Kinetics of the base-stacking reaction of N6-dimethyladenosine. An ultrasonic absorption and dispersion study

ARTICLE *in* THE JOURNAL OF PHYSICAL CHEMISTRY · AUGUST 1977

Impact Factor: 2.78 · DOI: 10.1021/j100532a002

3 AUTHORS, INCLUDING:

Maarten P Heyn
Freie Universität Berlin
119 PUBLICATIONS **4,907** CITATIONS
SEE PROFILE

Carlo Ulisse Nicola
University of Applied Sciences and Arts Nort…
11 PUBLICATIONS **99** CITATIONS
SEE PROFILE

decomposition over the observational times scales employed, and, in fact, "normal" Arrhenius behavior has been observed[24-29] for the reaction of OH radicals with a variety of olefins over the temperature range 210–500 K.

Acknowledgment. The authors gratefully acknowledge the financial support of NSF Grant No. CHE76-10447 and Environmental Protection Agency Grant No. 800649. The contents do not necessarily reflect the view and policies of the Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

References and Notes

(1) M. C. Dodge, Workshop on Mathematical Modeling of Photochemical Smog, Summary Proceedings, Report EPA-R4-73-010, Environmental Protection Agency, Washington, D.C., 1973.
(2) B. J. Finlayson and J. N. Pitts, Jr., *Science*, **192**, 111 (1976).
(3) E. D. Morris, Jr., and H. Niki, *J. Phys. Chem.*, **75**, 3640 (1971).
(4) D. D. Davis, W. Bollinger, and S. Fischer, *J. Phys. Chem.*, **79**, 293 (1975).
(5) D. A. Hansen, R. Atkinson, and J. N. Pitts, Jr., *J. Phys. Chem.*, **79** 1763 (1975).
(6) R. A. Perry, R. Atkinson, and J. N. Pitts, Jr., *J. Phys. Chem.*, **81**, 296 (1977).
(7) G. J. Doyle, A. C. Lloyd, K. R. Darnall, A. M. Winer, and J. N. Pitts, Jr., *Environ. Sci. Technol.*, **9**, 237 (1975).
(8) A. C. Lloyd, K. R. Darnall, A. M. Winer, and J. N. Pitts, Jr., *J. Phys. Chem.*, **80**, 789 (1976).
(9) G. Boocock and R. J. Cvetanovic, *Can. J. Chem.*, **39**, 2436 (1961).
(10) G. R. H. Jones and R. J. Cvetanovic, *Can. J. Chem.*, **39**, 2444 (1961).
(11) I. Mani and M. C. Sauer, Jr., *Adv. Chem. Ser.*, **No. 82**, 142 (1968).
(12) E. Grovenstein, Jr., and A. J. Mosher, *J. Am. Chem. Soc.*, **92**, 3810 (1970).
(13) R. A. Bonanno, P. Kim, J. H. Lee, and R. B. Timmons, *J. Chem. Phys.*, **57**, 1377 (1972).
(14) J. S. Gaffney, R. Atkinson, and J. N. Pitts, Jr., *J. Am. Chem. Soc.*, **98**, 1828 (1976).
(15) H. Akimoto, M. Hoshino, G. Inoue, M. Okuda, and N. Washida, 12th Informal Conference on Photochemistry, Gaithersburg, Md., June 28–July 1, 1976.
(16) R. Atkinson and J. N. Pitts, Jr., *J. Phys. Chem.*, **78**, 1780 (1974); **79**, 295 (1975).
(17) R. Atkinson and J. N. Pitts, Jr., *J. Phys. Chem.*, **79**, 541 (1975).
(18) S. W. Charles, J. T. Pearson, and E. Whittle, *Trans. Faraday Soc.*, **59**, 1156 (1963).
(19) D. G. L. James and R. D. Suart, *Trans. Faraday Soc.*, **64**, 2752 (1968).
(20) A. Amano, O. Horie, and N. H. Hanh, *Int. J. Chem. Kinet.*, **8**, 321 (1976).
(21) S. W. Benson, "Thermochemical Kinetics", Wiley, New York, N.Y., 1968.
(22) C. K. Ingold, "Structure and Mechanism in Organic Chemistry", Cornell University Press, Ithaca, N.Y., 1953.
(23) R. J. Cvetanovic, 12th International Symposium on Free Radicals, Laguna Beach, Calif., Jan 4–9, 1976.
(24) R. Atkinson and J. N. Pitts, Jr., *J. Chem. Phys.*, **63**, 3591 (1975).
(25) N. R. Greiner, *J. Chem. Phys.*, **53**, 1284 (1970).
(26) I. W. M. Smith and R. Zellner, *J. Chem. Soc., Faraday Trans. 2*, **69**, 1617 (1973).
(27) R. Atkinson, R. A. Perry, and J. N. Pitts, Jr., *Chem. Phys. Lett.*, **38**, 607 (1976).
(28) R. Atkinson, R. A. Perry, and J. N. Pitts, Jr., *J. Chem. Phys.*, **66**, 1197 (1977).
(29) R. Atkinson, R. A. Perry, and J. N. Pitts, Jr., *J. Chem. Phys.*, in press.
(30) J. A. Kerr, M. J. Parsonage, and A. F. Trotman-Dickenson, "Strengths of Chemical Bonds", in "Handbook of Chemistry and Physics", 56th ed, The Chemical Rubber Company, Cleveland, Ohio, 1975–1976.
(31) J. H. S. Green, *Chem. Ind.*, 1575 (1962).

