Kinetics of Base-Stacking Interactions and Proton Exchange of 6-Methylpurine Aqueous Solutions

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Acoustical relaxation properties of aqueous solutions of 6-methylpurine have been studied using broadband ultrasonic spectrometry in the frequency range 0.2−2000 MHz. Excess attenuation with relaxation characteristics was found near the lower limit of the measuring range and also around 100 MHz. The former relaxation process has been assigned to the proton exchange of the ampholytic 6-methylpurine solutions, yielding reasonable rate constants, e.g., 1.1×10^3 s^{-1} dm^3 mol^{-1} for the ionization and 6×10^8 s^{-1} dm^3 mol^{-1} for the neutralization at neutral pH. The high frequency relaxation process has been discussed in terms of a sequential and a random isodesmic reaction scheme of stack formation. Similar rate constants have been found for both models, e.g., 5×10^9 and 2.5×10^8 s^{-1} for the association and dissociation constant of the sequential reaction scheme, if cooperativity effects are neglected. There are, however, indications of weak cooperativity in the stacking.

Introduction

In water purine bases and mononucleosides associate, forming stacks of molecules, which are primarily stabilized by hydrophobic effects. Stacking is of considerable significance to the molecular basis of life because it constitutes the dominant interaction maintaining the secondary structure of DNA and other biopolymers.[1,2] Irrespective of its relevance in biology, stacking is also of substantial interest for our understanding of entropy-driven aggregation[3] in water in general. Stacking aggregation seems to be uniquely restricted to aqueous solutions.

The polyaromatic rings with hydrophilic groups substituted at the periphery represent a class of partly hydrophilic and partly hydrophobic molecules contrasting with the more common amphiphiles that form more or less globularly shaped micelles in water.[4−6] The latter are characterized by an effective number $f(i)$ of monomeric chains exposed to water ($i = 1, 2, ...$) that display a relative maximum at a certain aggregation number i. While $f(1) = 1$, initially $f(i)$ increases less than proportional with i because the hydrocarbon chains are protected from full contact with water due to aggregation. At high aggregation number, when the micelle reaches a spherical shape with the headgroups densely packed on the surface and with the hydrocarbon chains hidden, $f(i)$ decreases back to finally reach zero. With the polyaromatic rings, however, the entropy decrease, resulting in aqueous solution from a single disk or from a stack of disks, remains almost the same. This is particularly true in the extreme case when the peripheral cylindric surface of disk-shaped molecules is hydrophilic and the upper and lower faces are hydrophobic. In this case the function $f(i)$ would be constant, equal to 1. The shape of the function $f(i)$ determines the character of aggregation, which, basically, for both classes of molecules is treated in terms of an isodesmic reaction scheme

$$N_1 + N_i \underset{k_i^r}{\overset{k_i^f}{\rightleftharpoons}} N_{i+1} \qquad i = 1, 2, ... \tag{1}$$

where N_i denotes a multimer made of i monomers and k_i^f and k_i^r are the forward and reverse rate constants, respectively.

Acoustical spectrometry has proven to be a powerful tool to investigate the coupled reactions of the micelle formation/decay[7−9] and of the stacking aggregation/disintegration[10−15] as well. Ultrasonic attenuation measurements in the frequency range 10−100 MHz, aiming at the self-association of N^6,N^9-dimethyladenine in water ($K = 45.6$ dm^3 mol^{-1}, ref 10, $K = k_i^f/k_i^r$, $i = 2, 3, ...$), revealed excess spectra slightly broader than theoretically predicted for a Debye-type[16] relaxation with discrete relaxation time. The data have been discussed in terms of a sequential isodesmic scheme of coupled reactions (eq 1), assuming an identical equilibrium constant K for all steps except the dimerization.[10] Ultrasonic attenuation and sound velocity dispersion data, for aqueous solutions of N^6,N^6-dimethyladenosine ($K = 22.2$ dm^3 mol^{-1}, refs 10 and 17) measured between 0.5 and 36 MHz indicated a single relaxation term with discrete relaxation time.[13] It has been analyzed by assuming a random isodesmic association/dissociation model in which, different from eq 1, each aggregate is allowed to combine with any other one and in which the rate constants, reaction volumes, and enthalpy changes are assumed to be independent of the size of aggregates. Interestingly, self-association of aliphatic heterocyclic *p*-dioxane in water has been treated[18] in terms of the Romanov−Solov'ev model of local fluctuations in the concentration[19−21] rather than a reaction scheme based on stoichiometrically well-defined aggregates. Ultrasonic attenuation spectra in the frequency range 7−450 MHz of aqueous solutions of 6-methylpurine ($K = 6.7$ dm^3 mol^{-1}, refs 10 and 17) have been discussed,[12] again assuming a sequential isodesmic model with the equilibrium constant $K_1 = k_1^f/k_1^r$ different from that of the others ($K = K_i$, $i = 2, 3, ...$).

