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Conformation of adenosine-5′-triphosphate in the presence of Mg^{2+} at different pH

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Abstract

NOESY experiments have been performed on adenosine-5′-triphosphate (ATP) in the presence of 1:1 Mg^{2+} ($MgCl_2$) at different pH. The results show that H8–H1′ and H8–H2′ interproton NOE intensities change with pH, with nearly equal intensity at low pH (pH 1.9) and with H8–H2′ signal stronger than the H8–H1′ signal at higher pH (between 3.7 and 8.5). The H8–H1′ and H8–H2′ distances and the C4–N9–C1′–O4′ torsion angle (χ) have been measured from random structures of adenosine and ATP. It is concluded that the lower pH allows the formation of a P_γ–O–Mg^{2+}–N1 bridge and the torsion angle takes a *cis* conformation accordingly.

Keywords: Adenosine-5′-triphosphate (ATP); Mg(II); NOESY; Torsion angle (χ)

1. Introduction

It is well known that nucleic acid structures are strongly dependent on the conformation of individual nucleotide residues. A-form DNA differs from B-form DNA by different sugar puckering (C2′ endo for B-form DNA and C3′ endo for A-form DNA). In Z-form DNA, the glycosidic bond of guanosine takes a *syn* conformation, in contrast to the *anti* conformations in normal B- and A- form DNA [1]. Because the biological effects of nucleotides have a strong relation to their conformations, the conformational study of nucleotides, particularly in solutions, has attracted much attention. X-ray crystallography, NMR spectroscopy and CD (circular dichroism) have been widely used for this purpose [2–4]. The conformation of a nucleotide also influences the binding site when it binds to metal ions. It is believed that the glycosidic bond of most guanosines and adenosines takes the *anti* conformation and they bind to metal ions with N7 in the purine ring [5–7]. However, recent reports suggest that when pH is low, ATP (adenosine-5′-triphosphate) binds to metal ions with N1 and this would require the glycosidic bond to be in the *syn* conformation [8–10].

In order to see if the *syn* conformation of the glycosidic bond in ATP is natural, we performed NMR NOESY experiments on 1:1 ATP–Mg^{2+} solutions with varied pH. The results are reported in this paper and they show a rotation range from 20 to $-150°$ for the torsion angle C4–N9–C1′–O4′ (χ). This torsion angle range covers part of the *syn* conformation (defined by $\chi = 0 \pm 90°$ [11]) and part of the *anti* conformation ($\chi = 180 \pm 90°$). At very low pH (pH 1.9), the H8–H1′ and the H8–H2′ NOE cross peaks have nearly equal intensities, while at higher pH (pH $\geq$ 3.7), the H8–H2′ peak become stronger than the H8–H1′ peak. A quantitative relationship between the interproton distance and the base-to-sugar torsion angle is established with the help of molecular modelling, which shows that, in the presence of Mg^{2+}, at lower pH ATP takes a *syn* conformation, while at higher pH *anti* conformations predominate.

Conformations of nucleotides that are affected by pH have been well documented. Some new conformations of Pt complexes with nucleotides have been discovered in variable pH experiments [12–14]. Although the mechanism of the pH dependent conformation of nucleotides is not yet fully understood, the phenomenon

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has been suggested to be very important in biological activity of the nucleotides [15]. Since the ATP–Mg^{2+} complex is of great importance in biology, the pH dependent conformation of ATP in the presence of Mg^{2+} reported in this paper would provide some insight to the pH dependent binding site of Mg^{2+} on ATP.

2. Experimental

The Na salt of ATP was used in this study, which was purchased from Boehringer Mannheim, GmbH, Germany and used directly without further purification. Other chemicals ($MgCl_2 \cdot 6H_2O$, NaOH and HCl) were of analytic purity from Shanghai Chemical Company, China. The pH values were determined by a Cole–Parmer pH meter equipped with a Cole–Parmer glass-body combination pH electrode (Chicago). Buffers of pH 4.01, 6.86 and 9.18 were used to calibrate the pH meter.

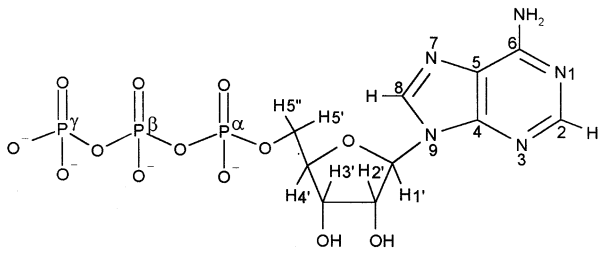

Fig. 1. The schematic structure of ATP.