Kinetics of the Base-Stacking Reaction of N^6,N^6-Dimethyladenosine. An Ultrasonic Absorption and Dispersion Study

M. P. Heyn,* C. U. Nicola, and G. Schwarz

Department of Biophysical Chemistry, Biozentrum der Universität Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland *(Received December 28, 1976)*

Publication costs assisted by the University of Basel

The kinetics of the self-association reaction of N^6,N^6-dimethyladenosine in water was studied by means of ultrasonic absorption and dispersion in the frequency range from 0.5 to 36 MHz. The self-association is believed to occur by vertical stacking of the purine rings. A single relaxation process was observed. This observation as well as the temperature and concentration dependence of the relaxation time and amplitude are in good agreement with the predictions based on a random isodesmic association model. At 25 °C the stacking recombination rate constant equals 1.74×10^9 M^{-1} s^{-1}. The magnitude and the temperature dependence of the recombination rate constant are in accordance with the values expected when the reaction is diffusion controlled. From the dissociation rate constant we obtain a value of 1.96×10^{-8} s for the lifetime of a stacked complex at 25 °C. In the evaluation of the amplitude data both the volume and enthalpy change have to be considered. For the volume change we find $\Delta V = -8.3$ mL/mol. The isodesmic association constant determined from the kinetic measurements is consistent with the value of 34 M^{-1} at 25 °C determined from equilibrium measurements.

Introduction

In contrast to the large number of equilibrium studies on base stacking, the kinetics has not received much attention.[1-3] This appears to be due to two factors. Since most of the bases and nucleosides have a low solubility and a small association constant, measurements can only be performed over a limited concentration range with small amplitudes. In the second place, the relaxation times are very short ranging from 50 to 3 ns and are thus experimentally not easily accessible. Some kinetic studies have been carried out with planar aromatic compounds, such as dyes, which in aqueous solution also self-associate by stacking.[4-7] The kinetics of linear self-association is furthermore of considerable importance for systems which can form hydrogen bonded aggregates[8] and for aggregates of proteins.[9,10]

Several theoretical treatments of the kinetics of linear self-association have recently appeared. The two most frequently discussed models are the sequential model, in which only monomers can add to or break off larger aggregates, and the two-state or random association model, in which association–dissociation reactions occur between any two aggregates. The sequential model was treated in great detail from the point of view of light-scattering

temperature-jump kinetics.[9,10] The relaxation time spectrum and the amplitudes were calculated for a number of weighed-in monomer concentrations.[9,10] As expected, this model led to a broad spectrum of relaxation times when the aggregation was sufficiently high. Most of the amplitude was associated with the longest relaxation time. The mean relaxation time was predicted to increase with increasing concentration and a clearly biphasic progress curve should be observed. The experimental data for the two purine derivatives investigated so far are characterized by an ultrasonic absorption spectrum which is somewhat broader than expected for a single relaxation process and by a decrease of τ with increasing weighed-in monomer concentration.[1-3] The sequential model appears to be inappropriate for these systems. In all of these considerations an isodesmic sequential model was assumed, in which the association constant is assumed to be independent of the length of the aggregate. In contrast to the sequential isodesmic model, which in general predicts a broad spectrum of relaxation times, the random isodesmic association model is equivalent to a dimerization model with only one relaxation time.[1,9]

The only two studies on the base stacking kinetics of nucleic acid components were carried out with N^6,N^9-dimethyladenine[1] and 6-methylpurine.[2] They were studied under conditions where base ionization reactions are negligible. In both cases ultrasonic absorption was used to determine the kinetic parameters. With the equipment presently available,[11,12] both the ultrasonic absorption and dispersion can be measured. About 40 frequency points are obtained per spectrum. In the previous studies typically eight or nine frequency points were measured, and only the absorption was determined. We are thus in a considerably better position to answer the important question whether only one relaxation time occurs. From equilibrium measurements alone it is not possible to distinguish between a sequential isodesmic model and a random isodesmic association model. This is in principle possible from kinetic measurements by measuring the number of relaxation times and the concentration dependence of the relaxation times and amplitudes. The accuracy of the present data should allow us to make such a distinction. The appropriate expressions for the relaxation time and amplitude as a function of the weighed-in monomer concentration for the random isodesmic association model are derived in the next section. Dimer models need not be considered, since molecular weight determinations using the ultracentrifuge showed that aggregation occurs much beyond the dimer stage.[13] In previous equilibrium measurements on the self-association of N^6,N^6-dimethyladenosine[13,14] we determined the association constant (34 M^{-1} at 25 °C) and the enthalpy change (–6.3 kcal/mol) for the isodesmic association model. It is of interest to compare the kinetically determined association constant with the value determined from the equilibrium measurements. Hydrophobic interactions are thought to be responsible for the stacking reaction. In connection with the various theories for these interactions there is considerable interest in the sign of the volume change for the reaction. From measurements of the ultrasonic absorption amplitude the sign of this volume change could be determined. N^6,N^6-Dimethyladenosine has the advantage of having no charged groups at neutral pH so that the contribution of protolytic reactions to the relaxation amplitude can be neglected.

Kinetics and Thermodynamics of the Random Isodesmic Association Model

In the random isodesmic association model each aggregate can combine with any other aggregate and the association constant, rate constants, volume change, and enthalpy change are assumed to be independent of the size of the aggregates. These assumptions will be discussed in more detail below. We consider the following set of reactions:

$$A_i + A_j \underset{k_D/\alpha_{ij}}{\overset{k_R/\alpha_{ij}}{\rightleftharpoons}} A_{i+j}$$

A_i represents a linear aggregate consisting of i stacked monomers. i and j may assume any integer value larger or equal to 1. k_R is the common recombination rate constant, k_D is the common dissociation rate constant. When $i = j$, $\alpha_{ij} = 2$ (on symmetry grounds), otherwise $\alpha_{ij} = 1$. The equilibrium association constant equals

$$\frac{k_R/\alpha_{ij}}{k_D/\alpha_{ij}} = \frac{k_R}{k_D} = K$$

and is the same for all reactions. Let c_n be the molar concentration of the species A_n. The differential equation describing the time dependence of c_n is then ($n \neq 1$)

$$dc_n/dt = \sum_{\substack{i=1 \\ i+j=n}}^{\infty} \sum_{j=1} (k_R/2)c_i c_j - \sum_{j=1}^{\infty} k_R c_n c_j - \sum_{\substack{i=1 \\ i+j=n}}^{\infty} \sum_{j=1} (k_D/2)c_{i+j} + \sum_{j=1}^{\infty} k_D c_{n+j} \quad (1)$$