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Considering the different models for stacking aggregation, the question arises whether the experimental accuracy and the available frequency band in the previous ultrasonic measurements have been adequate to clearly prove the underlying relaxation time distribution. This is particularly true because proton-transfer reactions may also contribute to the sonic spectra of the aqueous solutions of cyclic bases. The ultrasonic attenuation data for aqueous solutions of purine ($K = 2.1$ dm^3 mol^{-1}, refs 10 and 17) have been evaluated assuming a predominant protolytic reaction and only a small effect from solute dimerization.[11] Because of the far-reaching conclusions drawn from the ultrasonic spectra and in view of the more recent significant development in the experimental techniques,[22,23] it seemed interesting to perform a broadband acoustical relaxation study of the 6-methylpurine/water system. This partly hydrophobic and partly hydrophilic base clearly stacks due to hydrophobic effects only. Hydrogen bonds between 6-methylpurine molecules are impossible because of missing H-bond donating sites. To inspect potential contributions from proton exchange to the spectra, one 6-methylpurine solution has been additionally measured at small pH. Also spectra of a purine solution have been recorded at two hydrogen ion concentrations for comparison.

Experimental Section

Aqueous Solutions. 6-Methylpurine (>98%, Aldrich, Taufkirchen, FRG), purine (>99%, Fluka, Taufkirchen, FRG), and HCl (p.a., Merck, Darmstadt, FRG) have been used without further purification. Water was deionized by bed ion exchange, additionally distilled and sterilized by UV irradiation. Aqueous solutions were prepared by depositing preweighed amounts of the organic constituent into suitable flasks and adding water up to the fiduciary mark. Some samples have been obtained by dilution of more concentrated solutions. Acidic samples have been prepared by potentiometric titration. The density ρ of the liquids was determined pycnometrically. The shear viscosity η_s was measured using Ubbelohde capillary viscometers (Schott, Mainz, FRG), which had been calibrated against deionized and degassed water.

Ultrasonic Attenuation Spectrometry. The ultrasonic attenuation coefficient α of the liquids has been measured in the frequency range 200 kHz to 2 GHz using three different methods and nine different specimen cells. Between 200 kHz and 20 MHz a cavity resonator method, specially designed to small sample loss, was employed. This yields the liquid attenuation coefficient relative to a reference liquid of carefully adjusted sound velocity and density.[22,23] A new biconcave reflector resonator (0.2−1.5 MHz[24]) and three biplanar resonator cells (1−20 MHz[25]) were available at the DPI. These cells mainly differ from one another by the fundamental frequency of thickness vibrations (4, 8, and 10 MHz) of the quartz transducer disks used as transmitter and receiver. At the MPI another biplanar resonator was employed for measurements between 1 and 3 MHz.[26] In the frequency ranges from 7 to 30 MHz and 80 to 400 MHz a fixed path cell[27] and a variable path length cell,[28] respectively, were used at the MPI to determine α from the decay in the amplitude of pulse modulated waves through the sample. The former method again yields the attenuation coefficient relative to the reference, the latter allows for absolute α measurements. Absolute determinations of the attenuation coefficient at variable sample length were also performed at the DPI utilizing an ultrasonic cell in the frequency range 50−500 MHz[29] and a hypersonic cell at frequencies between 1 and 2 GHz.[30]

