The Interaction between Pyrimidine Nucleosides and Benzene in Aqueous Solution, studied by Proton Magnetic Resonance

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Changes caused in the PMR spectra of aqueous solutions of four pyrimidine nucleosides (uridine, deoxyuridine, cytidine and thymidine) containing benzene (0.12 molal) by the variation of concentration and temperature have been investigated. From the data obtained, the participation of the benzene molecules in the stacking of the nucleosides is deduced.

Two types of interaction yield the main contributions to the stability of the secondary structure of DNA and RNA molecules in aqueous solution:

1. Specific hydrogen bonding with stringent geometric requirements between the complementary base pairs in the two strands of the double helix of DNA or in helical regions of the RNA chain.

2. Unspecific hydrophobic interactions between the adjacent bases of the nucleosides, with less rigid geometrical requirements.

The second type also occurs in aqueous solutions of the monomeric nucleosides and nucleotides, and is responsible for the solubility enhancement of uncharged polycyclic aromatic compounds in such solutions as compared to pure water^{1, 2}. The self association has been investigated previously by PMR³⁻⁸. The shielding of the ring protons is influenced by the π -electron-clouds of the adjacent heterocyclic ring structure. A neighbouring aromatic molecule will cause similar effects and it should be possible to decide via changes in the chemical shifts, whether an aromatic molecule will be found preferably in the vicinity of other nucleosides or if it is distributed at random in the bulk solution. The limited sensitivity of high resolution PMR combined with the low solubility in water of most aromatic compounds containing no polar groups, restricted the investigation of the stacking properties to aromatic ring systems solubilized by an attached polar or even charged group as for instance ethidium bromide⁹. In these cases the attraction between the nucleoside and the aromatic molecule could be caused by electrostatic or dipole-dipole interactions as well. However, many of the

most powerful carcinogenic and mutagenic compounds, for instance 3,4-benzpyren and 20-methylcholanthren containing carbon and hydrogen only but no polar groups, are known to interact strongly with DNA and RNA¹⁰⁻¹². Benzene, the simplest aromatis, is sufficiently soluble in water to allow the observation of proton resonances in a single scan experiment and might serve as a model substance for the less soluble condensed aromatic compounds. Besides, benzene is a dangerous poison itself and its ways of physiological action on the living cell are far from completely understood¹³. In the following experiments it has been investigated whether benzene, dissolved in aqueous solutions of pyrimidine nucleosides, does take part in the association of the bases.

Experimental

Substances: The nucleosides were obtained from Papierwerke Waldhof-Aschaffenburg AG, Mannheim, BRD, and used without further purification. Heavy water (99.5% deuterated) and benzene (Uvasol) were purchased from E. Merck, Darmstadt, BRD, and the hexamethyldisiloxane (HMDS) (puriss.) used for external locking, from Fluka AG., Buchs, Switzerland. The heavy water was saturated with benzene by shaking it with a surplus of benzene in a sealed flask for at least 24 hours. The solutions were prepared immediately before use by mixing weighted amounts of the benzene solution with the appropriate quantities of nucleosides. Concentrations given are molalities. The concentration of the benzene was 0.12 molal (D₂O saturated at 20 °C) in all experiments. The concentration of the nucleosides was varied up to the solubility limit. In some cases it was possible to prepare supersaturated solutions that did not yield precipitates during the run of an experiment.

Spectra

The PMR-spectra were obtained in the CW mode at 100 MHz with a Varian XL-100-15 spectrometer

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equipped with the XL-100 variable temperature accessory in 12 mm tubings. Frequencies given in this paper are taken against external neat hexamethyldisiloxan (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 was ± 0.4 Hz. This scatter is mainly due to the limited accuracy of the variable temperature accessory, which on frequent checks showed variations up $\pm 2^\circ\text{C}$. The spectra were taken between 0° and 40°C in steps of 10°C .

Results

The spectra of four pyrimidine nucleosides were obtained: uridine (U) (0.1 to 1.2 molal), deoxyuridine (dU) (0.1 to 0.5 molal), cytidine (C) (0.1 to 0.4 molal), and thymidine (T) (0.05 to 0.25

molal). All resonances in the PMR spectra of these compounds have been assigned to the various protons¹⁴. In the substances investigated here the signals of the base protons and the proton at the C_1' -position of the sugar are well separated from the resonances of the residual protons of the sugar moiety. The latter are crowded around $\tau = 4$ ppm and their observation in heavy water is often rendered difficult by residual water protons. Figs. 1 to 8 give the positions of the resonance signals of

Fig. 1. Concentration and temperature dependence of the chemical shift of the H_5 -proton resonance in a solution of uridine (U) 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_6 -proton resonance in a solution of uridine (U) in deuterium oxide, with 0.12 molal benzene added. (Shifts are taken against HMDS as an external standard.)

the benzene protons, the H_6 -protons, the H_5 -protons (in the case of T: the methyl-protons) and the H_1' -protons of the four nucleosides dissolved in a 0.12 molal solution of benzene in deuterium oxide as a function of concentration at temperatures between 0° and 40°C . In addition, solutions with the same concentrations of nucleosides but without benzene were examined. Comparison of the shifts observed at equal temperatures and concentrations in these

Fig. 2. Concentration and temperature dependence of the chemical shift of the H_5 -proton (in the case of thymidine CH_3 -protons) in solutions of deoxyuridine (dU), cytidine (C) and thymidine (T) in deuterium oxide, with 0.12 molal benzene added. (Shifts are taken against HMDS as an external standard.)