The first and fourth sum describe the formation of A_n from smaller aggregates and from larger aggregates, respectively. The second and third terms describe the decrease in c_n due to growth into larger aggregates and break up into smaller aggregates. For $n = 1$ the first and third sums are absent. The equilibrium concentrations will be denoted by $\bar{c}_n$. At equilibrium the left-hand side of eq 1 is zero for every n, and the solution of the set of equations is

$$\bar{c}_n = K^{n-1}\bar{c}_1{}^n \quad (2)$$

The equilibrium concentrations are thus the same as in the sequential isodesmic model,[4,15] as expected. With equilibrium methods it is not possible to distinguish between the two isodesmic models. Let $\bar{c}_0$ be the total weighed-in monomer concentration and let $\bar{c}_m$ be the molarity. With the help of (2) $\bar{c}_0$ and $\bar{c}_m$ can be expressed in terms of $\bar{c}_1$:

$$\bar{c}_0 = \sum_{i=1}^{\infty} i\bar{c}_i = \frac{\bar{c}_1}{(1 - K\bar{c}_1)^2} \quad (3)$$

and

$$\bar{c}_m = \sum_{i=1}^{\infty} \bar{c}_i = \frac{\bar{c}_1}{1 - K\bar{c}_1} \quad (4)$$

The sum in (3) converges since it represents mass conservation. Using the customary dimensionless variables

$$s = K\bar{c}_1 \quad \text{and} \quad L = K\bar{c}_0$$

we can write (3) as

$$L = s/(1 - s)^2 \quad (5)$$

Inverting and choosing the correct root, we have

$$s = \frac{2L + 1 - (4L + 1)^{1/2}}{2L} \quad (6)$$

Substituting in (4), we find

$$K\bar{c}_m = \frac{s}{1-s} = \frac{2L + 1 - (4L+1)^{1/2}}{(4L+1)^{1/2} - 1}$$
$$= {}^1/_2[(1+4L)^{1/2} - 1] \quad (7)$$

The variable L can be changed experimentally by varying $\bar{c}_0$ or the temperature. It is the natural dimensionless variable of the problem. Equations 3–7 also apply for a sequential isodesmic model. The fraction f_n of weighed-in monomers that is incorporated in aggregates of length n is given by

$$f_n = \frac{n\bar{c}_n}{\bar{c}_0} = \frac{nK^{n-1}\bar{c}_1{}^n}{\bar{c}_0} = n\frac{s^n}{L}$$
$$= \frac{n}{L}\left[\frac{2L + 1 - (4L+1)^{1/2}}{2L}\right]^n \quad (8)$$

It is convenient to introduce the concept of a stacking bond. A linear aggregate consisting of i monomers contains $(i-1)$ stacking bonds. The total concentration of stacking bonds $\bar{c}_{st}$ is thus

$$\bar{c}_{st} = \bar{c}_2 + 2\bar{c}_3 + 3\bar{c}_4 + \ldots = \sum_{i=1}^{\infty}(i-1)\bar{c}_i \quad (9)$$

Clearly

$$\bar{c}_{st} + \bar{c}_m = \sum_{i=1}^{\infty} i\bar{c}_i = \bar{c}_0$$

Thus

$$K\bar{c}_{st} = K\bar{c}_0 - K\bar{c}_m = \frac{s}{(1-s)^2} - \frac{s}{(1-s)} = \frac{s^2}{(1-s)^2}$$
$$= (K\bar{c}_m)^2 \quad (10)$$

or

$$\bar{c}_{st}/\bar{c}_m{}^2 = K \quad (11)$$

K can thus be viewed formally as the equilibrium constant for the reaction between stacking surfaces at the ends of aggregates (concentration $\bar{c}_m$) to form stacking bonds (concentration $\bar{c}_{st}$). A stacking bond can be formed by the recombination of any two aggregates. A stacking bond can be broken at any site within an aggregate. If the stacking rate constants, the volume change, and the enthalpy change are independent of the size of the stack and if the random association model is assumed, we can kinetically observe only changes in $\bar{c}_{st}$. Consequently the relaxation time spectrum will be completely degenerate with only one relaxation time remaining. Using eq 1 we will now derive the differential equation for c_{st}. Multiplying (1) by $n-1$ and summing over n we obtain

$$\frac{dc_{st}}{dt} = \frac{k_R}{2}\sum_{n=1}^{\infty}(n-1)\sum_{\substack{i=1\\ i+j=n}}\sum_{j=1}c_ic_j - k_R\sum_{n=1}^{\infty}(n-1)\sum_{j=1}^{\infty}c_nc_j$$
$$-\frac{k_D}{2}\sum_{n=1}^{\infty}(n-1)\sum_{\substack{i=1\\ i+j=n}}\sum_{j=1}c_{i+j} + k_D\sum_{n=1}^{\infty}(n-1)\sum_{j=1}^{\infty}c_{n+j}$$

Taking the first two terms and the last two terms together one obtains after some manipulation

$$\frac{dc_{st}}{dt} = \frac{k_R}{2}\sum_{i=1}^{\infty}\sum_{j=1}^{\infty}c_ic_j - \frac{k_D}{2}\sum_{i=1}^{\infty}\sum_{j=1}^{\infty}c_{i+j}$$

This result can also be written as

$$\frac{dc_{st}}{dt} = \frac{k_R}{2}\left(\sum_{i=1}^{\infty}c_i\right)^2 - \frac{k_D}{2}\sum_{n=1}^{\infty}(n-1)c_n$$

or as

$$\frac{dc_{st}}{dt} = \frac{k_R}{2}c_m{}^2 - \frac{k_D}{2}c_{st}$$

The rate equation for stacking bond formation is thus

$$\frac{dc_{st}}{dt} = \frac{k_R}{2}(c_0 - c_{st})^2 - \frac{k_D}{2}c_{st} \quad (12)$$