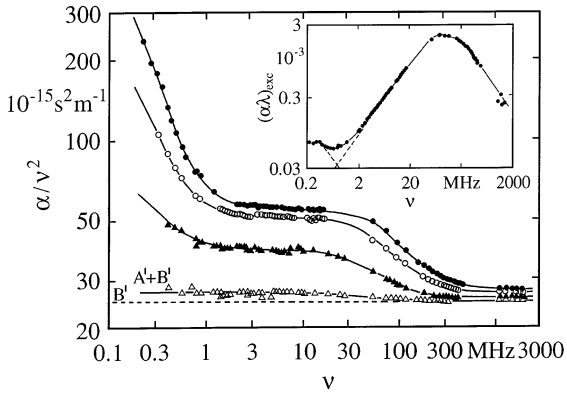

Figure 1. Frequency normalized ultrasonic attenuation spectra for aqeuous 6-methylpurine solutions (20 °C) at four concentrations: (△) 0.025 mol dm^{-3}; (▲) 0.145 mol dm^{-3}; (○) 0.4 mol dm^{-3}; (●) 0.6 mol dm^{-3}. For the last solution the data are also displayed as excess attenuation per wavelength in the inset.

Sound Velocity Measurements. At frequencies $\nu < 20$ MHz the sound velocity c_s of the samples has been derived from subsequent resonance frequencies and the sonic path length of the cells, taken into account the nonharmonic resonance frequencies.[22] These data have been confirmed by results from the traveling wave transmission method at small transducer separation, resulting in a characteristic waviness of the output signals due to multiple reflections.

Experimental Errors. The temperature T of the samples was controlled to within ± 0.03 K and it was measured with an accuracy of ± 0.02 K. Temperature gradients and differences in the temperature of different cells resulted in an estimated error of less than 0.1% in the α data of the liquids. Fluctuations in the frequency ν of the sonic signals were smaller than 0.01%. Their effect in the α values has been neglected, and in the regression analysis of the measured spectra the ν values have been considered to be accurately known. Ultimately, the experimental errors of the attenuation coefficient data depend on the frequency and on the α values as well. From repeated measurements of the same samples and from overlaps of segments of spectra obtained with different methods and cells they may be globally characterized to be

ν (MHz)	0.1−0.3	0.3−3	3−20	20−30	50−300	300−500	1000−2000
$\Delta\alpha/\alpha$	0.1	0.05	0.02	0.05	0.01	0.03	0.01

The relative errors in the sound velocity are $\Delta c_s/c_s = 0.005$.

Results and Analytical Description of Spectra

In Figure 1 α/ν^2 spectra for 6-methylpurine solutions at four concentrations are shown at 20 °C. To accentuate its high frequency part, the spectrum at the highest 6-methylpurine content is also presented in the format $\alpha\lambda = (\alpha/\nu^2)(c_s\nu)$. Excess attenuation

$$(\alpha\lambda)_{exc} = \alpha\lambda - B\nu \qquad (2)$$

is shown in the diagram because the asymptotic high frequency contribution $B\nu$ to the spectra is of less interest here.

All α/ν^2 spectra approach a frequency independent high frequency value $B' = \lim_{\nu\to\infty}(\alpha/\nu^2) = B/c_s$ at $\nu > 1$ GHz. At lower frequencies the α/ν^2 values exceed B', clearly indicating relaxation phenomena. This is even true at the lowest 6-methylpurine concentration ($c = 0.025$ mol dm^{-3}) for which the low frequency data $\lim_{\nu\to 0}(\alpha/\nu^2)$ exceed B' by $A' = 2 \times 10^{-15}$ s^2 m^{-1} (Figure 1). At higher base content two dispersion phenomena ($d(\alpha/\nu^2)/d\nu < 0$) emerge, one with a relaxation frequency

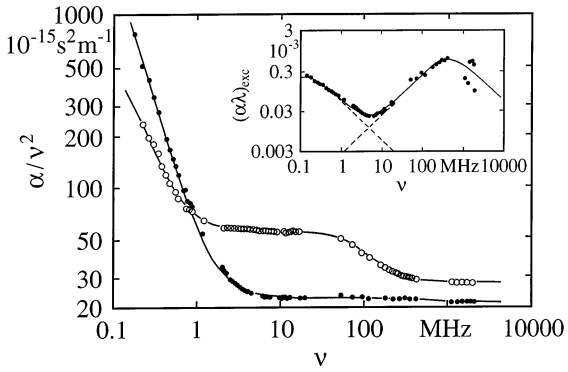

Figure 2. Frequency normalized ultrasonic attenuation spectra for aqeuous solutions of purine (●, 0.625 mol dm^{-3}, 25 °C) and 6-methylpurine (○, 0.6 mol dm^{-3}, 20 °C). In the inset, the spectrum of the purine solution is also shown in the format $(\alpha\lambda)_{exc}$ vs ν.