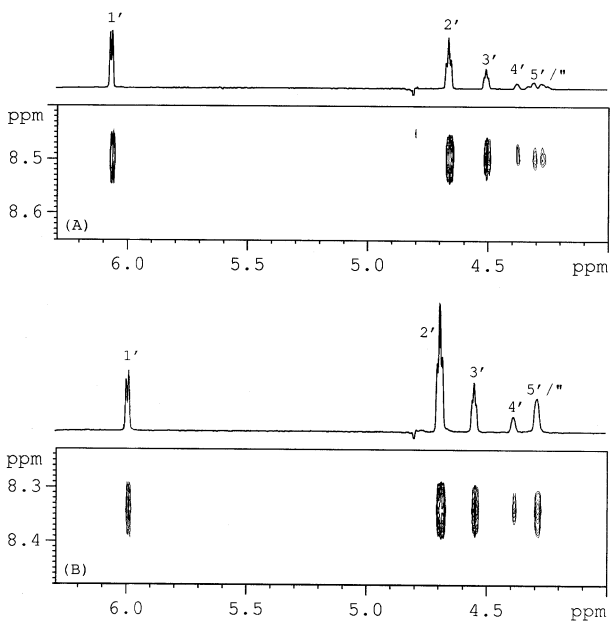

Fig. 2. Regional NOE spectrum of ATP in the presence of equimolar $MgCl_2$ at pH 1.9 (A) and pH 8.5 (B), showing the NOE correlation between H8 (8.51 ppm at pH 1.9 and 8.35 ppm at pH 8.5) and the sugar protons. The slices are shown at the top as projections.

ATP solutions, with and without equimolar $MgCl_2$, were prepared by quantitatively dissolving the chemical(s) in H_2O with 10% D_2O for field lock purpose. The pH values were adjusted by adding tiny drops of NaOH and HCl. The concentrations of ATP and $MgCl_2$ were all kept at 0.2 mol l^{-1}.

NMR experiments were performed on both ATP and ATP–Mg^{2+} samples on a Bruker ARX-500 spectrometer at 298.2 K. Two-dimensional phase-sensitive NOESY spectra were recorded with $2048(\omega 2) \times 256(\omega 1)$ data points and 16 scans per FID. The mixing time was set to 500 ms for all experiments. Water suppression was accomplished by low power, continuous wave presaturation. All 2D FID were apodized in both dimensions with a $\pi/2$ shifted, squared sine-bell function and zero filled to give a 2048×512 matrix before Fourier transformation. The water signal serves as the reference (4.8 ppm).

Molecular modelling was performed on a silica graphics workstation with the CNS version 1.0 (Crystallography and NMR System, http://cns.csb.yale.edu) software package. InsightII module was used for molecule display. In CNS, a single stranded DNA consisting of 63 adenine residues was generated. The 63 residues had random conformations, and their interproton distances and torsion angles were measured. In the meantime, molecular dynamics were performed on an ATP molecule using simulation annealing protocol. Sixty dynamics structures were sampled.

3. Results and discussion

For clarity we present the ATP structure in Fig. 1, with the atoms numbered. We have measured NOESY spectra of ATP in the presence of equimolar Mg^{2+} at four pH values (pH 1.9, 3.7, 5.0 and 8.5). Fig. 2 shows the H8 slices of the NOESY spectra for the ATP–Mg^{2+} system at pH 1.9 and 8.5, where H8 correlates mainly with H1′ (6.06 ppm at pH 1.9 and 5.99 ppm at pH 8.5) and H2′ (4.66 ppm at pH 1.9 and 4.69 ppm at pH 8.5). Since the other protons (H3′, 4.50–4.55 ppm; H4′, 4.38 ppm; H5′ and H5″, 4.26–4.32 ppm) are also in the proximity of H8 with the distance under 0.5 nm, the NOE peaks correlating with those protons also appear, partly due to a spin diffusion effect or a multiple-step NOE transfer effect during the long mixing time (500 ms). However, the nearly equal intensity of the H8–H1′ and H8–H2′ cross peaks implies that H8 has nearly equal distances to H1′ and to H2′. The cross peak intensities relative to the diagonal peak of H8 are listed in Table 1. For comparison, the NOE data for ATP solutions free of Mg^{2+} are also presented. The data in Table 1 suggest that the interproton distances are dependent on pH.