At equilibrium $dc_{st}/dt = 0$ and we obtain the familiar result (11). By the assumption of random isodesmic association, the equilibrium and kinetic problems have been reduced formally to those of a dimerization reaction of stacking surfaces (c_m) into stacking bonds (c_{st}). Equation 12 can be solved and the resulting relaxation time τ is given by

$$1/\tau = k_R\bar{c}_m + (k_D/2) \quad (13)$$

Substituting for $\bar{c}_m$ from (7), we can transform this result into a more useful and familiar form in terms of the variable L:

$$1/\tau = (k_R/2K)(4L+1)^{1/2} \quad (14)$$

Squaring we have

$$1/\tau^2 = (k_R{}^2/4K^2)(4L+1) = (k_D{}^2/4)(4K\bar{c}_0 + 1)$$
$$= k_Dk_R\bar{c}_0 + (k_D{}^2/4) \quad (15)$$

For a single relaxation process characterized by the relaxation time τ the ultrasonic excess absorption $\alpha\lambda$ and the ultrasonic velocity $v(\omega)$ depend on the angular frequency ω of the sound wave in the following way

$$\frac{\alpha\lambda}{\pi} = g\frac{\omega\tau}{1+(\omega\tau)^2} \quad (16)$$

$$\frac{2(v(\infty) - v(\omega))}{v(\infty)} = g\frac{1}{1+(\omega\tau)^2} \quad (17)$$

g is the amplitude of the relaxation process. $v(\infty)$ is the high frequency limit of the velocity. For the single Debye absorption curve (16), maximum absorption occurs at the angular frequency $\omega = 1/\tau$ or at the frequency $f = 1/2\pi\tau$. The amplitude g is related to the absorption maximum by

$$g = 2(\alpha\lambda)_{max}/\pi \quad (18)$$

Alternatively g can be determined from the ultrasonic dispersion:

$$g = \frac{2(v(\infty) - v(0))}{v(\infty)} \quad (19)$$

The amplitude g is related to the reaction volume ΔV and the reaction enthalpy ΔH in the following way:

$$g = \frac{\Gamma}{RT\varkappa}\left(\Delta V - \frac{\alpha_p}{\rho c_p}\Delta H\right)^2 \quad (20)$$

In (20) $\varkappa$ is the adiabatic compressibility, T the absolute temperature, R the gas constant, ρ the density, α_p the thermal expansion coefficient, and c_p the specific heat at constant pressure. In using this expression for the case of random association we are implicitly assuming that ΔV and ΔH are the same for all possible aggregation reactions. The factor Γ is defined by

$$\Gamma = K(\partial\bar{c}_{st}/\partial K) \quad (21)$$

and depends only on the equilibrium properties of the

system. From (10) and (5) it is easy to see that

$$\bar{c}_{st} = \bar{c}_1 L = \bar{c}_0 s$$

Using (6) we thus obtain the following expression for Γ

$$\Gamma = \bar{c}_0 L \frac{ds}{dL} = \frac{2L + 1 - (4L + 1)^{1/2}}{2K(4L + 1)^{1/2}} \tag{22}$$

Rearranging (22), we obtain

$$\Gamma = -\frac{1}{2K} + \frac{1}{2K} \frac{2L + 1}{(4L + 1)^{1/2}} \tag{23}$$

Plotting Γ vs. $(2L + 1)/(4L + 1)^{1/2}$ thus appears to be a useful way to present the amplitude data. The data at a fixed temperature and with varying $\bar{c}_0$ should lie on a straight line intersecting the horizontal axis at $(2L + 1)/(4L + 1)^{1/2} = 1$. The slope and the intercept with the vertical axis are equal to $1/(2K)$ and $-1/(2K)$, respectively, and depend on T via K. Since $(2L + 1)/(4L + 1)^{1/2}$ depends both on $\bar{c}_0$ and K, K should be known in advance. A second way to present the amplitude data is obtained by dividing eq 22 by $\bar{c}_0$:

$$\frac{\Gamma}{\bar{c}_0} = \frac{2L + 1 - (4L + 1)^{1/2}}{2L(4L + 1)^{1/2}} \tag{24}$$

When the normalized dimensionless amplitude data $\Gamma/\bar{c}_0$ are plotted vs. L, all the data points both with varying $\bar{c}_0$ and varying T should lie on the curve (24). This function has its maximum at $L = (1 + 2^{1/2})/2 = 1.207$, is zero at $L = 0$, and decreases monotonically to zero for large L (see Figure 4). It is in principle possible to determine $K(T)$ from the $\bar{c}_0$ value at which $\Gamma/\bar{c}_0$ has its maximum. Since the maximum is very broad and the data are of limited accuracy, this is not an accurate way of determining K. As an order of magnitude check it is useful though. In order to determine the maximum it is necessary to collect data at L values well below 1.207.

We will now briefly discuss the assumptions made in the random isodesmic association model. The sequential model, in which only monomers can add to or break off an aggregate, may be criticized on physical grounds. It is not reasonable to exclude reactions between aggregates of various sizes since the major part of the interaction between the bases is short ranged. In the random association model this sequential restriction is removed. The isodesmic feature, namely, that the chemical parameters describing the reaction between any two aggregates are the same, is certainly a simplification. We assumed that the association constant, the rate constants, the enthalpy change, and the volume change are independent of the sizes of the two interacting aggregates. If the reaction is diffusion controlled, the recombination rate constant k_R for the self-association of two neutral aggregates A_i and A_j depends on the size of the reacting partners in the following way

$$k_R = \frac{4\pi N}{1000}(D_i + D_j)(R_i + R_j) \tag{25}$$

in which D_i is the diffusion constant and R_i is the radius of an aggregate containing i monomers. N is Avogadro's number. The diffusion constant D_i depends on the size of the particle. For a spherical particle of radius R_i we have

$$D_i = kT/6\pi\eta R_i \tag{26}$$

in which k is Boltzmann's constant, η the viscosity of the solution, and T the absolute temperature. In this case the expression for the rate constant simplifies to

$$k_R = \frac{2NkT}{3000\eta} \frac{(R_i + R_j)^2}{R_i R_j} \tag{27}$$

With R_i proportional to i, we note that k_R is approximately independent of i and j as long as i and j do not differ too much. This is certainly the case when both i and j are small. Using (8) we can calculate the fraction of monomers incorporated in aggregates containing i monomers. With the highest L values employed in our experiments we find that more than 75% of the monomers are in aggregates with lengths less than 6. Thus in our experiments the aggregates are so small that the approximation of an i-independent rate constant appears to be justified.

