Anomalous Base-Stacking of the N_1-Oxide of AMP

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The intermolecular association in aqueous solution of the N_1-oxide nucleotide analog of adenosine-5′-monophosphate has been investigated by ^{1}H-NMR spectroscopy over the concentration range 0.0006–0.6 M. The concentration profile of individual chemical shifts provides evidence for an unusual stacking pattern compared to that of the natural nucleotide AMP, involving only the imidazole moiety of the adenine base. The different stacking pattern of this nucleotide analog suggests that the exact stacking geometry of natural nucleotides interacting with nucleotide-depending enzymes, is achieved by adjusting to the geometry of the individual receptor molecule.

Introduction

The selective recognition of individual nucleotides by macromolecules such as proteins and enzymes plays an important role in the regulation of metabolic pathways and is involved at all levels of biological activity [1]. Besides localized point interactions like electrostatic interactions or hydrogen bondings, there are also delocalized interactions involved in these specific binding processes, such as base stacking interactions between the base part of an individual nucleotide and suitably located heteroaromatic aminoacid residues of the enzyme. By now, the capacity of natural nucleotides to engage in such base stacking interactions has been well recognized [2–9]. For such interaction studies it is convenient to use chemically modified analogs. While we were testing N_1-oxide adenine nucleotide analogs as substrates and/or as allosteric effectors of various phosphotransferases, it was suggested that base stacking must play a major role in the nucleotide interaction with allosteric enzymes [10–12]. The present investigation shows, that indeed the stacking pattern of N_1-oxide nucleotide analogs, as revealed by their concentration dependent NMR-spectra, is different from that of natural adenine nucleotides. This, at least in part, could explain the different biological responses.

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Materials and Methods

Adenosine-N_1-oxide 5′-monophosphate (o^1AMP) was prepared through a gentle oxidation of 5′-AMP (Boehringer, Mannheim) with permaleic acid at neutral pH in aqueous solution [10]. Deuterated AMP-[^{2}H-8] and o^1AMP-[^{2}H-8] were obtained on heating 0.2 M AMP (o^1AMP) in D_2O at pD 12 for 24 hours at 60 °C. The deuterium incorporation was about 80% and could be increased to 99% by prelonged heating up to 5 days at 60 °C. There was no ^{1}H-NMR evidence of decomposition during this time at this temperature.

The ^{1}H-NMR spectra were taken with a Varian XL-100 spectrometer operating at 100 MHz in the pulse Fourier transform mode at a probe temperature of 30 °C. Samples were made up from 99.8% D_2O in 5 mm NMR tubes and the pH adjusted with NaOD or DCl to a "pD" (meter reading) of 8.5. Individual concentrations were accurately determined from the characteristic UV absorbance at 233 nm ($\varepsilon_{max} = 40800$). Depending on the dilution of the samples, between 10 and 10000 transients were accumulated at 4 seconds intervals following pulses at 58 μs. To avoid interaction with an internal standard, chemical shifts were measured relative to an external concentric $CHCl_3$ capillary and then converted to the TMS scale.

All calculations for stacking pattern evaluation were done with a PDP-8/I computer.

Results

Fig. 1 shows the concentration dependence of ^{1}H-NMR chemical shifts for the nonexchangeable protons H(8), H(2) and H(1′) in o^1AMP. As dis-

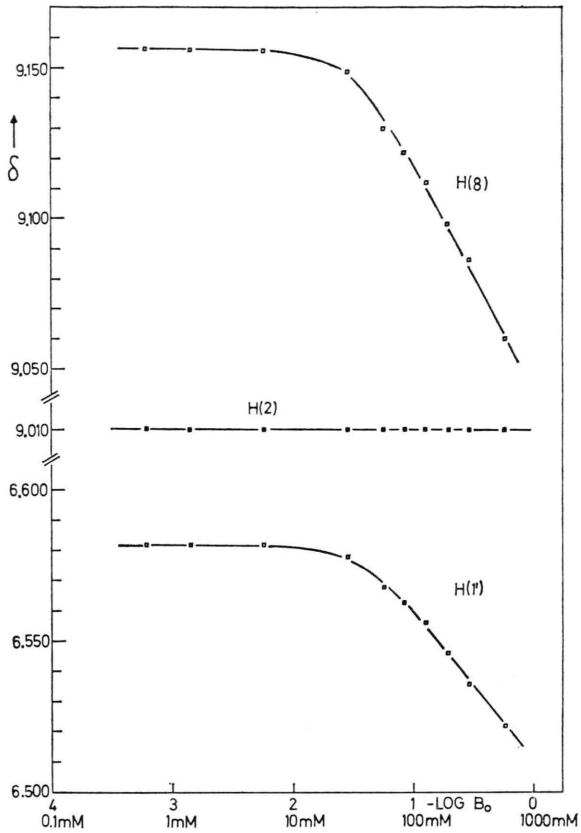

Fig. 1. Semilogarithmic plot of the concentration dependence of ^{1}H-NMR chemical shifts (in ppm downfield from external TMS) for the H(8), H(2) and H(1′) protons in adenosine-N_1-oxide-5′-monophosphate (o^{1}AMP).