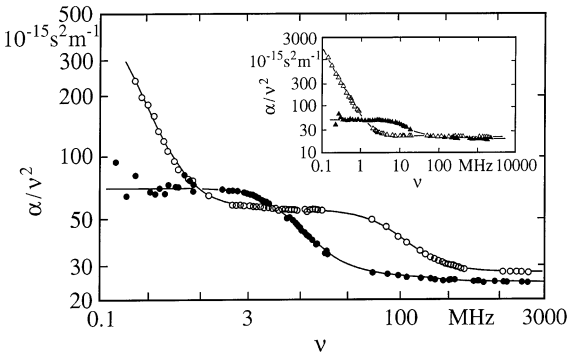

Figure 3. Comparison of ultrasonic spectra for aqueous solutions with (closed symbols, pH = 2) and without (open symbols, pH = 6.8) HCl added: 6-methylpurine, 20 °C, (○) 0.6 mol dm^{-3}, (●) 0.55 mol dm^{-3}; purine, 25 °C, (△) 0.625 mol dm^{-3}, (▲) 0.50 mol dm^{-3}.

ν_{R2} at around 100 MHz, the other one with relaxation frequency ν_{R1} in the low frequency part of our measuring range or even below this range. Both relaxation regions are also revealed by relative maxima in the excess attenuation per wavelength spectrum.

Figure 2 shows the α/ν^2 spectrum of the most concentrated 6-methylpurine solution (c = 0.6 mol dm^{-3}), in conjunction with that of the purine solution with an additional similar base content (c = 0.625 mol dm^{-3}). The latter spectrum likewise displays two relaxation regions, as also illustrated by the plot of data in the $(\alpha\lambda)_{exc}$ format given in the inset of Figure 2. The amplitude of the low frequency relaxation, however, seems to be larger for purine than for its derivative, whereas that of the high frequency relaxation is significantly smaller for purine than for 6-methylpurine.

The effect of an enhanced hydrogen ion concentration is displayed in Figure 3 where the α/ν^2 spectra for 6-methylpurine and purine (insert) solutions without and with HCl added are shown. For both cyclic bases the reduction in the pH leads to a significant reduction in the amplitude A_1 and relaxation time $\tau_1 = (2\pi\nu_{R1})^{-1}$ of the low frequency relaxation term. Simultaneously, the amplitude of the high frequency relaxation term in the spectrum of the 6-methylpurine system decreases substantially.

To apply for the above findings, the ultrasonic attenuation spectra have been represented by a sum of two Debye-type relaxation terms and the asymptotic high frequency term

$$\alpha\lambda = \frac{A_1\omega\tau_1}{1+\omega^2\tau_1^2} + \frac{A_2\omega\tau_2}{1+\omega^2\tau_2^2} + B\nu \qquad (3)$$

TABLE 1: Sound Velocity c_s and Parameters of the Relaxation Spectral Function (Defined by Eq 3) for Aqueous Solutions of 6-Methylpurine at Different Temperatures and Concentrations

T/°C	c, mol dm^{-3}	c_s, m/s ±0.5%	10^6A_1 ±25%	τ_1, ns ±50%	10^3A_2, ±2%	τ_2, ns ±20%	B, ps ±0.3%
20	0.025	1483.4			0.13	3.8	37.22
20	0.040	1484.0			0.27	3.4	37.22
20	0.070	1485.1			0.51	3.3	37.75
20	0.145	1488.1	14	687	1.13	2.9	37.72
20	0.201	1490.4	31	947	1.48	2.7	38.35
20	0.40	1499.5	59	809	2.80	2.1	40.20
20	0.50	1504.8	84	743	3.61	1.8	40.38
20	0.60	1510.7	133	985	4.16	1.6	41.49
25	0.40	1510.5	44	211	3.05	1.4	34.18
30	0.40	1521.3	52	338	3.10	1.2	29.66
			+HCl, pH = 2				
20	0.55	1530.3	584	18	0.54	1.0	37.75