Experimental Section

Materials and Methods. N^6,N^6-dimethyladenosine (Merck-Schuchardt and Koch-Light) was found to be chromatographically pure by thin-layer chromatography. Elemental analysis confirmed this result (theoretical values in brackets): 48.88 (48.80) C, 5.73 (5.80) H. The substance was used without further purification. All solutions were prepared in twice distilled water on a weight per volume basis. Concentrations were determined spectrophotometrically using an extinction coefficient for the unstacked molecule of 18 800 M^{-1} cm^{-1} at 275 nm.[16]

The construction of the 5-mL ultrasonic cell and the methods to determine both the absorption and dispersion have been described previously.[11,12] For ultrasonic absorption measurements the solution densities have to be known very accurately. A minimum of four significant figures is required. The densities were determined with a digital measuring device DMA 02 C (Anton Paar KG, Graz, Austria). A NaCl solution of the same density as the N^6,N^6-dimethyladenosine solution was used as a reference. This choice appears to be justified, since NaCl does not absorb in this frequency range and since the ultrasonic velocities in a NaCl solution and in an N^6,N^6-dimethyladenosine solution of the same density are almost the same. Since the relaxation amplitudes are small for N^6,N^6-dimethyladenosine, scattering from small air bubbles presented a serious problem in the 0–2 MHz range. This problem has been discussed elsewhere.[17] In order to remove most of these bubbles the N^6,N^6-dimethyladenosine solutions were heated for 1.5 h at 65 °C. The bubbles then become so large that they can be removed by gentle shaking. This treatment did not change the concentration of the solution and no hydrolysis seems to occur. To fill the cell a special syringe was used which allows a very small rate of filling.

Results

Measurements were performed for weighed-in concentrations $\bar{c}_0$ ranging from 3.02×10^{-2} to 17.4×10^{-2} M at the four temperatures 15, 25, 35, and 45 °C. The excess absorption and dispersion data for $\bar{c}_0 = 0.115$ M at 15 °C are shown in Figure 1. A simultaneous least-squares fit was made of both the absorption and dispersion data to eq 16 and 17 using g and τ as common parameters. Figure 1 shows that it is indeed possible to fit both sets of data assuming a single relaxation process characterized by a relaxation time τ of 14.57 ns and an amplitude g of 8.60×10^{-4}. As expected for a single Debye curve, the log–log plot of Figure 1 has a slope of +1 on the low frequency side of the maximum and of −1 on the high frequency side. The dispersion and excess absorption data can be used to construct a Cole–Cole plot. The resulting curve is circular with center on the dispersion axis, again indicating that

TABLE I: Association and Rate Constants for the Self-Association of N^6,N^6-Dimethyladenosine in Aqueous Solution as a Function of the Temperature

T, K	k_R, M^{-1} s^{-1}	k_D, s^{-1}	$k_R\eta/T$, erg mol^{-1} deg^{-1}	K,[a] M^{-1}	K,[b] M^{-1}
288	$(1.26 \pm 0.07) \times 10^9$	$(0.28 \pm 0.01) \times 10^8$	0.50×10^5	49 ± 2.0	47 ± 2.8
298	$(1.74 \pm 0.10) \times 10^9$	$(0.51 \pm 0.02) \times 10^8$	0.52×10^5	34 ± 1.4	33 ± 2.0
308	$(2.12 \pm 0.13) \times 10^9$	$(0.89 \pm 0.04) \times 10^8$	0.50×10^5	24 ± 1.0	24 ± 1.4
318	$(2.37 \pm 0.14) \times 10^9$	$(1.33 \pm 0.05) \times 10^8$	0.45×10^5	17 ± 0.7	16 ± 1.0

[a] Equilibrium measurements. [b] Amplitude measurements.

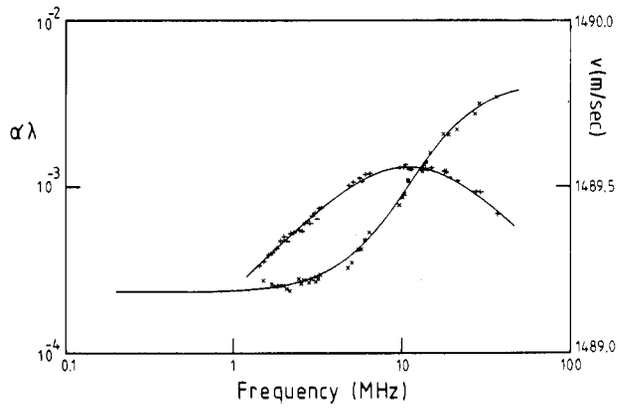

Figure 1. The sound velocity $v(x)$ and the excess sound absorption coefficient $\alpha\lambda$ (+) of a 0.115 M aqueous solution of N^6,N^6-dimethyladenosine at 15 °C as a function of the frequency. The continuous curves are least-squares fits to eq 16 and 17 for a single relaxation process with parameters $\tau = 14.57$ ns and $g = 8.60 \times 10^{-4}$.