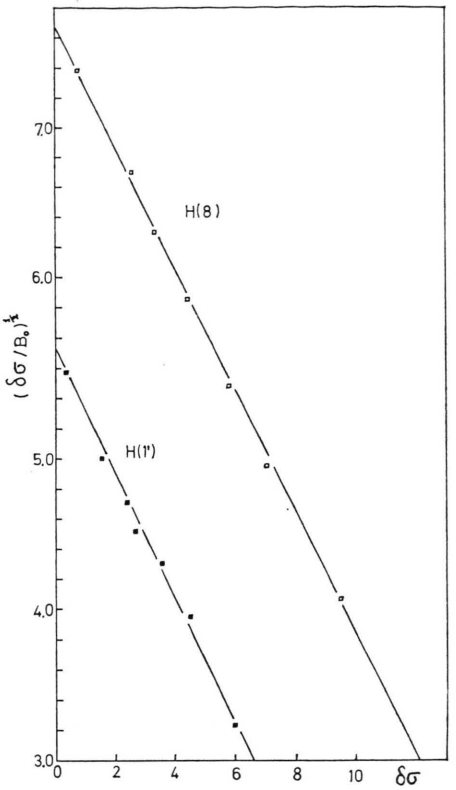

Fig. 2. Self-association of adenosine-N_1-oxide-5′-monophosphate as a plot of $(\delta\sigma/B_0)^{1/2}$ versus $\delta\sigma$. According to Dimicoli and Hélène [14] the dimer shift is obtained from the x-axis intercept and the microscopic association constant from the product of the intercept and the square of the slope.

played in this semilogarithmic plot, above concentrations of 10 mM, the chemical shifts for the H(8) and H(1′) protons are concentration dependent, *i.e.*, they move upfield with increasing contratration. By increasing the o^{1}AMP concentration from 0.6 mM to 578 mM, the corresponding upfield shifts are 9.6 Hz and 6.0 Hz for the H(8) and H(1′) protons, respectively. Under comparative conditions the upfield shift for 500 mM AMP are 19 Hz and 16 Hz for H(8) and H(1′), respectively. Such concentration profiles are typical for stacking interactions. The most striking difference between o^{1}AMP and AMP is that in AMP the largest concentration-dependent upfield shift occurs for the H(2) proton (38 Hz), while the H(2) chemical shift in o^{1}AMP is virtually concentration independent. This indicates a different stacking geometry.

The observed monomer chemical shifts for o^{1}AMP at "pD" 8.5 are 9.156 ppm H(8), 9.010 ppm H(2) and 6.582 ppm H(1′), while the corresponding shifts for the natural nucleotide AMP, obtained at infinite dilution [13], are: 9.055 ppm H(8), 8.717 ppm H(2) and 6.587 ppm H(1′). The distinction between the H(8) and H(2) chemical shifts in the AMP and o^{1}AMP spectra was based upon deuteration of the H(8) position. As shown by these data, the monomer chemical shifts for the ribose H(1′) protons in AMP and o^{1}AMP are identical within experimental error while those of the H(8) and H(2) base protons occur in o^{1}AMP at 0.1 and 0.3 ppm downfield, respectively from those in AMP. It is interesting to note the far reaching influence of the N_1-oxygen on the H(8) proton in the imidazole moiety.

The concentration-dependent chemical shift data were analysed by the isodesmic model of Dimicoli and Hélène [14] to obtain both the apparent equilibrium constant characterizing the intermolecular

association and the dimer chemical shifts. According to this method a plot of $(\delta\sigma/B_0)^{1/2}$ versus $\delta\sigma$ ($\delta\sigma$ being the chemical shift difference between the individual chemical shifts at concentration B_0 and the chemical shift at infinite dilution) gave straight lines (Fig. 2), from which the microscopic association constant at 30 °C was calculated to be $K^{30\,°C} = 2.86\ M^{-1}$ from the H(8) proton and $K^{30\,°C} = 2.10\ M^{-1}$ from the H(1′) proton. The proton-averaged apparent equilibrium constant for the self-association of o¹AMP is $K^{30\,°C} = 2.48\ M^{-1}$. The chemical shifts for the dimer were determined to be 10.2 Hz and 7.8 Hz upfield from the corresponding H(8) and H(1′) monomer shifts. No association constant could be obtained from the H(2) proton chemical shift since it is concentration independent.

