The Association between Purine Nucleosides and Benzene in Aqueous Solution, Studied by Proton Magnetic Resonance and Nuclear Overhauser Enhancements

Hans-Dietrich Lüdemann and Eberhard v. Goldammer

Fachbereich Biologie, Lehrstuhl für Physik, Universität Regensburg

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Nucleosides, benzene, water, PMR, NOE

The dependence of the PMR spectra of three purine nucleosides (inosine, adenosine and deoxyadenosine) on temperature, concentration and addition of benzene (0.12 molal) has been investigated in aqueous solution. The interaction of benzene with the nucleosides is deduced from the spectra. Enthalpies of self association have been determined as 2.2 ± 0.1 kcal·mole^{-1} for dA and I. The corresponding values obtained for hetero association with benzene are 2.7 ± 0.2 kcal·mole^{-1} for dA and 2.3 ± 0.2 kcal·mole^{-1} for I. The formation of edge to face complexes is concluded. Additional evidence for this type of hetero association has been obtained from the concentration and solvent dependence of the nuclear Overhauser enhancements (NOE) of solutions of the nucleosides. The enhancement factors of aqueous solutions of dA and I depend on the concentrations of the nucleosides. In solutions containing I the NOE found previously in DMSO and the NOE observed in D_2O differ significantly [1]. The results obtained can be explained by a change of the average glycosyl torsion angle of approximately 10° to 15° in the two solvents.

Aqueous solutions of monomeric nucleosides can serve as models for studies of the self and hetero association occuring in polymeric DNA and RNA. Proton magnetic resonance (PMR) shifts are a very sensitive probe in this type of investigations, and have been used frequently [2–6].

Coplanar face to face stacking as it is found in aqueous solutions of nucleic acids has been observed as the predominant type of association between aromatic nucleic acid constituents and other aromatic compounds carrying polar groups such as purine, tryptophane, or ethidium bromide [7–10]. However, a different type of complex may be formed between nonpolar aromatic molecules like benzene, and aromatic partners carrying polar groups resulting in a rectangular edge to face configuration. In this structure the plane of the nonpolar molecule is placed at right angles relative to the planar polar molecule in close contact with a polar side group. The system is stabilized by charge transfer between the aromatic ring of the former and the polar group of the latter.

So far PMR investigations of such complexes are lacking although these should be of great potential interest, *e. g.* as a possible path of reaction for the carcinogenic action of aromatic compounds. The barrier of low solubility in water which handicaps such experiments can be overcome by application of a sensitive PMR spectrometer. This is demonstrated in the present work on aqueous solutions of nucleic acid constituents and benzene which was used as a nonpolar model substance.

A basis for discrimination between alternative structures is provided by measurements of PMR-shifts. Whereas the effects of aromatic ring currents on the shifts of ring protons are reciprocal between compounds stacked face to face onto each other, a different result is expected for rectangular complexes. On the basis of such differences the existence of the complexes in aqueous solution has been concluded. Supporting evidence has been furnished by measurements of nuclear Overhauser enhancements (NOE).

The interaction between benzene and some pyrimidine nucleosides has been investigated by measurements of PMR-shifts in a preceding paper [11]. These experiments are now extended to the three purine nucleosides adenosine (A), deoxyadenosine (dA), and inosine (I). In comparison to pyrimidine nucleosides the purine derivatives are much less soluble but the effect on the benzene protons at equal concentrations and the variation of chemical shifts of the base protons with concentration is about one order of magnitude higher, facilitating work on the latter.

Requests for reprints should be sent to Dr. H.-D. Lüdemann, Lehrstuhl für Physik, Fachbereich Biologie der Universität Regensburg, *D-8400 Regensburg*, Universitätsstraße 31.

Another way to avoid the poor solubility in water is to use other polar solvents. However, application of the resulting structural data to aqueous systems which has been practised occasionally is not justified generally. In the case of inosine, substantial conformational differences in D_2O and DMSO are deduced from the experimental data due to a change of the average glycosyl torsion angle of about 15°.