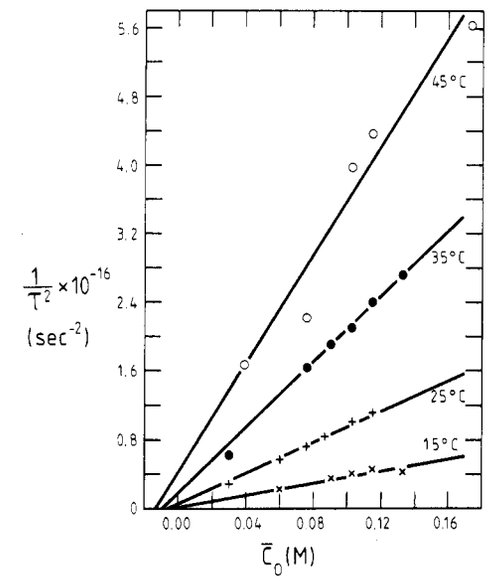

Figure 2. The square of the reciprocal relaxation time, $1/\tau^2$, plotted vs. $\bar{c}_0$, the weighed-in concentration of N^6,N^6-dimethyladenosine: (○) 45 °C; (●) 35 °C; (+) 25 °C; (×) 15 °C. The rate constants determined from the straight lines are collected in Table I.

only a single relaxation process contributes. At each temperature and concentration the relaxation time and amplitude were determined in this way. The relaxation time data for the four temperatures are plotted according to eq 15 in Figure 2. In accordance with the theory for the random isodesmic association model the data at 15, 25, and 35 °C lie on straight lines. Although the data at 45 °C show considerable scatter they are not inconsistent with a straight line. The experimental error in τ is estimated to be ±2%. According to eq 15 the slope of the straight line equals $k_R k_D$, the intercept on the vertical axis equals $1/4k_D^2$, and the intercept on the horizontal axis equals $-1/4K$. These three parameters are temperature dependent. The intercepts on the vertical axis are small and thus difficult to determine accurately. The slope can be determined more reliably. The data were therefore evaluated in the following way. The intercepts on the horizontal axis, $-1/4K(T)$, were fixed by using the value of the association constant obtained previously.[13,14] The slope of the straight lines and the intercept with the vertical axis were then used to determine k_R and k_D. In this way only one kinetic constant was obtained from the kinetic data. An independent "kinetic" association constant cannot be determined in this way. Figure 2 shows, however, that the kinetic data are consistent with the values for the association constant from the equilibrium measurements, since it is possible to draw straight lines through the kinetic data and the horizontal intercepts determined by the equilibrium measurements. The values of the kinetic constants obtained in this way are collected in Table I. From the way we analyzed the data, it follows that the ratios k_R/k_D must be approximately equal to the association constants determined by equilibrium methods, as is indeed the case. The value of the recombination rate constant k_R may be compared with the prediction from eq 25 for a diffusion controlled reaction. Values for the diffusion constants of various adenine derivatives at 25 °C are available.[18] A value of 4.8×10^{-6} cm^2 s^{-1} appears to be reasonable for N^6,N^6-dimethyladenosine at 25 °C. For the encounter distance $(R_i + R_j)$ we take 3.4 Å, the value determined from X-ray diffraction measurements for the distance between the bases in a stacked dinucleotide. Using these values we obtain a predicted recombination rate constant at 25 °C of 2.47×10^9 M^{-1} s^{-1}. This should be compared with the experimental value of 1.74×10^9 M^{-1} s^{-1}. In view of the approximations made the agreement must be considered to be satisfactory. According to eq 27, the product $k_R(T)\eta(T)/T$ must be temperature independent if the reaction is diffusion controlled. This is also the case if the particles are not spherical. The values for this product are collected in Table I. We note that these values are indeed approximately independent of the temperature. The value at 45 °C is a little lower than the others. The error in k_R at 45 °C is however quite large compared to the error in k_R at the three other temperatures. An alternative way to present these data is to plot ln k_R vs. $1/T$. In this way an approximately linear plot is obtained with an apparent activation energy of 4.1 kcal/mol. This value is in good agreement with the value of 4.4 kcal/mol at 35 °C calculated on the basis of eq 27 using the temperature dependence of the viscosity of water. We conclude that the reaction is essentially diffusion controlled.

The amplitude data were evaluated with eq 18, 19, and 20. From (20) it is apparent that at a single temperature only the absolute value of $[\Delta V - (\alpha_p/\rho c_p)\Delta H]$ can be determined. We will call this absolute value Δ. Δ was calculated from eq 20 using the experimental g values and using the Γ values calculated from (22) with the association constant K from equilibrium measurements. For a fixed temperature the Δ values so determined are approximately independent of the weighed-in monomer concentration $\bar{c}_0$. Using the value of $\Delta H = -6.3$ kcal/mol previously determined from equilibrium measurements,[13,14] the contribution of the ΔH term to Δ is about 2.7 mL/mol at 45

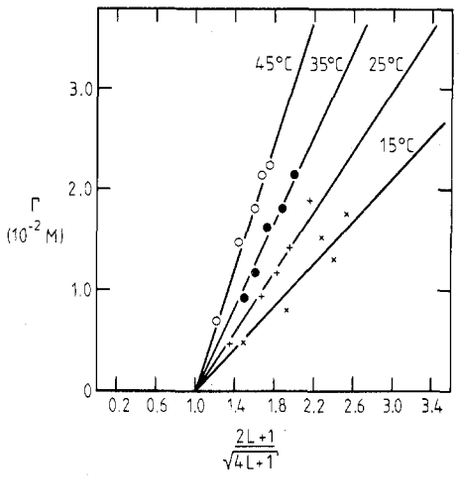

Figure 3. The amplitude of the relaxation process Γ plotted vs. $(2L + 1)/(4L + 1)^{1/2}$: (O) 45 °C; (●) 35 °C; (+) 25 °C; (×) 15 °C. For an isodesmic random self-association process the data at one temperature are predicted to lie on a straight line intersecting the horizontal axis at 1 (eq 23).

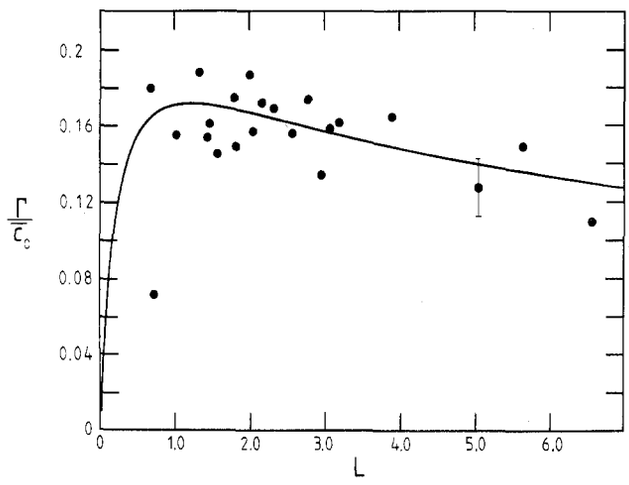

Figure 4. The amplitude Γ divided by the weighed-in monomer concentration $\bar{c}_0$ plotted vs. $L = K\bar{c}_0$. The continuous curve is the prediction for the isodesmic random self-association model (eq 24). The error bar gives an estimate of the error in $\Gamma/\bar{c}_0$.

