{\max}/\epsilon_T, \times 10^8$	f_r, MHz
10 ^a	1.08	22
25 ^a	4.11	40
25 ^b	4.41	40
35 ^{a,b}	4.7	55
49 ^b	4.16	83

^a This work. ^b Reference 7. ^c The value of α_0/f^2 was taken to be that of water.

required for eq 5 we have applied the data treatment described previously. The results of this analysis are shown in Table II. In addition to thermodynamic re-

Table II. Calculated Enthalpies, Equilibrium Constants, Entropies, and Rate Constants for the Syn–Anti Isomerization

$T, ^\circ\text{C}$	$\Delta \bar{H}, \text{kcal/mol}^a$	K	$\Delta S, \text{eu}$	$k_f \times 10^{-8}, \text{sec}^{-1}$	$k_r \times 10^{-7}, \text{sec}^{-1}$
10	10.8 ± 3	1.4 ± 0.4	39 ± 20	0.8	5.8
25 ^b	11.3 ± 3	4.5 ± 1.4	40 ± 20	2.0	4.6
25 ^c	11.6 ± 3	4.0 ± 1.2	40 ± 20	2.0	5.1
35 ^{b,c}	10.6 ± 3	7.9 ± 2.4	40 ± 20	3.1	3.9
49	9.9 ± 3	15.9 ± 4.8	40 ± 20	4.9	3.1

^a ΔH (from temperature dependence of K) = 11.4; ΔS = 41 eu. The best fit of $\Delta \bar{H}$ calculated and ΔH from the $\ln K$ vs. $1/T$ plot was obtained using $\Delta V = -0.3 \text{ cm}^3$. ^b This work. ^c Reference 7.

sults, kinetic results are also presented. These are calculated from the relationship

$$\tau^{-1} = 2\pi f_r = k_f + k_r = k_f(1 + K^{-1})$$

The results are subject to rather large errors. These are due mainly to the fact that both f_r and the magnitude of the effects are difficult to measure *via* conventional pulse techniques when the effects are this small. The entire process has been computerized and we are able to determine sources of error. In Table II it can be seen that the reverse rate constant decreases with temperature.

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This cannot be correct. It simply reflects the fact that accumulated errors in f_r and the calculated K values as functions of temperature have made the calculation of this rate constant meaningless. It is to be expected also that the forward rate constant will be in error and only its order of magnitude is certain. However the thermodynamic functions are much better defined since small errors in μ_{\max} do not bring about very large changes in the calculated results. This is best seen in Tables I and II where the present estimate of μ_{\max} and the literature value differs by 7.2%. Taken together as an average value or individually these two sets of data lead to virtually the same ΔH and the difference in equilibrium constants is about 12%. This is, however, the effect of changing only a single point and hence cannot reveal the effect of altering the temperature dependences. In order to see this effect we *arbitrarily* changed the values of the relaxation frequencies from the values in Table I to the ones in Table III, while

Table III. Influence of Errors in f_r upon Calculated Parameters. Limiting Values of the Relaxation Frequency^a

$T, ^\circ\text{C}$	f_r, MHz	$\mu_{\max}/C_T, \times 10^8$
25	35	3.60
25	35	3.86
35	55	4.77
35.5	55	4.61
49.0	87	4.36

^a These values are our estimates of the maximum variation of f_r which are at all consistent with the absorption data.

retaining the same magnitude. These values were chosen as being within the realm of possible variation in f_r given that these values are likely to be subject to errors of 5% or more in view of the small magnitudes of the effects. Larger variations are unlikely. The calculated results for this system are shown in Table IV.

Table IV. Calculated $\Delta H, K, \Delta S, k_f$, and k_r Using Data from Table III^a

$T, ^\circ\text{C}$	$\Delta \bar{H}, \text{kcal/mol}$	K	$\Delta S, \text{eu}$	$k_f \times 10^{-8}, \text{sec}^{-1}$	$k_r \times 10^{-7}, \text{sec}^{-1}$
25 ^b	8.6 ± 2.25	3.6 ± 1.1	31 ± 15	1.7	4.7
25 ^c	8.8 ± 2.25	3.2 ± 1.0	31 ± 15	1.7	5.2
35 ^b	8.8 ± 2.25	5.1 ± 1.5	31 ± 15	2.9	5.7
35.5 ^c	8.7 ± 2.25	5.5 ± 1.7	31 ± 15	2.9	5.3
49 ^c	8.2 ± 2.25	10.2 ± 3.1	31 ± 15	5.0	4.9

^a ΔH (from temperature dependence of K) = 8.6, ΔS = 31 eu. Best fit of $\Delta \bar{H}$ calculated and ΔH from $\ln K$ vs. $1/T$ plot was obtained using $\Delta V = 0.5 \text{ cm}^3$. ^b This work. ^c Reference 7.