Experimental

Substances

Inosine, adenosine and deoxyadenosine (I, A and dA) were purchased from Papierwerke Waldhof-Aschaffenburg AG., Mannheim, BRD, and used without further purification. Heavy water (99.5% deuterated), dimethylsulfoxide (99.5% deuterated), and benzene (Uvasol) were bought from E. Merck, Darmstadt, BRD, and hexamethyldisiloxane (HMDS) (puriss.), the external locking substance, from Fluka AG., Buchs, Switzerland. The solutions used in the shift measurements were prepared as described previously [11]. The nuclear Overhauser enhancements were measured with solutions in sealed tubes degassed with a minimum of five freeze-pump-thaw cycles at a pressure of maximal $5 \cdot 10^{-5}$ Torr. The nucleosides used in these experiments were dissolved in heavy water and freeze-dried, in order to remove the exchangeable protons. In order to minimize a proton deuteron-exchange of the base protons, the frozen solutions were stored at −20 °C until use.

Spectra

The proton shifts were obtained in the CW-mode at 100.1 MHz with a Varian XL-100 variable temperature accessory in 12 mm tubings. Frequencies given in the following are taken against external neat HMDS contained in a coaxial capillary tubing. They were measured to ± 0.1 Hz with a U-4410 frequency counter. Bulk magnetic susceptibility corrections were not applied. The maximum deviations of the frequencies measured in the same solution in different runs is ± 0.5 Hz. As discussed previously this scatter is mainly due to the temperature variations in the probe volume. The nuclear Overhauser enhancements were taken at normal probe temperature (38 °C), the spectrometer locked to the deuterons of the solvent. The saturation frequency was supplied by a Varian spin decoupler. In a series of preliminary experiments it was checked, that the peak heights observed are proportional to the peak areas within the limits of error inherent to the integration process. Consequently, in the experiments only the relative differences of the peak heights were taken for the NOE.

The output of the decoupler unit was increased in steps of 5 dB and the spectra were recorded with the decoupler frequency alternatively on the resonance to be saturated and 200 Hz off resonance. A plot of the enhancement factors *versus* decoupler output was obtained from this data. In this plot the region where the resonance under investigation is saturated shows up as a plateau. At low power outputs experimental points are low because of incomplete saturation of the spin system, whereas at high RF-levels they run either to higher or to lower enhancements due to interference of the decoupler output with the observation field and/or because of saturation of other protons close to the signal to be saturated. After having established the plateau region, the experiments were repeated as described, above the central range of this region, increasing the RF in steps of 2 to 3 dB. The enhancements given in the Table below, are the average values of 20 to 30 determinations. They are judged as reliable to ± 0.005. The enhancements are usually given as $f_d(s)$-values [30], d standing for detected and s for saturated. The $f_d(s)$-values are the factors by which the intensity of the proton resonance at position d is modified by the second radio frequency field in resonance with the proton spin at position S.

Results

Shifts

Due to the high sensitivity of the XL-100-15 system, spectra in the concentration range from 0.01 molal to the limit set by the solubility of the compounds could be obtained in single sweep experiments. In some cases crystallization of the supersaturated solutions occurred sufficiently slow to allow recording of spectra. Inosine (I) (0.01 to 0.1 molal), adenosine (A) (0.01 to 0.05 molal), and deoxyadenosine (d) (0.01 to 0.133 molal) in 0.12 molal solutions of benzene in heavy water were studied. The line positions as function of concentration and temperature for the H(8), H(2), H(1′), and benzene protons are given in Figs. 1 to 4. In a second set of experiments the same nucleosides were dissolved in pure heavy water. Compared to the benzene-containing solutions, the shifts in this case are displaced approximately 0.5 to 1.0 Hz downfield, an effect similar in size and sign to the results with the pyrimidine nucleosides [11]. Contrary to this small effect close to the limit of reproducibility, the