Fig. 4. Concentration and temperature dependence of the chemical shift of the H_6 -proton in solutions of deoxyuridine (dU), cytidine (C) and thymidine (T) in deuterium oxide, with 0.12 molal benzene added. (Shifts are taken against HMDS as an external standard.)

Fig. 5. Concentration and temperature dependence of the chemical shift of the $H_{1'}$ -proton resonance in a solution of uridine (U) in deuterium oxide, with 0.12 molal benzene added. (Shifts are taken against HMDS as an external standard.)

Fig. 6. Concentration and temperature dependence of the chemical shift of the $H_{1'}$ -proton in solutions of deoxyuridine (dU), cytidine (C) and thymidine (T) in deuterium oxide, with 0.12 molal benzene added. (Shifts are taken against HMDS as an external standard.)

solutions to those containing benzene showed, that the resonances of the base protons and the $H_{1'}$ -protons are displaced to higher fields by 0.2 to 0.8 Hz on the addition of benzene. These differences are rising with decreasing temperature and concentration. The observed effects are beyond the experimental uncertainties, but considering the reproducibility of ± 0.4 Hz a detailed discussion is not attempted.

Discussion

The high field shifts observed with increasing concentrations of solute for protons bound to an aromatic ring system are attributed to shielding ef-

fects caused by the π -electron-clouds of neighbouring molecules arranged in face to face stacks¹⁵⁻¹⁷. The magnitude and geometric conditions of this effect have been calculated by JOHNSON and BOVEY¹⁵. Lately this calculations have been extended by GIESSNER-PRETTRE, and PULLMAN¹⁸ to some naturally occurring pyrimidine and purine bases. The latter calculations contrary to earlier statements¹⁹

Fig. 7. Concentration and temperature dependence of the chemical shift of the benzene-protons resonance in a solution of uridine (U) in deuterium oxide with 0.12 molal benzene added. (Shifts are taken against HMDS as an external standard.)

Fig. 8. Concentration and temperature dependence of the chemical shift of the benzene-protons in solutions of deoxyuridine (dU), cytidine (C) and thymidine (T) in deuterium oxide with 0.12 molal benzene added. (Shifts are taken against HMDS as an external standard.)

reveal that the difference of shifts expected for a completely stacked monomer in solution compared to the isolated molecule in the case of the pyrimidine bases is almost one order of magnitude smaller than for benzene. A comparison of the concentration and temperature dependence of the shifts, obtained for protons at the same position in different nucleosides, shows the following similarities: Changes of the chemical shift of the H_6 -proton are always smaller than changes of the H_5 -position. The concentration dependence of the H_1 -proton shift is higher in the deoxyribosides than in the ribosides, while for the temperature dependence the reverse is true.

Concentration dependence

The association of the bases in stacks has been measured by osmometric methods^{20, 21}. According to these results the association increases in the sequence $U < C < T$. The concentration dependence of the chemical shift of the base protons (Fig. 1, 2 H_6 and Fig. 3, 4 H_5 , respectively, $-CH_3$ in T) has the sequence $U < T < C$. Only the benzene protons (Figs. 7, 8) are shielded with increasing concentration in the sequence suggested by the osmometric data for self-association. Crystallographic data show²² that the H_6 -proton of the pyrimidines in the anti conformation with respect to the glycosidic bond, is very close to the O_5' of the sugar moiety. An intramolecular hydrogen bond connecting C_6 via the H_6 to O_5' is suggested for this geometry. It is reasonable to assume a similar sterical arrangement in the dissolved molecules²³ especially under consideration of the recent PMR results obtained by BARRY et al.²⁴ who used lanthanide shift reagents for an analysis of the pre-

ferred conformation of mononucleotides in solution. The anti conformation, with, or without a hydrogen bond, would inhibit the approach of any other molecule, solvent or nucleoside, to the vicinity of H_6 compared to a proton or methyl group in the 5-position of the pyrimidine bases, the latter being freely exposed to the surrounding medium. This assumption may be supported by comparison of the H_6 -shifts observed in thymidine and cytidine. Though the first is known to associate stronger²⁵ than the second, the concentration dependence of the shifts is significantly greater in cytidine than in thymidine, since presumably the bulkier methyl group of the thymidine is a more effective obstacle to approaching molecules than the H_5 -proton of cytidine. The shifts of the uridine protons (Figs. 1, 3, 5) show the smallest concentration dependence. The curves obtained for H_6 (Fig. 1) seem to pass through a shallow minimum while the H_5 (Fig. 3) resonance is even shifted to lower fields with increasing concentration. Both observations do not contradict the statements given above.