It is evident that the calculated enthalpy has changed by 22.5%. The calculated values of the equilibrium constant show variations of up to 30%. Since the assumed relaxation frequencies represent reasonably large uncertainty in the data, we can conclude that our calculated enthalpy is accurate to within 25% and equilibrium constants are accurate to within 30%. The error limits included in Table II have been estimated in this way. Note that in Table IV no value is given for 10°. This point was excluded from the calculations using arbitrary data since the relaxation frequency predicted for 10° from said data was grossly inconsistent with the

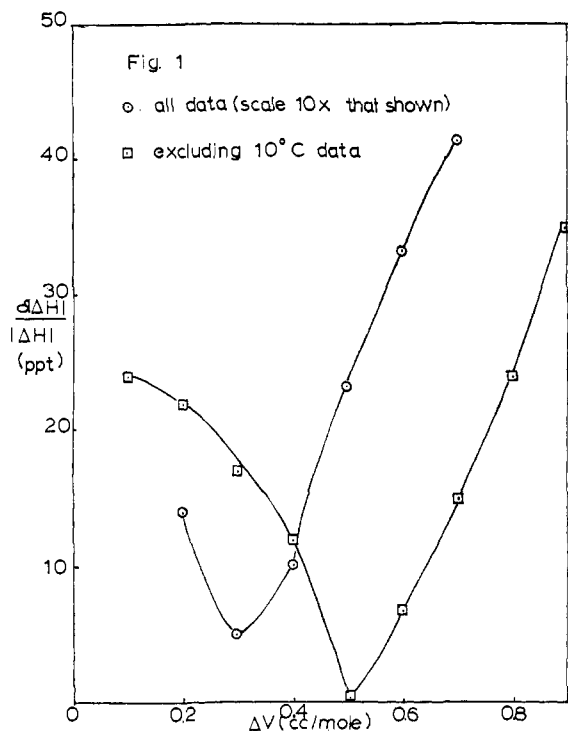


Figure 1. Plot of the difference between ΔH calculated in parts per thousand and ΔH from $\ln K$ vs. $1/T$ plot vs. ΔV .

experimental results. Hence this arbitrary plot is most likely an overestimate of possible variation in relaxation frequency. The arbitrary data do have one interesting feature, however. Using a ΔV of -0.7 cm^3 or more to fit the thermodynamic functions (this is, however, not the value which gives the best fit between ΔH calculated and ΔH from $\log K$ vs. $1/T$ plots) leads to reverse rate constants which increase with temperature. The final problem to be considered is the error in ΔV . By hypothesis the correct ΔV is the one which makes ΔH calculated from each point and the ΔH from a $\log K$ vs. $1/T$ plot equal. We can therefore plot the relative difference between these two values of ΔH vs. ΔV and look for a minimum at the correct value of ΔV . Such a plot is shown in Figure 1. It can be seen that satisfactory minimization occurs. We know that the point at 10° is most subject to error and hence we treated the data excluding this point. Minimization occurs at a different value of ΔV and with a much smaller relative error. In view of the difference in ΔV between these two data treatments ΔV cannot be considered to be known to better than ± 0.2 cm^3 . It is useful to note that excluding the 10° point lowers ΔH to 10.0 kcal/mol but changes the K values by negligible amounts. The sign of all thermodynamic functions was determined such that the anti form is the dominant one at high temperature in accord with the nmr results. (The sign of ΔH cannot be determined by ultrasonics. However, the

sign of ΔS and ΔH must be the same while ΔH and ΔV must have opposite signs.)

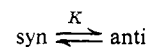
We are confident that by improving the quality of the data the errors in all thermodynamic functions and rate constants can be markedly reduced. However, the conventional pulse technique for sound absorption measurements is probably inadequate for systems such as the present one due to the small excess absorption. Other ultrasonic measurement techniques can be applied to such systems in order to reduce errors. Such work is currently in progress in this laboratory.

Conclusion

The high resolution nmr data strongly suggest the predominance of the anti conformation at 30° or higher.^{5a} Since the rate of conformational syn-anti interconversion is very fast on the nmr time scale,⁷ in this "fast exchange" limit the observed chemical shift is a weighted average of the syn and anti resonances, *i.e.*

$$\nu_{\text{obsd}} = n_{\text{anti}}\nu_{\text{anti}} + n_{\text{syn}}\nu_{\text{syn}}$$

where ν_{obsd} , ν_{anti} , and ν_{syn} are the chemical shifts of the time average mixture and "pure" conformational signals, respectively, and n_{anti} and n_{syn} are the mole fractions of the anti and syn populations. According to our results at 30° the K is already of a magnitude which within experimental error would only indicate the properties of the predominant species. Assuming the predominant species to be the anti at high temperature the thermodynamic properties indicate a K



as indicated in Table II. The implication is that both the enthalpy change and the entropy change are positive for this direction. If there is an intramolecular N-3-O-5'-H hydrogen bond in the syn conformation, then ΔH and ΔS values are reasonable. But this intramolecular hydrogen bond may not be a prerequisite for this phenomenon. We should emphasize again that the NOE measurements implied the coexistence of significant syn and anti populations of adenosine near ambient temperatures.⁶

With our finding of the strong temperature dependence of K , it should be feasible to derive chemical shifts for the conformationally "pure" rotamers by extrapolating to infinite dilution at each temperature here quoted. With a knowledge of the high temperature conformer from other measurements (*e.g.*, nmr) our method is capable of determining all the important thermodynamic and kinetic parameters of such isomerizations.

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