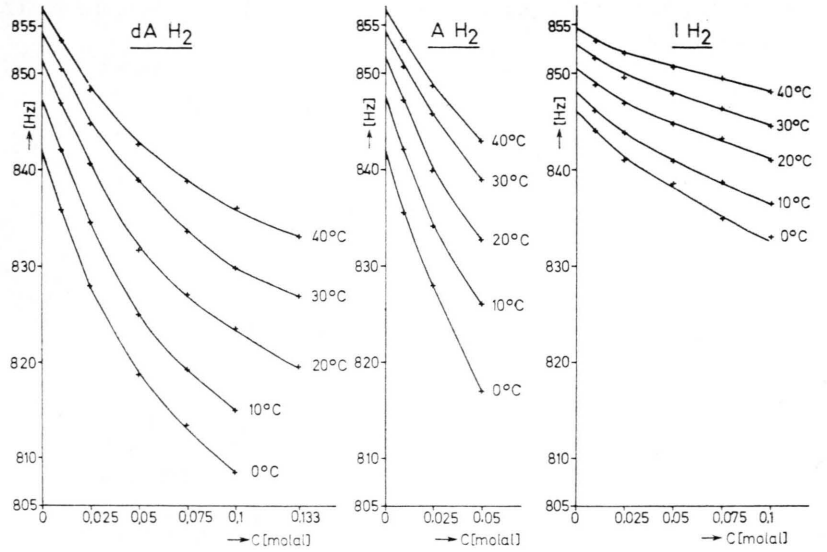

Fig. 1. Concentration and temperature dependence of the chemical shift of the H(2)-proton resonance in solutions of deoxyadenosine (dA), adenosine (A) and inosine (I) in deuterium oxide with 0.12 molal benzene added. (Shifts are taken against HMDS as an external standard.)

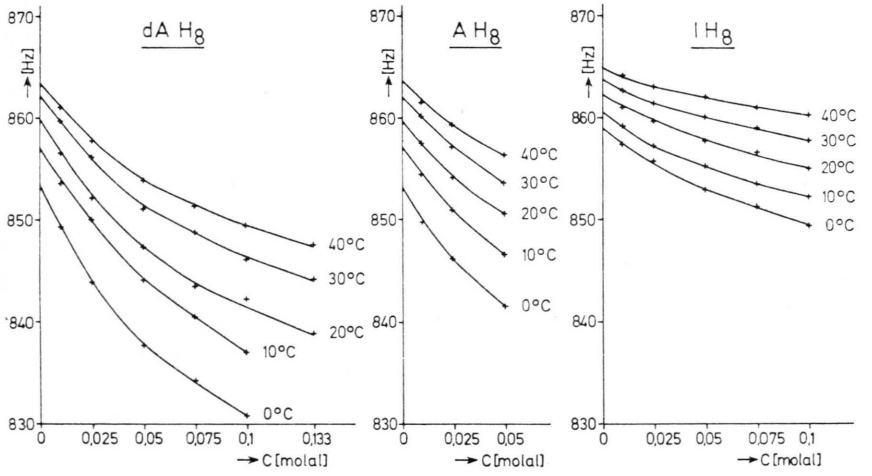

Fig. 2. Concentration and temperature dependence of the chemical shift of the H(8)-proton resonance in solutions of deoxyadenosine (dA), adenosine (A) and inosine (I) in deuterium oxide with 0.12 molal benzene added. (Shifts are taken against HMDS as an external standard.)

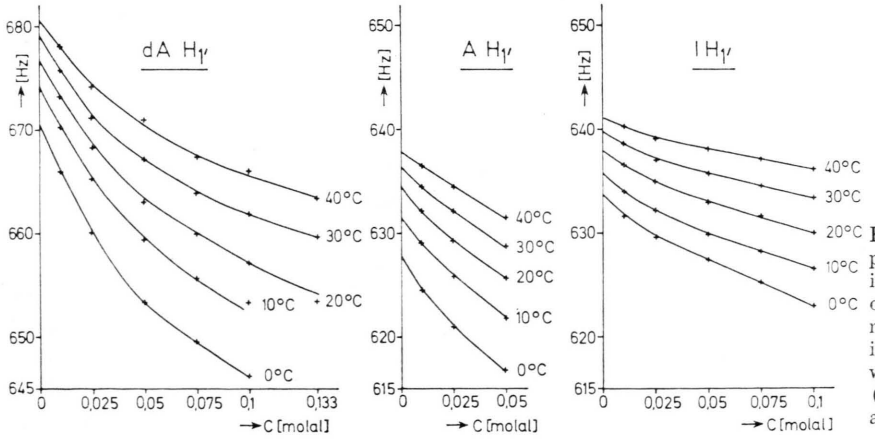

Fig. 3. Concentration and temperature dependence of the chemical shift of the H(1')-proton resonance in solutions of deoxyadenosine (dA), adenosine (A) and inosine (I) in deuterium oxide with 0.12 molal benzene added. (Shifts are taken against HMDS as an external standard.)

Table I. Nuclear Overhauser enhancements of the solutions of some purine nucleosides in D_2O and DMSO, obtained at 38 °C.