The ring currents in uracil according to the calculations of GIESSNER-PRETTRE and PULLMAN¹⁸ are weaker than in cytosine. Consequently, the influence of the ring currents on the shifts in aqueous uridine solutions can easily be masked by minor changes in the bulk magnetic susceptibility.

A similar purely sterical explanation might be given for the different concentration dependence of the H_1 -proton shift in the ribosides and deoxyribosides (Figs. 5, 6). Here the replacement of the hydroxyl-group at the 2'-position by a hydrogen exposes the H_1 more freely to the surrounding medium.

Shift of the benzene protons: Even minor changes in the structure of the nucleosides investigated here, show up in differences in the chemical shift of the benzene protons (Figs. 7, 8, 9). This proves,

Fig. 9. Shift of the benzene-protons as a function of temperature in a 0.12 molal solution in deuterium oxide (C_6H_6) and with 0.25 molal uridine (U), deoxyuridine (dU), cytidine (C) and thymidine (T) added (left scale), and temperature dependence of the methyl-protons of a tetramethylammoniumchloride solution ($c=1.0\%$) in deuterium oxide (right scale). (Shifts are taken against HMDS as an external standard.)

together with the fact, that no similar effects were found for solutions of equal concentration in dimethylsulfoxide, the participation of benzene in the association equilibria of the mononucleosides. Benzene however, can produce shielding effects an order of magnitude higher than those caused by the face to face approach of the pyrimidine bases. Still, the influence of the ring currents in the nucleosides on the benzene protons at comparable concentrations is greater than the effect of benzene on the base protons. Two likely explanations might be given for this observation; 1) In the case of face to face stacking between benzene and the nucleosides the center of the benzene ring is preferably found above or below the nitrogen atoms or carbonyl groups of the pyrimidine rings. The arrangement would bring some benzene protons into the regions of the highest diamagnetic shift of the pyrimidine bases while leaving the H_5 and H_5' protons of the bases rather unaffected by shielding from the benzene.

2) if attachment of the benzene rings perpendicular to the pyrimidine bases occurs the benzene ring centering on one of the carbonyl groups would form a kind of a charge-transfer-complex. For both ways of interaction the influence on the ben-

zene and base protons will be similar. No decision can be made between these two possibilities on the basis of proton shift measurements alone.

Effects of temperature

Decreasing temperature results in a high field shift of all proton resonances. At first sight, one is tempted to explain this increase of shielding in the same way as the concentration effects. If, however, the shifts were caused by association of the bases, the difference between two signals obtained at different concentrations should become smaller with decreasing concentration and at infinite dilution all isotherms should join.

Since obviously the isotherms in the Figs. 1 to 8 with small but significant deviations run parallel, the assumption given above does not hold. In some of their investigations KREISHMAN and CHAN²⁶ used tetramethylammoniumchloride as an water soluble "inert" reference substance. In a preliminary experiment during this work the chemical shift of the tetramethylammoniumchloride protons in a 1% aqueous solution was measured against an external lock capillary filled with HMDS. Cooling the solution in 10 °C steps from 40 °C to 0 °C gave a high field shift of 7.0 Hz. Calculations of the change of the differences between the magnetic volume susceptibilities with temperature under the most unfavorable assumptions for the coefficients of thermal expansion yielded a maximum shift of 2.5 Hz. In Fig. 9 the shifts of the benzene protons (concentration 0.12 molal) in pure heavy water and in aqueous 0.25 molal solutions of U, dU, C and T are plotted as functions of temperature. In the lower part of the figure the resonance of the methyl protons of a 1% tetramethylammoniumchloride aqueous solution is plotted. This compound, completely ionized in water, can certainly neither associate nor produce any ring current effects. The explanation should consequently be searched for in the surrounding hydration sphere. A decrease in temperature will allow the water molecules surrounding the hydrophobic parts of a dissolved molecule to arrange more regularly and this may lead to a slightly higher electron density at the proton. This qualitative explanation is further corroborated by comparing the change in chemical shift with temperature among the different protons observed here. The H_5 , H_1' and the benzene protons situated on positions in their molecules exposed to the surrounding water

are shifted between 40 °C and 0 °C approximately 10 Hz upfield, while the H₆-protons, which in the anti conformation of the nucleosides are hidden in a recess of the molecule, can be influenced much less by the solvent. Accordingly the shifts due to a temperature change of 40 °C vary by 3 to 5 Hz only.

From the data presented here, one can deduce that benzene dissolved in aqueous solutions of pyrimidine nucleosides does take part in the stacking or association of the bases. It does not seem sensible to attempt the determination of the thermodynamic parameters governing the association process from the shifts observed, mainly because the effects observed are on the limit of detectability and because

of the impossibility to ascribe them quantitatively to definite structures and phenomena. The investigation of the variation of the spin-lattice relaxation times of the different nucleoside protons and the benzene protons as functions of concentration and temperature, by application of the Fourier-transform-nuclear magnetic resonance and the observation of nuclei other than hydrogen will probably yield more detailed insight into the stacking interaction among nucleosides and aromatic hydrocarbons, such investigations are under way in this laboratory.

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