Herein A_i and $\tau_i = (2\pi\nu_{Ri})^{-1}$, i = 1, 2, are the relaxation amplitudes and relaxation times, respectively, and $\omega = 2\pi\nu$. The parameter values of eq 3 have been obtained from nonlinear least-squares fitting procedures, minimizing the reduced variance

$$\chi^2 = \frac{1}{N-1-P}\sum_{n=1}^{N} w_n^2[(\alpha\lambda)_{meas}(\nu_n) - (\alpha\lambda)_{model}(\nu_n,\xi_p)]^2 \qquad (4)$$

Herein ν_n (n = 1, ..., N) are the measurement frequencies, ξ_p (p = 1, ..., P) are the adjustable parameters, $(\alpha\lambda)_{meas}$ is the measured value, $(\alpha\lambda)_{model}$ corresponds to the model relaxation spectral function, and $w_n = 1/\Delta(\alpha\lambda)(\nu_n)$ is a weighing factor, assumed to be inversely proportional to the experimental errors. Along with the sound velocities the parameter values for the 6-methylpurine system, as found by the nonlinear regression analysis of the spectra, are presented in Table 1.

Discussion

Proton Exchange. Previous studies of the stacking kinetics of purine bases in water[10−13] have revealed ultrasonic relaxations in the 10−100 MHz range, corresponding to our high frequency relaxation term ("2"). Because stacking aggregation is characterized by a unimodal size distribution in which, contrary to micellar systems, the dip for small (oligomeric) aggregates is missing,[3] no low frequency relaxation due to the formation and decay of aggregates is thus expected to exist. Such relaxation, with relaxation time on the order of milliseconds and seconds, reflects the formation and decay of aggregates in the spectra of micelle solutions,[31−32] in addition to the fast monomer exchange with relaxation time on the order of nanoseconds and microseconds. For this reason, we suggest that the low frequency relaxation term in our spectra, emerging below 1 MHz in the neutral solutions (Figures 1−3), represents a proton transfer reaction of the ampholytic solute. Though such reaction is of minor interest here, proton exchange is briefly discussed to verify this assignment.

In principle, 6-methylpurine (MP) is capable of an anionic ($MP^{\ominus}$) and a cationic ($MP^{\oplus}$) form, as illustrated by Figure 4. In aqueous solutions the scheme of coupled protolysis, hydrolysis, and proton exchange reactions governs the conversion of these species. Because of the small content of hydronium and hydroxyl ions of the neutral 6-methylpurine solution, its kinetics is dominated by proton exchange between the ampholytic molecules. For simplicity, we consider this reaction largely decoupled from the protolysis and hydrolysis at pH = 6.8 and neglect activity corrections even at rather high solute concentrations. Using the nomenclature of Figure 5 the ultrasonic

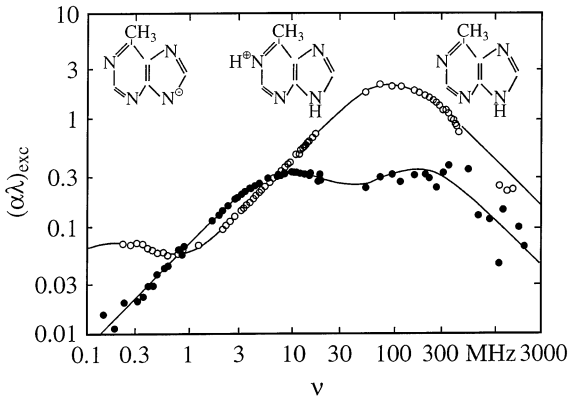

Figure 4. Ultrasonic excess attenuation spectra of the 6-methylpurine solutions given in Figure 3: (○) pH = 6.8; (●) pH = 2. Also shown are the anionic, cationic, and neutral forms of the ampholytic heterocyclic solute.