°C and cannot therefore be neglected if $|\Delta V|$ ranges between 5 and 10 mL/mol. Under such circumstances Δ is expected to be temperature dependent since the thermal expansion coefficient α_p varies considerably between 15 and 45 °C. For water α_p changes from 1.51×10^{-4} deg^{-1} at 15 °C to 4.23×10^{-4} deg^{-1} at 45 °C. We observed that Δ decreased from 7.3 mL/mol at 15 °C to 6.3 mL/mol at 45 °C. Since the contribution of the ΔH term to Δ is positive and increasing with increasing temperature, and since Δ decreases with increasing temperature, one concludes that ΔV must be negative. Correcting the Δ values at the four temperatures for the contribution of the ΔH term (using $\Delta H = -6.3$ kcal/mol[13,14] and the thermal expansion coefficient of water), an approximately temperature independent ΔV value is obtained. A small temperature dependence remains, which is most likely due to experimental error. At 25 °C we find $\Delta V = -8.3$ mL/mol. The sign of ΔV is in agreement with the concentration dependence of the apparent molar volume which can be calculated from the solution density. The apparent molar volume decreases from 205.9 mL/mol at a weighed-in monomer concentration of 0.0101 M to 202.3 mL/mol at a concentration of 0.0876 M. Making the assumption that the volume change is independent of the stack length a ΔV value of −8.2 mL/mol at 25 °C can be calculated from these data. Although the agreement with the value determined from the amplitude is fortuitous, there can be little doubt that the concentration dependence of the density is only consistent with a negative ΔV. Since we have confirmed the value of ΔV, independently of the ultrasonic absorption data, we may conversely use it together with the experimental g values to compute Γ values and to plot them according to eq 23. The results are shown in Figure 3. Equation 23 predicts that for each temperature the data at various concentrations should lie on a straight line with a slope of $1/2K(T)$ and passing through the point $(2L + 1)/(4L + 1)^{1/2} = 1$. One observes that the data are in reasonable agreement with this prediction and that the slope increases with increasing temperature as should be the case since ΔH is negative. The association constants determined from the slopes are collected in the last column of Table I. Comparing with the association constants determined from the equilibrium measurements[13,14] (fifth column of Table I), we note that the agreement is quite good. This is not so surprising since the equilibrium association constant was used in calculating the horizontal coordinate. What the table shows however is that the K values from the amplitude measurements are consistent with those from the equilibrium measurements. In principle it is possible to determine K from these amplitude data independently of the equilibrium data by fitting all the data to eq 23 using as parameters ΔH and K (25 °C). These parameters could be varied until the data at the four temperatures lie on four straight lines with slopes obeying van't Hoff's law. This was not attempted in view of the limited accuracy of the data. An alternative way to present all the amplitude data on a single curve is provided by eq 24. Such a plot of $\Gamma/\bar{c}_0$ vs. L is shown in Figure 4. The continuous curve is the prediction for the isodesmic random association model (eq 24). The horizontal coordinate L was calculated using the association constant from the equilibrium measurements. Here again a least-squares fit of all the data would yield a ΔH and a K (25 °C) value. Due to the very broad maximum at $L = 1.207$ and the very considerable scatter in the experimental points, it is however not possible to determine K accurately in this way. One may conclude however that the amplitude data are at least consistent with the equilibrium association constant of 34 M^{-1} at 25 °C. An association constant which is larger or smaller by a factor of 2 is unlikely to be correct. The qualitative features predicted by the model, a decrease of the amplitude for large and small L values with a broad maximum around $L = 1.207$, are in agreement with the experimental data. Accurate data for small L values are difficult to obtain because at such concentrations the amplitude is very small. To give an idea of typical excess absorption values, α/f^2 at the resonance frequency equals 338×10^{-17} s^2/cm for a solution with $\bar{c}_0 = 0.06$ M at 25 °C ($L = 2.0$). This should be compared with the value of 22×10^{-17} s^2/cm for water at 25 °C.

Discussion

In the previous ultrasonic absorption studies on the self-association of N^6,N^9-dimethyladenine[1] and 6-methylpurine,[2] it was concluded that the absorption curves were somewhat broader than would be expected for a single relaxation process. Due to the limited accuracy, these data were not inconsistent with a random isodesmic association model,[1] which predicts only one relaxation time. They left room however for an interpretation based on models with several relaxation times. The results for 6-methylpurine for instance were explained using a sequential model with attenuated association constants,[3] in which the association

constant for the formation of an aggregate consisting of n monomers equals K/n. These authors claim that their model leads to a narrow relaxation time spectrum and an absorption curve consistent with a slightly broadened Debye. The present data with typically 40 frequency points per curve, both for the absorption and the dispersion, show no broadening with respect to a single Debye curve and thus lend strong support to the random isodesmic association model. It is possible that the previously observed broadening was due to experimental inaccuracy. The sequential isodesmic association model leads under certain conditions also to a single relaxation process (complete degeneracy).[8] These conditions are not satisfied in the present system. A simple dimerization, which would also lead to a single relaxation time, can be excluded on the basis of molecular weight determinations.[13] In two ultrasonic absorption studies on purine nucleosides evidence was presented for absorption in the 2–4-ns range.[19,20] The relaxation times were independent of the concentration. This fact in conjunction with other evidence led these authors to attribute the absorption to the intramolecular anti–syn conformational equilibrium of the glycosidic bond. The association constants of the nucleosides studied by these authors are so low that it is indeed likely that a contribution due to self-association could not have been observed at the concentrations employed. In our experiments we found no evidence for the presence of such a second relaxation process which should have occurred at frequencies close to the upper end of the range available to us.

