New Data on the Conformation of Nucleoside 5'-Di- and 5'-Triphosphates Obtained by Roesy Experiments

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Abstract: The conformation and flexibility of four nucleotides commonly used as ATPase substrates, ADP, ATP, AMPPCP, and AMPPNP, have been investigated by using ROESY and T_2 experiments, in the absence and presence of Mg ions. This work, based on ROESY techniques, provides new data about the conformation of nucleosides 5'-di- and 5'-triphosphates. It is shown that ADP exhibits a conformation similar to that observed for the nucleoside 5'-monophosphates[8,11] and that, in contrast, one particular conformational region is predominant for the nucleoside 5'-triphosphates: in the presence of a third phosphate group, high anti conformations are predominant. The data relative to AMPPCP indicate that this base conformation is associated with a base–phosphate stacking, which could be a general feature of the nucleoside 5'-triphosphates. T_2 measurements at two frequencies and concentration dependence ROESY experiments show that the conformational properties derived from ROESY spectra are due to intramolecular interactions and, in addition to NOE and ROE values, suggest that a model of an isotropic motion governed by a single τ_c is not sufficient to characterize the molecular dynamics of the nucleosides triphosphates.

Introduction

In order to understand the enzymatic mechanisms at a molecular level, a common approach consists of studying the enzyme–substrate interaction process when the substrate is replaced by an inhibitor. The case of ribonucleotides, especially nucleoside 5'-di- and 5'-triphosphates, is of particular interest since they are major substrates of numerous enzymatic reactions, and because a lot of analogues are available. It seems therefore interesting to characterize their conformational properties. In this field, NMR† spectroscopy is a powerful tool for elucidating the conformation of bound and free substrates.[1-5] Recent development of two-dimensional (2D) techniques gives supplementary advantages. Among these techniques, ROESY experiments, first described by Bothner-By et al.,[6] appeared as a possible source of interesting data on substrate conformations, especially those of nucleotides, which are common cofactors of enzymatic reactions. The conformation of the nucleotide base around the glycosidic bond is efficiently determined by intramolecular base–sugar dipolar interactions as shown in previous works:[7,8] analysis of 1D NOE between the H8 base proton and the ribose ring protons, and analysis of T_1 measurements, provided essential information about the nucleotide conformation and the syn–anti equilibrium; in particular, the conformation of various free nucleoside 5'-monophosphates in solution has been extensively studied.[7-11] Surprisingly, whereas the conformation of ADP, ATP, and AMPPNP bound to enzymes has been investigated by transferred NOE experiments,[3,4] no proton–proton relaxation data on the free nucleosides 5'-di- and 5'-triphosphates are available to our knowledge; only one work[12] based on paramagnetic relaxation measurements involving free and bound $Co(NH_3)_4$-ATP has been reported. The present work shows that the study of proton–proton dipolar interactions in nucleotides may be significantly improved by using 2D ROESY experiments and describes the results obtained for a set of free nucleoside di- and triphosphates commonly used as ATPase substrates or inhibitors, ADP, ATP, AMPPCP, and AMPPNP, both in the absence and presence of Mg ions. Transverse relaxation time measurements at two frequencies, combined with concentration dependence experiments, were performed in order to complete the interpretation of the results obtained by the ROESY experiments.

Results

I. Prior Data Obtained by *J*-Coupling Analysis. It is well-known that a simple analysis of chemical shifts and spin-coupling constants of nucleotides can provide useful information about the nucleotide conformation,[10] in particular that of the ribose. The following section briefly reports the main points deduced from the corresponding data.

(i) As far as the base and sugar ring proton chemical shifts are concerned, no significant difference is detected between the various studied compounds. In the presence of Mg ions, the H5' and H5'' resonances of all the nucleotides display a loss of magnetic nonequivalence. Such an effect is a well-known feature of nucleotides and has already been reported in the literature.[10]

(ii) In the absence of Mg ions, all the nucleotides exhibit the same J(H1'–H2') coupling constant value, i.e., 5.8 ± 0.1 Hz, indicating similar C3' endo ↔ C2' endo equilibria of the ribose ring. The J(H1'–H2') value relative to the three nucleoside 5'-triphosphates (ATP, AMPPCP, AMPPNP) does not change after addition of Mg ions, whereas the value corresponding to ADP is decreased from 5.9 to 5.2 Hz, revealing a decrease of the C2' endo proportion and an equilibration of the conformer populations.