I.1 0.1 dA + 0.12 ⌀ in DMSO, Proton saturated

Proton observed	H(8)	H(2)	H(1′)	Benzene
H(8)	—	0.012	0.153	0
H(2)	0.010	—	0.029	0
H(1′)	0.164	0.032	—	0
Benzene	0	0	0	—

I.2 0.1 dA + 0.12 ⌀ D_2O, Proton saturated

Proton observed	H(8)	H(2)	H(1′)	Benzene
H(8)	—	0.074	0.170	0.016
H(2)	0.070	—	0.048	0.016
H(1′)	0.187	0.034	—	0.017
Benzene	pos.	0.014	0.015	—

I.3 0.1 dA in D_2O, Proton saturated

Proton observed	H(8)	H(2)	H(1′)
H(8)	—	0.065	0.168
H(2)	0.067	—	0.053
H(1′)	0.180	0.031	—

I.4 0.025 dA in D_2O, Proton saturated

Proton observed	H(8)	H(2)	H(1′)
H(8)	—	0.010	0.165
H(2)	0.013	—	0.028
H(1′)	0.181	0.024	—

I.5 0.1 I in DMSO, Proton saturated

Proton observed	H(8)	H(2)	H(1′)
H(8)	—	0.015	0.141
H(2)	0.012	—	0.031
H(1′)	0.137	0.036	—

I.6 0.1 I in D_2O, Proton saturated

Proton observed	H(8)	H(2)	H(1′)
H(8)	—	0.036	0.186
H(2)	0.041	—	0.028
H(1′)	0.210	0.034	—

I.7 0.025 I in D_2O, Proton saturated

Proton observed	H(8)	H(2)	H(1′)
H(2)	—	0.010	0.198
H(1′)	0.012	—	0.048
H(8)	0.229	0.41	—

I.8 * 0.025 A in D_2O, Proton saturated

Proton observed	H(8)	H(2)	H(1′)
H(8)	—	0.012	0.197 (0.18)
H(2)	0.013	—	
H(1′)	0.207 (0.20)	0.043	0.032 —

* Values in parentheses were obtained by Davis and Hart [1] in 0.25 m DMSO solution.

influence of the purine nucleosides on the benzene protons is almost an order of magnitude larger compared to the effect of the pyrimidine derivatives (see Fig. 4). Extending the measurements to a minimum concentration of 0.01 molal nucleoside allows an extrapolation of the data to infinite dilution, with an accuracy equal to the experimental error given, and to separate, at least at this concentration, the influence of temperature on the shifts from the association effects.

Nuclear Overhauser enhancements

Table I contains the enhancements of six solutions of dA, A and I in heavy water. For comparison the results obtained for two solutions of dA and I in DMSO are included. The enhancements for I in DMSO at a slightly higher concentration (0.25 molal) have in part been published previously [1]. The data, where comparable, agree within the limits of experimental error. Temperature variation has not

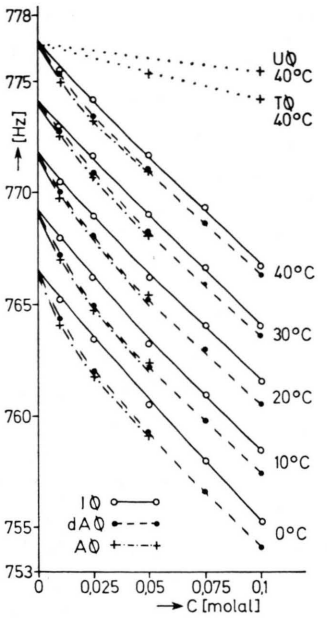

Fig. 4. Concentration and temperature dependence of the chemical shift of the benzene-protons in solutions of deoxyadenosine (dA), adenosine (A) and inosine (I) in deuterium oxide with 0.12 molal benzene added. (Shifts are taken against HMDS as an external standard.)

been attempted in this type of experiments, since the flow of cold nitrogen keeping the sample at low temperatures destroys the extreme long term stability prerequisite for this kind of investigations.

Discussion

In A and dA the concentration and temperature dependence of H(2) is the same. The H(8) and H(1′) protons in the two nucleosides show equal changes in the infinite dilution shifts, whereas at all temperatures the concentration dependence is higher in dA than in A by a mean factor of 1.6 at H(1′) and 1.3 at H(8). The effects observed with the different protons of I are approximately one third to one half of those found in A and dA.