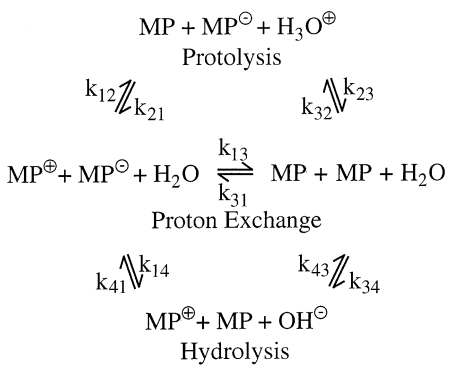

Figure 5. Reaction scheme accounting for the different proton exchange processes of ampholytes in water.

relaxation rate is then related to the concentrations of the species as

$$\frac{1}{\tau_1} = k_{13}([MP^{\oplus}] + [MP^{\ominus}]) + k_{31}2[MP] \tag{5}$$

where $[MP^{\oplus}] = [MP^{\ominus}]$ ($\ll[MP] \approx c$). With the equilibrium constant

$$K_1 = k_{13}/k_{31} = [MP]^2/[MP^{\oplus}]^2 \tag{6}$$

the reverse rate constant may be expressed as

$$k_{31} = (2[MP]\tau_1(1 + [MP]/[MP^{\oplus}]))^{-1} \tag{7}$$

The amplitude A_1 of the ultrasonic relaxation process is related to the isentropic reaction volume $\Delta V_{S1} = \Delta V_{T1} - a_p\Delta H_1/\rho c_p$ as

$$A_1 = \frac{\Delta V_{S1}{}^2 \pi \Gamma_1 c_{s\infty} \rho}{RT} \tag{8}$$

where ΔV_{T1} is the isothermal volume change and ΔH_1 is the enthalpy change accompanied by the process. Furthermore, a_p denotes the coefficient of thermal expansion, c_p the specific heat at constant pressure, $c_{s\infty}$ the sound velocity at frequencies well above the relaxation frequency, and Γ a stoichiometric factor which, for the reaction under consideration, is given by

$$\Gamma_1{}^{-1} = 2([MP^{\oplus}]^{-1} + [MP]^{-1}) \tag{9}$$

We do not know the volume change associated with the proton exchange process. For an estimate we use $\Delta V_S{}^2 = 1100$ cm^6/mol^2 as resulted for the protolysis of triethylamine $N(CH_2)_3N$ at 25 °C,[33] in close agreement with $\Delta V_S{}^2 = 720$ cm^6 mol^2 for the dissociation/neutralization of water.[34] On this conditions, $\Gamma_1 = 4 \times 10^{-4}$ mol dm^{-3} results and thus $[MP^{\oplus}] = [MP^{\ominus}] = 8 \times 10^{-4}$ mol dm^{-3}, yielding the reasonable values $K_1 = 5.6 \times 10^5$, $k_{31} = 1.1 \times 10^3$ s^{-1} dm^3 mol^{-1}, and $k_{31} = 6 \times 10^8$ s^{-1} dm^3 mol^{-1} for the equilibrium constant and the rate constants, respectively.

At pH = 2 protolysis may be considered to be decoupled from the other reactions of the complete scheme. Because the concentration of the anionic species $MP^{\oplus}$ is only small, step

$$MP^{\oplus} + H_2O \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} MP + H_3O^{\oplus} \tag{10}$$

has to be taken into account. Hence

$$\frac{1}{\tau_1} = k_{21}([H_3O^{\oplus}] + [MP]) + k_{12} \tag{11}$$

where

$$K_2 = k_{21}/k_{12} = [MP^{\oplus}]/([H_3O^{\oplus}][MP]) \tag{12}$$

and

$$\Gamma_2{}^{-1} = [MP^{\oplus}]^{-1} + [H_3O^{\oplus}]^{-1} + [MP]^{-1} \tag{13}$$

Analogous application of the above procedure for the proton exchange, using the same isentropic reaction volume, yields $K_2 = 1.5 \times 10^5$ dm^3 mol^{-1}, $k_{21} = 1.5 \times 10^{11}$ dm^3 mol^{-1} s^{-1}, and $k_{12} = 10^6$ s^{-1}, which is again a reasonable result.