The microscopic association constant for AMP was evaluated on the basis of the same isodesmic model using the data of Evans and Sarma [15]. As obtained from the individual chemical shift differences the results are $K^{30.5\,°C} = 2.19\ M^{-1}$ from H(8), $K^{30.5\,°C} = 2.07\ M^{-1}$ from H(2) and $K^{30.5\,°C} = 1.97\ M^{-1}$ from H(1′). The corresponding proton averaged apparent equilibrium constant is $K^{30.5\,°C} = 2.08\ M^{-1}$. The dimer shifts for AMP are 20.7 Hz, 23.2 Hz and 48.0 Hz upfield from the corresponding H(1′), H(8) and H(2) monomer shifts.

Discussion

The mechanism of self-association of nucleic acid bases, nucleosides and nucleotides in aqueous solution has been investigated extensively; the existence of vertical intermolecular base stacking interactions was inferred from different experiments such as vapor pressure osmometry, microcalorimetry, sedimentation equilibrium, ultraviolet hypochromism measurements and especially ¹H-NMR studies. This field was recently reviewed by Ts'o [2, 3]. It is generally agreed that the aggregation via vertical stacking of bases proceeds beyond the dimer stage.

The literature results for adenine derivatives have been interpreted in terms of two different base stacking geometries. While Ts'o and coworkers [2, 3] and Evans and Sarma [15] have proposed a head-to-head arrangement, which places the N(9) ribosyl moieties on the same side of the stack, other authors [6, 7, 16–18], based mainly on Nuclear Overhauser Effect studies, favour a head-to-tail arrangement, which results in ribosyl placement on opposite sides of the stack.

As shown by the concentration dependent self-association of o¹AMP, this nucleotide analog also is able to undergo stacking, but in contrast to AMP, the pyrimidine moiety in o¹AMP is not involved in this stacking. This surprising result must be due to the effect of the oxygen substituent at the N_1 which carries a partial charge and has an enhanced degree of solvation thus preventing this part from stacking. It could be rationalized by considering the arrangement shown in Fig. 3 which places the N(9) ribosyl residues opposite each other, but on the same face of the stacking units. The charged hydrophilic N – O groups are not only far apart, but they also reach out from the hydrophobic region of overlapping bases. The only way to construct a space-filling model of vertically interacting o¹AMP molecules, such that the imidazole moieties should overlap while the pyrimidine moieties should be at least 4 Å apart (an inter-ring distance of less than 4 Å is necessary for a significant stacking interaction [18]), would be a head-to-tail arrangement as shown in Fig. 3. In such an arrangement, the phosphate charge repulsion, the repulsion between the permanent dipoles and steric hindrance are minimized, making the model most plausible. Assuming that the stacking proceeds beyond the dimer stage represented in Fig. 1, a rosette like helicoidal aggregate could be assumed, centered around an axis through the overlapping imidazole rings.

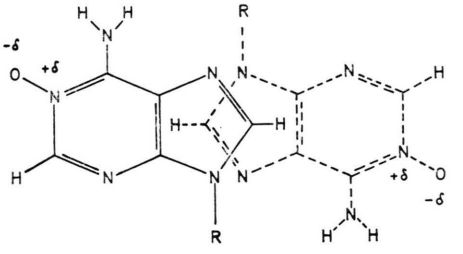

Fig. 3. Suggested base stacking pattern of adenosine-N_1-oxide 5′-monophosphate, R = ribosyl 5′-monophosphate.

It is surprising, however, to find almost identical apparent equilibrium constants for AMP ($2.08\ M^{-1}$) and o¹AMP ($2.48\ M^{-1}$), which indicates a similar degree of stacking association of these two nucleotides, regardless of their different stacking patterns.

The foregoing experiment with the synthetic nucleotide analog o¹AMP shows that the exact stacking geometry of interacting adenine nucleotides could be different and depend on conditions. It

further provides evidence that in aqueous solutions, adenine nucleotides must not possess a unique structure such as the well-described solid-state stacking pattern [19], but due to an increased conformational flexibility about the glycosidic bond, the adenine moiety may be able to assume a geometry which optimizes both in-plane hydrogen bondings and out-of-plane stacking interactions with a given receptor macromolecule.

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