Temperature dependence of shifts

The data given in the Figs. 1 to 4 were obtained with an external HMDS lock. As stated before, no bulk magnetic susceptibility corrections were applied. Changes of the difference between the bulk magnetic susceptibilities of the sample and lock liquids with temperature should be considered as the most plausible origin for the effects observed. However, under the most unfavourable assumptions for the coefficients of thermal expansion in the two liquids, this effect can account at most for a change of -2.5 to -3.0 Hz for a temperature variation from 40 °C to 0 °C *.

The H(2) resonances in A and dA are shifted 15 Hz downfield when the temperature is decreased from 40 °C to 0 °C, while H(1′) and H(2) are only lowered by 10 Hz. The corresponding values for I are H(2): 9 Hz, H(8): 6 Hz and H(1′): 7.5 Hz (Figs. 1 – 2). The shifts of the benzene protons in 0.12 molal solution in D_2O decrease by 10 Hz in the same temperature interval (Fig. 4).

These observations can be explained qualitatively by the influence of the solvation sphere surrounding the nucleoside molecule. Some of the molecules in the immediate vicinity of the base will form hydrogen bonds with the ring nitrogenes and the ligand at the 6-position. The degree of formation of hydrogen bonds depends on the temperature and can indirectly influence the electron densities at the three protons observed here. In addition, the structure of the whole hydration sphere around the nucleoside changes with temperature, and consequently may also modify the local magnetic susceptibilities or the electron densities at the base protons.

Similar temperature dependencies have been found previously in the systems dioxane-water and pyridine-water [12]. Both effects discussed above should lead to upfield shifts with decreasing temperature, and explain the even higher changes observed at H(2) of A and dA. Of all protons observed, H(2) is closest to the amino group at the 6-position. Fratiello has examined the concentration dependence of the amino protons in a series of amides [13]. From his results it is evident, that around the NH_2-group a strong hydration sphere is built up. The change of this structure with temperature is thought to be responsible for the effects reported here.

Concentration dependence of shifts

Nucleosides

The high field shifts observed with increasing concentration are explained by the effects of the aromatic ring currents in the nucleosides [14–16]. In

* This value is close to the result obtained for the smallest temperature variation in a pyrimidine nucleoside published in a previous paper [11]: Between 40 °C and 0 °C the observed change of shift is -2.9 Hz for H(6) of U at infinite dilution.

water, the nucleosides associate strongly [17]. They form collision complexes in which the bases are preferably ordered in "face to face" stacks. This sterical arrangement brings neighbouring molecules into regions, where the diamagnetic part of the ring currents weakens the effective magnetic field and accordingly the resonance signals are shifted to higher frequencies.

In all three nucleosides the H(2)-resonance is most sensitive to the increase of concentration. Since even in purine [18] the change of the resonance signal of H(8) with concentration is only about half the one observed at H(2), the sterical hindrance of approaching molecules due to the sugar moiety cannot be the only source of this effect. Sterical arguments should however be considered in comparison of the shifts of H(8) and H(1′) in A and dA. As stated before, the H(2) signals in these nucleosides show the same temperature and concentration dependence within experimental limits of error. The other two resonances are shifted considerably less in A than in dA. This can be explained most plausibly by a change in the sterical arrangements of the ribose and deoxyribose groups, respectively. A changed mean glycosyl torsion angle and the bulky 2′-hydroxyl group are regarded as the most likely explanation for these findings. The concentration dependence of all nucleoside protons observed here becomes more pronounced with decreasing temperature, as is to be expected, if the association process is exothermic.

Benzene

The shift of the benzene protons in pure aqueous solution is not concentration dependent. Spectra run with benzene, 0.005 molal in heavy water, yielded the same signal position as in saturated 0.12 molal solution. This proves that the average environment around the benzene molecules is not altered by increasing dilution. In the experiments with the pyrimidine nucleosides [11] it appeared rather puzzling that the influence of the benzene molecules on the nucleosides is much smaller than the effect of the nucleosides on the benzene resonance. The same applies for the present case (Fig. 4). Although the influence of the purine nucleosides on the benzene resonances is approximately five times stronger than found in the earlier experiments with the pyrimidine nucleosides, changes in the nucleoside resonances remain at the limit of detectability. The effects of the two purine derivatives on the benzene resonances are almost equal, the adenine nucleosides causing slightly greater changes. The results seem to rule out the participation of the benzene molecule in face to face stacking processes. The most likely explanation is the formation of a collision complex between the benzene molecules and the substituent in the 6-position, the plane of the benzene ring being preferably perpendicular to the plane of the purine bases. This "edge to face" arrangement would expose part of the benzene protons to the regions of high diamagnetic fields [16] while leaving the nucleosides rather unaffected. This explanation is in accordance with the findings of Ledaal [19] with aromatic solvent induced shifts in a series of simpler polar organic compounds. Further support for this explanation can be drawn from the influence on the benzene shift exerted by purine riboside lacking a polar substituent at the 6-position. At equal concentrations the effect observed is approximately 50% of the shifts found for dA and I [20].