We conclude that the low frequency relaxation of the neutral solution of 6-methylpurine and of purine in water may be assigned to the proton exchange between the molecules of ampholyte. If HCl is added to the solutions, protolysis dominates the reaction kinetics and because of the considerable concentration of hydronium ions at pH = 2, the relaxation rate $\tau_1{}^{-1}$ of the proton-transfer becomes much larger (eq 11). Therefore the low frequency relaxation process is shifted toward higher frequencies (Figure 3, Table 1). Brennan and Kustin, studying the hydrolysis branch of the coupled reaction scheme (Figure 5) at pH = 9.88, found even higher relaxation frequencies.[11] We mention that a full account of the hydrolysis of 6-methylpurine and purine has to also consider coupling of the proton exchange reaction to the stacking process and, additionally, the fact that the ampholytes offer three nitrogen lone electron pairs for the association of protons. For this reason, the equilibrium (10), in principle, has to be completed:

$$\begin{aligned} MP + 3H_3O^{\oplus} &\rightleftharpoons MP^{\oplus} + 2H_3O^{\oplus} + H_2O \\ &\rightleftharpoons MP^{2\oplus} + H_3O^{\oplus} + 2H_2O \\ &\rightleftharpoons MP^{3\oplus} + 3H_2O \end{aligned} \tag{14}$$

Because of the repulsive forces of electrically charged 6-methylpurine molecules, however, formation of $MP^{2\oplus}$ and particularly of $MP^{3\oplus}$ is unlikely.[34]

Stacking. Essentially two different models of stacking kinetics of heterocyclic bases have been discussed in the literature. In the sequential model[10] only monomers can associate to or dissociate from larger aggregates, as represented by the isodesmic reaction scheme given in eq 1. Identical equilibrium constants $K = K_i$, $i = 2, 3, \ldots$, have been assumed for all steps

in the coupled scheme of reactions, except the dimerization ($i = 1$). For this particular step, to account for cooperativity effects, allowance was made for a modified stability constant βK, where β is a cooperativity parameter. The sequential model has been extended for the effect of "attenuated" equilibrium constants, varying as

$$K_i = K/i \qquad i \geq 2 \tag{15}$$

again allowing the association constant of the dimerization to vary independently.[12] The attenuated equilibrium constant model predicts a relaxation time distribution in the acoustic spectra. It has been particularly introduced because the available data pointed at an underlying relaxation time distribution in the ultrasonic spectra of solutions of 6-methylpurine in water.[34] The broadening in the measured spectra was probably due to experimental errors, because later measurements on *N*,^{6}*N*^{9}-dimethyladenine[10] and on *N*,^{6}*N*^{6}-dimethyladenosine[13] in water, like ours on aqueous solutions of 6-methylpurine, did not reveal indications of an underlying distribution of relaxation times. The high frequency relaxation in our broadband ultrasonic attenuation data can be well represented by a discrete relaxation time, thus suggesting one degenerate sequential association model ($\beta = 1$).

Alternatively, a random isodesmic association model has been proposed,[13] which includes the formation and decay of an aggregate from or into, respectively, any two smaller aggregates

$$N_i + N_j \underset{k_{ij}^{r}}{\overset{k_{ij}^{f}}{\rightleftharpoons}} N_{i+j} \qquad i, j = 1, 2, ... \tag{16}$$

The random isodesmic model has been shown to also predict ultrasonic spectra with a discrete relaxation time. If $k_{ij}^{f,r} = k_{f,r}$ at $i \neq j$ and $k_{ii}^{f,r} = k_{f,r}/2$, it is related to the forward and reverse rate constant as[13]

$$\tau_2^{-2} = k_f k_r c + k_r^2/4 = k_r^2(1 + 4Kc)/4 \tag{17}$$

The sequential isodesmic model (eq 1) yields[10]

$$\tau_2^{-1} = 2k_f c_m + k_r \tag{18}$$

where $k_f = k_i^f$, $k_r = k_i^r$, $i = 2, 3, ...$, and

$$c_m = \sum [N_i] = [N_1](1 - \beta) + \frac{\beta[N_1]}{1 - K[N_1]} \tag{19}$$

The total 6-methylpurine concentration is given by

$$c = [N_1](1 - \beta) + \frac{\beta[N_1]}{(1 - K[N_1])^2} \tag{20}$$

Hence, if $\beta = 1$ is assumed and thus $k_f = k_i^f$, $k_r = k_i^r$, $i = 1, 2, ...$,

$$c_m = ((1 + 4Kc)^{1/2} - 1)/(2K) \tag{21}$$

follows and

$$\tau_2^{-2} = 4k_f k_r c + k_r^2 = k_r^2(1 + 4Kc) \tag{22}$$

The squared relaxation rates τ_2^{-2} of the high frequency relaxation of the 6-methylpurine solutions are displayed versus solute concentration c in Figure 6. Up to 0.4 mol dm^{-3} the experimental data in fact define a linear relationship, yielding