Table 1
Integration volumes of the H8–H1′ and H8–H2′ NOE peaks of ATP at different pH, referenced to the H8 diagonal peak in each spectrum

pH	With Mg^{2+}		Without Mg^{2+}	
	H8–H1′	H8–H2′	H8–H1′	H8–H2′
1.9	0.075	0.073	−0.003	−0.043
3.7	0.148	0.278	−0.000	−0.050
5.0	0.116	0.279	−0.010	−0.069
8.5	0.073	0.190	−0.014	−0.064

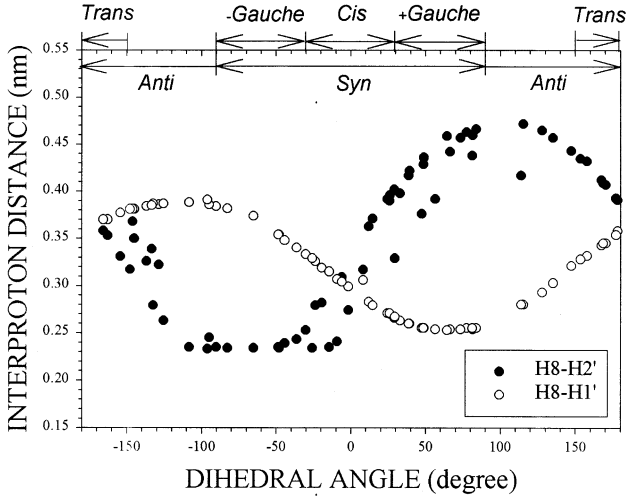

Fig. 3. Plot of H8–H1′ and H8–H2′ distances versus the torsion angle χ for adenine.

The relative intensities of the NOE peaks should be an indication of the relative distances of the involved spins. It is true that the mixing time in our experiments is rather long (500 ms), compared to those used in macrobiomolecules. But since ATP is a small molecule, the spin diffusion effect should not be too serious and stronger NOE peaks should correspond to shorter distances, although the spin diffusion effect probably has broken the r^{-6} rule for quantitative analysis. In solutions, the ATP molecule can take all possible conformations, but at a given pH, the molecule should have a certain average conformation, and this average conformation can be pH dependent, because pH can change the charge distribution of the molecule. As a result, the H8–H2′ and H8–H1′ distances are also pH dependent.

The curves presented in Fig. 3 are helpful to our understanding of the conformation-dependent interproton distances, where the H8–H1′ (open circles) and H8–H2′ distances (filled circles) are plotted versus the torsion angle χ, i.e. the dihedral angle C4–N9–C1′–O4′. The data were taken from 63 random CNS structures for adenosine. Similar curves have also been obtained from DISCOVER structures of ATP sampled in molecular dynamics calculations. The H8–H1′ curve is a perfect sine function, but the H8–H2′ curve is not so smooth as the H8–H1′ curve. In generating the CNS structures and in the molecular dynamics calculation, the ribose sugar can take random conformations or puckers (C3′ endo, C2′ endo, etc.). Since the H8–H1′ distance, or r(H8–H1′), is not affected by the sugar pucker, the curve is smooth, which shows a sine function of the torsion angle in a full rotation cycle (from -180 to 180°). As a contrast, the H8–H2′ distance, or r(H8–H2′), is strongly dependent on the sugar pucker, so at a given χ angle, the H8–H2′ distance can have two or more values. Even so, the H8–H2′ curve can still be approximated by a sine function. In biochemistry, a full rotation of the glycosidic bond can be described by *anti* and *syn* conformations which are defined by $\chi = 0 \pm 90°$ and $\chi = 180 \pm 90°$, respectively. In the meantime, another set of notations are also in current use: *cis* ($\chi = 0 \pm 30°$), *trans* ($\chi = 180 \pm 30°$), *+gauche* ($\chi = 60 \pm 30°$) and *−gauche* ($\chi = 300 \pm 30°$, or $\chi = -60 \pm 30°$) [11], but in this set of notations names are not given to $\chi = 120 \pm 30°$ and $\chi = 240 \pm 30°$ ($-120 \pm 30°$). For clarity, the two sets of notations are indicated in Fig. 3. When χ is close either to 0° (*cis* conformation) or to $\pm 180°$ (*trans* conformation), the two distances do not differ much. Their differences reach maxima when χ is close to $\pm 90°$. Fig. 3 indicates that when χ falls in the range from 0 to $-180°$, the r(H8–H1′) distance is longer, while when χ is between 0 and 180°, r(H8–H2′) is longer. Thus, NOE experiments can indicate, at least qualitatively, the conformation of the glycosidic bond in ATP.