Thermodynamic considerations

It is current practice in studying solutions containing compounds able to form weak molecular complexes to derive from the temperature and concentration dependence of the chemical shifts the thermodynamic parameters governing the association processes and deduce stoichiometric models for the products of association. The significance of such procedures has been investigated by Deranleau [21, 22] by application of the principles of information theory, leading to the result, that in most cases were the stoichiometry of the reaction and the chemical shifts of all complexes involved is unknown in advance a proof for a specific model can hardly be obtained from data taken in only a limited range of the degree of association. Association constants, or apparent association constants derived from the commonly applied procedures [23–25] are parameters of little physical significance. The first derivative of the apparent association constant *versus* temperature however can yield the correct reaction enthalpy [22].

The solubility of the purine-nucleosides of which three of the most soluble were used in our work, limits the investigation at and below room temperature to concentrations smaller than 0.1 molal. Although the slopes of the curves in Figs. 1 to 4 decrease at higher concentrations, the data are in-

sufficient for any safe extrapolation to the plateau of complete association.

In order to compare the shift values measured at different temperatures, and separate the influence of the solvation phenomena and susceptibility changes from the changes of shift caused by association, it was assumed that the solvation effects are not dependent on the degree of association. That this approximation is reasonable, is supported by a comparison of the "reduced shifts" $\delta_{red}^{H(A)}$ for the three protons observed

$$\delta_{red}^{H(A)} = (\Delta_T^{\infty} - \Delta_T^{C})/(\Delta_{40°C}^{\infty} - \Delta_{40°C}^{0,1}).$$

Δ_T^{∞} = Frequency at infinite dilution and temperature T;

Δ_T^{C} = frequency at concentration C and temperature T.

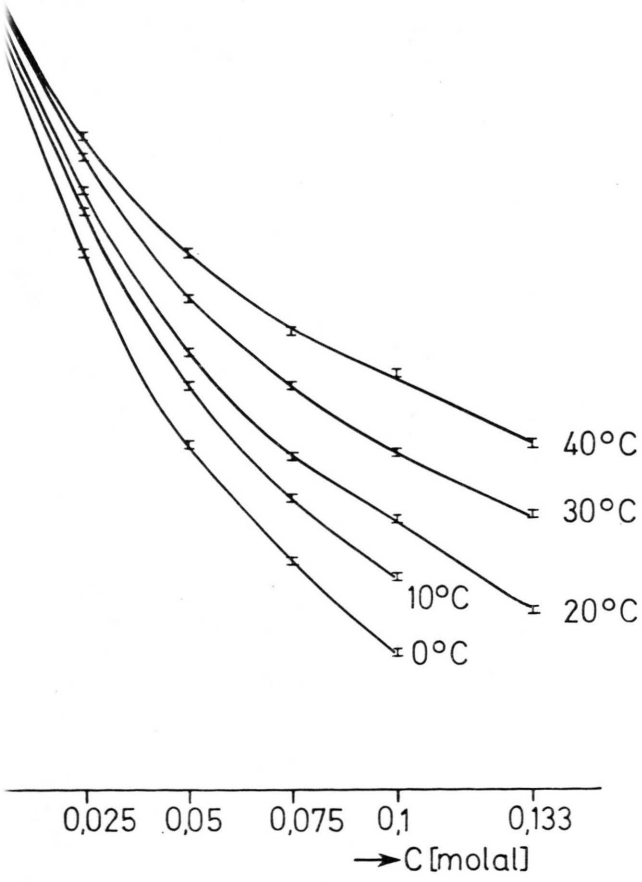

Fig. 5. Concentration dependence of the average values of the "reduced shifts" δ_{red} of the H(2), H(8) and H(1′) proton resonances in deoxyadenosine in deuterium oxide solutions with 0.12 molal benzene added.