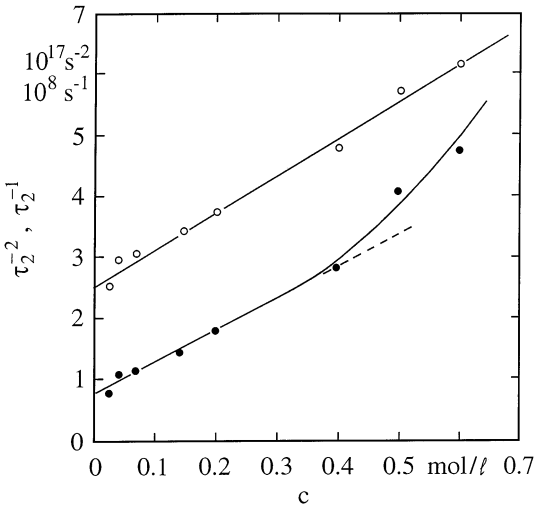

Figure 6. Squared relaxation rates τ_2^{-2} (●) and relaxation rates τ_2^{-1} (○) of the high frequency relaxation process versus 6-methylpurine concentration c.

$k_r = 5 \times 10^8$ s^{-1}, $K = 8$ dm^3 mol^{-1}, and $k_f = 4 \times 10^9$ dm^3 mol^{-1} s^{-1} if the random isodesmic model (eq 17) is assumed, and $k_r = 2.5 \times 10^8$ s^{-1}, $K = 20$ dm^3 mol^{-1}, and $k_f = 5 \times 10^9$ dm^3 mol^{-1} s^{-1} on assumption of the sequential isodesmic model. The τ_2^{-1} versus c relation also given in Figure 6 exhibits a linear behavior up to the highest concentration of this study. This finding possibly indicates a cooperativity factor smaller than one, because c_m tends toward c if β approaches zero and, consequently, eq 18 predicts a linear dependence of τ_2^{-1} upon c. A β value smaller than 1 implies cooperativity effects in the formation of larger stacks because the formation of the dimer is less favored than the other steps in the coupled reactions.

Conclusions

The Debye-type relaxation terms in the broadband ultrasonic spectra of aqueous solutions of 6-methylpurine reflect the proton exchange between the ampholytic polyaromatic ring molecules and their formation of stacks. At neutral pH the ionization and neutralization rate constant of the proton exchange reaction have been estimated as $k_{31} = 1.1 \times 10^3$ s^{-1} dm^3 mol^{-1} and $k_{13} = 6 \times 10^8$ s^{-1} dm^3 mol^{-1}, respectively, corresponding with an equilibrium constant $K_1 = k_{13}/k_{31} = 5.6 \times 10^5$. At low pH the proton exchange between the ampholytic molecules is masked by protolysis, characterized by the significantly larger rate constants $k_{21} = 1.5 \times 10^{11}$ dm^3 mol^{-1} s^{-1} and $k_{12} = 10^6$ s^{-1} as well as by the equilibrium constant $K_2 = k_{21}/k_{12} = 1.5 \times 10^5$ dm^3 mol^{-1}. The relaxation term that reveals the stacking can be discussed in terms of a sequential isodesmic reaction model and also of a random isodesmic model. If cooperativity effects are neglected, similar forward rate constants follow for both association models: $k_f = 5 \times 10^9$ dm^3 mol^{-1} s^{-1} if the sequential model is assumed and $k_f = 4 \times 10^9$ dm^3 mol^{-1} otherwise. The reverse rate constants differ by a factor of 2, $k_r = 2.5 \times 10^8$ s^{-1} for the former and $k_r = 5 \times 10^8$ s^{-1} for the latter model, yielding $K = k_j/k_r = 20$ dm^3 mol^{-1} and $K = 8$ dm^3 mol^{-1}, respectively. There are some indications for cooperativity, favoring the formation of large aggregates compared to the formation of dimers.

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