The data in Table 1 show that at pH 1.9, the H8–H1′ and H8–H2′ NOE peaks have nearly equal intensities, with the implication that the torsion angle is around 0 or 180°. As pH is increased, the H8–H2′ signal becomes stronger than the H8–H1′ signal, corresponding mainly to the *−guache* conformation. We have shown that for solutions where ATP interacts with Mg^{2+}, Zn^{2+} and Fe^{3+}, N1 binds with the metal ions [8–10,15]. Therefore, it is reasonable to propose that, in the pH range we are studying in this paper, the torsion angle of ATP is in the range from 20 to $-150°$. The conformation of the ATP molecule changes from *cis* to *trans* through *−guache*, as the pH of the solution is increased. At lower pH, the ATP molecule are more likely to take the *cis* conformation, while at higher pH, the *trans* conformation is more stable, in full agreement with the ^{1}H–^{15}N HMBC experiments reported recently [15].

The data for the pure ATP solutions show a much narrower rotation range for the glycosidic bond, most probably in the *−gauche* conformation. The negative sign in the intensities for pure ATP shown in Table 1 is the indication of the fast motion limit. The much wider conformation range for ATP in the presence of Mg^{2+} than in the absence of Mg^{2-} suggests that the ATP–metal interaction is easily affected by pH. At lower pH, there is a binding competition between metal ion and

H^+. Since the charge of the metal ions (Mg^{2+}, Zn^{2+} and Fe^{3+}, etc.) is stronger than H^+, the metal ions usually win the competition. In the meantime, the γ-phosphate can become negatively charged even when pH is very low. A P_γ–O–M–N1 bridge is likely to form in the solution, where M denotes the metal ion. In this case the ATP molecule can easily take the *cis* conformation. When pH is increased from 1.9 to 5, the binding ability of the metal ion should be reduced, since the hydrated metal ion could form hydroxo species. As a result, N1 loses connection with the metal ion, leading to $-$*gauche* conformations, which are the most common structure for ATP and adenosine in DNA. In the absence of the metal ions, the hydrogen cannot build a bridge between P_γ and N1. So only the $-$*gauche* conformation can be found.

Our NMR experiments do not show any evidence for the existence of the $+$*gauche* conformation either in the presence or in the absence of the Mg^{2+} ion, although the CNS-generated structures can have all conformations. Intuitively, $+$*gauche* conformations have higher tension than $-$*gauche* conformations, since in $+$*gauche* conformations the six-member ring of the adenine is directly on the top of the sugar. It should be pointed out that not all *anti* conformations have the lowest energy; the torsion angle range between 30 and 90° (i.e. $+$*gauche* conformation) which is just in the *anti* conformation range, has not been found in our experiments, probably due to the very high tension. But since the structures generated by CNS and sampled in molecular simulation represent molecules in vacuum rather than in solutions, all structures have similar energy. In vacuum, the dissociation (or association) of the phosphate protons and the protonation (or deprotonation) of N1, N7 and the amine nitrogen (N6) are hardly taken into account.

From Fig. 3 it can be seen that both $r(\mathrm{H8–H2}') > r(\mathrm{H8–H1}')$ and $r(\mathrm{H8–H2}') < r(\mathrm{H8–H1}')$ can happen in *syn* conformation range as well as in *anti* conformations. Thus, it is better to use the *cis*, *trans* and $\pm$*gauche* notations to describe the conformation of ATP and adenosine when NOE intensity is involved, although the notations are not complete.

4. Conclusion

The change of the relative NOE intensities with pH, as has been observed in 1:1 ATP–Mg^{2+} solutions, suggests that, as the pH increased, the conformation of the torsion angle χ of ATP changes from *cis* to *trans* over $-$*gauche*. The *cis* conformation is probably due to a bridge between P_γ and N1 built by a Mg^{2+} ion. At higher pH such a bridge is broken, because hydration of metal ions is strengthened and $-$*gauche* conformations predominate.

Acknowledgements

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