Though $(\Delta_{40°C}^{\infty} - \Delta_T^{\infty})$ varies up to 50% for the different protons within the same nucleoside, the reduced shifts are identical, within the experimental limits of error and do not show any systematic deviation. In Fig. 5 the average values of $\delta_{red}^{H(A)}$ for the three protons of dA are given (this quantity will be symbolized by δ_{red} in the following). The obvious conclusion to be drawn from this results is that the shifts of the three protons are influenced by the same equilibria, but react with different sensitivity to changes in their environment. In other words, the apparent association constants calculated from the shift values of any of the three protons must be identical, and only the values of $(\Delta_T^{\infty} - \Delta_T^{ass})$ (Δ_T^{ass} being the resonance frequency for a completely associated nucleoside solution) can be different for the three protons. In recent publications on the association equilibria between tryptamine, respectively tryptophane, and a series of nucleotides [10, 26] this conclusion was not drawn, leading to the result that the apparent association constants calculated for the different protons in the same nucleotide show a maximal variation by a factor of two. In the calculations described in the following, only the average of the three values of $\delta_{red}^{H(A)}$ is discussed.

Since the addition of the benzene does not seem to influence the self association of the purine nucleosides markedly, the data for the benzene-nucleoside-solutions given in the Figs. 1 to 3 were used for the plot of Fig. 5. Plots of the results from the pure nucleoside solutions are very similar and the qualitative conclusions drawn from these are the same. It is generally agreed, that osmometric and NMR data from the aqueous solutions of the nucleosides can only be explained by multistep equilibria [27, 28]. According to the discussion given by Deranleau the Scatchard-plot (δ_{red}/c *versus* δ_{red}) [23] will show the greatest deviation from linearity, if the microscopic equilibrium constants in a multiple equilibria are not identical. In Fig. 6 this plot is given for the data on dA. At all temperatures the curves show a negative curvature. This curvature is to be expected, if the microscopic equilibrium constant for the first step of association is smaller than the same constants of consecutive steps. The curvature of the lines shown in Fig. 6 do not allow to determine the intercept with the ordinate and the slope for $\delta_{red} \rightarrow 0$ with any accuracy, and render the determination of values for the apparent macroscopic association

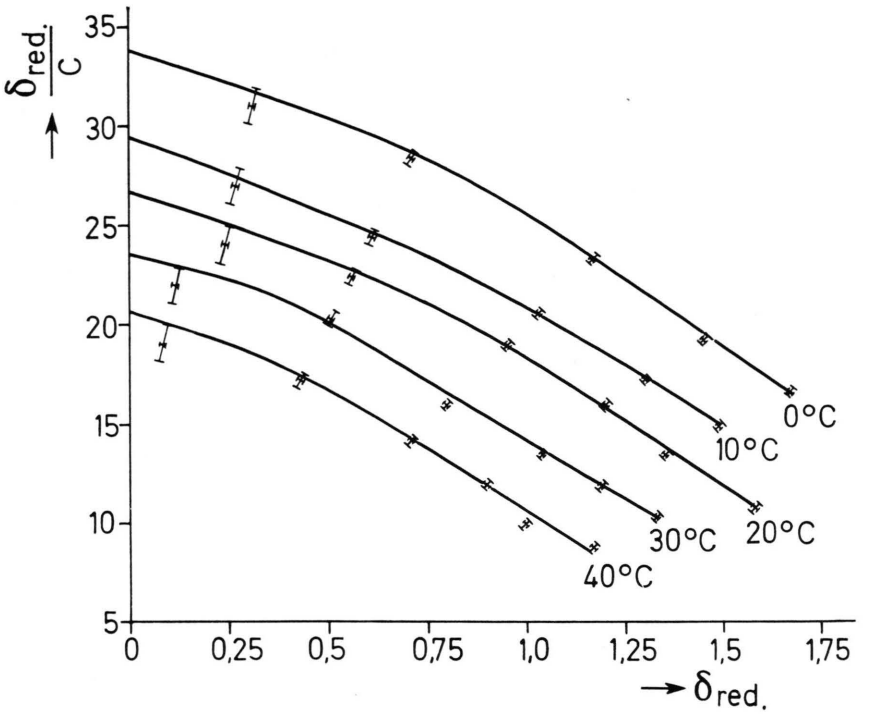

Fig. 6. Scatchard plot [(δ_{red}/c_{dA}) *versus* δ_{red}] of the mean "reduced shifts" of the H(2), H(8) and H(1′) proton resonances in deuterium oxide solutions with 0.12 molal benzene added. (Experimental points are given with error bars.)

constants and the temperature derivatives of these constants highly uncertain. However, a Benesi-Hildebrand-plot [24] of the data yields straight lines. From this graph K_{ass}^{app} was taken for the five different temperatures applied. From a Van't Hoff-plot of the constants an enthalpy of self association for dA of 2.2 ± 0.1 kcal·mol^{-1} was determined.