The dependencies of $1/\tau^2$ and Γ on temperature and concentration are also in good agreement with the predictions from the random isodesmic association model. Although no completely independent "kinetic" association constants could be determined, the relaxation time data are consistent with the association constant from equilibrium measurements, which was also obtained using the isodesmic model. The results for k_R in Table I show that base stacking is a very fast process. From the magnitude of k_R and its temperature dependence we concluded that the reaction is diffusion controlled. At 25 °C, we obtained $k_R = 1.74 \times 10^9$ M^{-1} s^{-1}. This value should be compared with the corresponding values of 9.3×10^8 M^{-1} s^{-1} for N^6,N^9-dimethyladenine[1] and 1.16×10^9 M^{-1} s^{-1} for 6-methylpurine.[3]

The amplitude data were evaluated using a bootstrap procedure. The scale of the horizontal coordinate in Figure 3 and 4 depends on K. The association constants determined from equilibrium measurements were used for this purpose. The slopes of the amplitude plots in Figure 3 can be used to determine "kinetic" association constants. The fact that these were within experimental error equal to the equilibrium values (Table I) shows the self-consistency of the equilibrium and kinetic data. From the amplitude and density data an approximately temperature independent ΔV value of −8.3 mL/mol was determined. For the closely related compound N^6,N^9-dimethyladenine a temperature dependent volume change was found with $\Delta V = -6.8$ mL/mol at 25 °C.[1] The decrease of ΔV with increasing temperature was rationalized by correlating it with a decrease in the water structure.[1] The ΔH contribution to the amplitude was neglected. In view of the fact that for N^6,N^9-dimethyladenine $\Delta H = -8.7$ kcal/mol,[1,13] it is likely that at least a part of the apparent temperature dependence of ΔV can be accounted for by the contribution of the ΔH term to Δ. Using $\Delta H = -8.7$ kcal/mol, we obtained a ΔV value of −8.9 mL/mol at 25 °C for N^6,N^9-dimethyladenine which is about the same as the value of −8.3 mL/mol we found for N^6,N^6-dimethyladenosine. For two other self-associating purine derivatives values are known for the volume of the reaction. For 9-methylpurine a value of −4.0 mL/mol was determined,[21] for 6-methylpurine a value of −4.5 mL/mol was obtained.[3] It is interesting to note that for these last two compounds which have rather small association constants, the ΔV values are about half of those of the two methylated adenine derivatives, which have association constants which are at least a factor of 5 larger. The sign of ΔV has also been determined for a number of purine derivatives by dilatometry.[22] For all of these compounds a negative ΔV was found. It thus appears likely that the volume change for the stacking reaction of all purine derivatives is negative. This negative sign appears not to be in conflict with current thinking on the theory of hydrophobic interactions.[21,23]

Acknowledgment. We are indebted to Dr. A. Labhardt for valuable advice. We wish to thank A. Lustig for performing the density measurements.

References and Notes

(1) D. Pörschke, and F. Eggers, *Eur. J. Biochem.*, **26**, 490 (1972).
(2) F. Garland and R. C. Patel, *J. Phys. Chem.*, **78**, 848 (1975).
(3) F. Garland and S. D. Christian, *J. Phys. Chem.*, **79**, 1247 (1975).
(4) B. H. Robinson, A. Seelig-Löffler, and G. Schwarz, *J. Chem. Soc., Faraday Trans. 1*, **71**, 815 (1975).
(5) D. H. Turner, R. Yuan, G. W. Flynn, and N. Sutin, *Biophys. Chem.*, **2**, 385 (1974).
(6) D. H. Turner, G. W. Flynn, S. K. Lundberg, L. D. Faller, and N. Sutin, *Nature (London)*, **239**, 215 (1972).
(7) G. G. Hammes and C. D. Hubbard, *J. Phys. Chem.*, **70**, 1615 (1966).
(8) J. Rassing, *Adv. Mol. Relaxation Processes*, **4**, 55 (1972).
(9) D. Thusius, P. Dessen, and J.-M. Jallon, *J. Mol. Biol.*, **92**, 413 (1975).
(10) D. Thusius, *J. Mol. Biol.*, **94**, 367 (1975).
(11) A. Labhardt and G. Schwarz, *Ber. Bunsenges. Phys. Chem.*, **80**, 83 (1976).
(12) A. Labhardt, Ph.D. Thesis, University of Basel, 1975.
(13) R. Bretz, A. Lustig, and G. Schwarz, *Biophys. Chem.*, **1**, 237 (1974).
(14) M. P. Heyn and R. Bretz, unpublished results.
(15) M. P. Heyn and R. Bretz, *Biophys. Chem.*, **3**, 35 (1975).
(16) L. B. Townsend, R. K. Robins, R. N. Loeppky, and N. J. Leonard, *J. Am. Chem. Soc.*, **86**, 5320 (1964).
(17) E. Meyer and E.-G. Neumann, Physikalische und Technische Akustik, F. Vieweg und Sohn, Braunschweig, 1967, p 121.
(18) W. J. Bowen and H. L. Martin, *Arch. Biochem. Biophys.*, **107**, 30 (1964).
(19) L. M. Rhodes and P. R. Schimmel, *J. Am. Chem. Soc.*, **96**, 2609 (1974).
(20) F. I. Braginskaya, *Sov. Phys. Acoust.*, **20**, 424 (1975).
(21) U. Gaarz and H.-D. Lüdemann, *Ber. Bunsenges. Phys. Chem.*, **80**, 607 (1976).
(22) D. D. Kasarda, *Biochem. Biophys. Acta*, **217**, 535 (1970).
(23) P. O. P. Ts'o, in "Basic Principles in Nucleic Acid Chemistry", Vol. 1, P. O. P. Ts'o, Ed., Academic Press, New York, N.Y., 1974, p 558.