The presentation of the δ_{red} values of I in a Scatchard plot results in straight lines. This was to be expected, since I is known to associate less than A and dA [26] and in the experimental results obtainable the change in degree of association is considerably smaller. The enthalpy of self association again was found to be 2.2 ± 0.1 kcal·mol^{-1}.

Interaction between benzene and the nucleosides

Scatchard-plots of $(\Delta_T - \Delta_T^C)$ for the benzene protons in solutions of dA and I show a positive curvature which is more pronounced in solutions containing dA. This can be explained by the assumption, that the self association of the purine nucleosides is competetive to the formation of collision complexes between benzene and the bases. ΔH of the hetero association was obtained as described above and yielded $\Delta H = 2.7 \pm 0.2$ kcal·mole^{-1} in the dA solutions and

$$\Delta H = 2.3 \pm 0.2 \text{ kcal} \cdot \text{mole}^{-1}$$

in solutions of I.

Nuclear Overhauser enhancements

Even a qualitative comparison of the concentration dependence of the shift values obtained with different purine nucleosides is rendered difficult, since the intensity of the aromatic ring currents is strongly influenced by the groups attached to the bases [16]. Therefore, an independent method to find approximate values for the degree of association would place the discussion on safer ground. In Table I the NOE-values for eight purine nucleoside solutions are reported. In a given spin system the NOE is determined only by the correlation times and the spatial arrangement of all the spins interacting. The enhancement between two spins will decrease with the inverse sixth power of the distance [29, 30]. Intermolecular effects will accordingly only be observed, if spins of two molecules approach each other to a distance comparable to the intramolecular distances of spins.

The NOE of A, I and their 2′, 3′, isopropylidene derivatives dissolved in DMSO have been published

and the preferred average glycosyl torsion angle was calculated from the data [1]. Nucleosides do not associate in this solvent and the undisturbed intramolecular effects can be observed at much higher concentrations than in water.

A comparison of the data for 0.1 molal solutions of I in DMSO (Table I.5) with the values given by Davis and Hart for 0.25 molal solutions shows no change of any NOE outside the experimental errors. The data of Table I.3 and I.4 for heavy water solutions of dA and I.6 and I.7 for the data of I obtained at concentrations of 0.1 and 0.025 molal on the contrary reveal significant differences. The $f_2(8)$ and $f_8(2)$ factors decrease with concentration. The 0.025 molal solutions show practically the same results as found in DMSO, while in the 0.1 molal solutions the $f_d(s)$ are higher by approximately 0.05 in dA and 0.025 in I. This part of the $f_d(s)$ is caused by intermolecular interaction of the base protons arranged in face to face stacks. The effect found in I is one half of the effect observed in dA, the same difference was found in the concentration dependence of the shift values.

The interaction between the benzene molecules and dA in aqueous solutions is clearly demonstrated by a comparison of Tables I.1 and I.2. While no measurable influence is found in DMSO, the enhancements found between benzene and the nucleoside protons in heavy water are positive proving that the benzene molecules in this solvent are preferably found in the vicinity of the bases.

The distances between H(1′) and H(8) depend critically on the glycosyl-torsion-angle, consequently the values of $f_{1'}(8)$ and $f_8(1')$ can yield valuable information on the preferred conformation in solution not obtainable by any other experimental technique [1, 30]. While these enhancements are the same in dA and A in the two solvents investigated here (Tables I.1, I.2, I.3, I.4, I.8) the results found in the solutions of I (Tables I.5, I.6, I.7) show a pronounced solvent dependence.

If it is assumed, that the correlation times of the purine nucleosides are the same in the two solvents (this assumption is corroborated by the fact, that $f_8(1')$ and $f_1(8)$ are not solvent dependent in A and dA), the differences observed can be explained by a change of the mean glycosyl-torsion-angle. Applying the calculations of the $f_d(s)$ values by Noggle and Schirmer [30] to aqueous solutions of I this effect would correspond to an increase of this angle by approximately 15° to $Y \approx 310 - 315°$ and remove most of the differences in conformation between the two nucleosides calculated by Davis and Hart [1] for the DMSO solutions of I and A.

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