(iii) Similar conclusions can be derived from the J(H4'–H5') and J(H4'–H5'') coupling constants analysis. The *gg* conformation for rotation about the exocyclic C5'–C4' bond is largely predominant (72 ± 2%), and the main variation of the *gg* rotamer proportion (from 72 to 66%) is observed for ADP after addition of Mg ions.

The *J*-coupling analysis shows that, apart from MgADP, all the studied nucleotides exhibit nearly the same ring puckering and exocyclic group conformation.

II. ROESY Experiments. (1) ADP Conformation. Figure 1 shows the one-dimensional 500-MHz spectrum of ADP sugar

† Abbreviations. ADP, adenosine 5'-diphosphate; ATP, adenosine 5'-triphosphate; AMPPCP, adenosine 5'-[β,γ-methylene]triphosphate; AMPPNP, adenosine 5'-[β,γ-imido]triphosphate; EDTA, ethylenediaminetetraacetate; NOE, nuclear Overhauser effect; NMR, nuclear magnetic resonance; ROESY, rotating-frame Overhauser enhancement spectroscopy.

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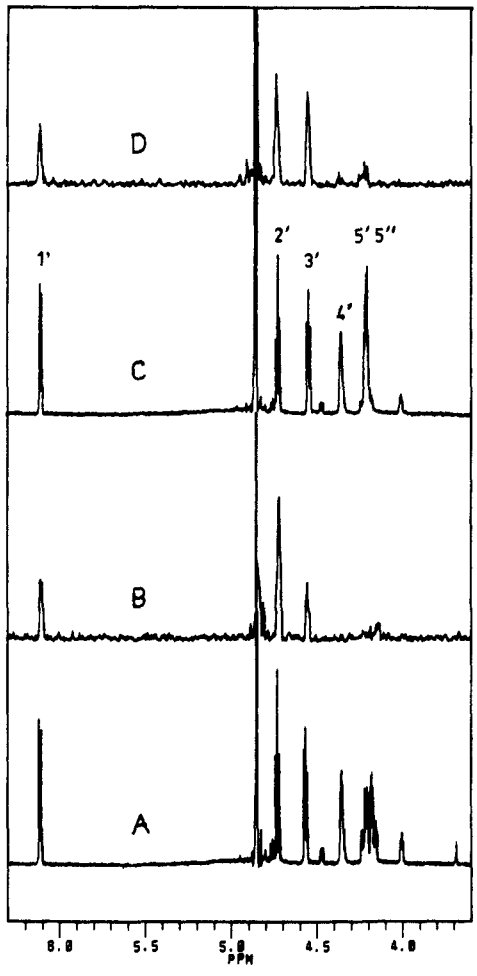

Figure 1. 500-MHz 1D spectrum and corresponding 2D ROESY matrix column at the H8 frequency of ADP (5 mM) ribose protons at 15 °C in the absence (A, B) and presence of Mg ions (10 mM) (C, D).

protons in the absence (Figure 1A) and presence (Figure 1C) of Mg ions. Each 1D spectrum is accompanied by the corresponding ROESY matrix column at the frequency of the H8 base proton (Figure 1B and D). These columns (or rows since the ROESY matrix is symmetrical) contain the H8 resonance as the diagonal peak (not shown) and the cross peaks located at the resonances of the ribose protons dipolar-coupled to the H8 proton. In the presence or absence of Mg ions, three intense cross peaks are observed and correspond to the H1', H2', and H3' protons. In the absence of Mg ions the volume ratios of the H8–H1', H8–H2', and H8–H3' connectivities are 25:50:25, whereas addition of Mg ions tends to equalize the contributions of the H2' and H3' protons. The 500-MHz ROESY spectra displayed in Figure 1B and D illustrate the quality of the information obtained for a nucleotide: a high selectivity combined to intense cross peaks.

These results are in agreement with previous studies of nucleotides based on 1D NOE and T_1 measurements[7-11] and show the flexibility of the base around the glycosidic bond: the intense and comparable H8–H1' and H8–H3' cross peaks indicate that the base explores both syn and anti conformational domains. The ADP base conformation is close to that observed for the purine nucleoside 5'-monophosphates where the syn and anti proportions were found to be equivalent.[9] The effect of Mg ions can be related to the change of the C3' endo ↔ C2' endo conformational equilibrium of the ribose ring monitored by the decrease of the J(H1'–H2') value and described in the previous section. The C3' endo conformation gives shorter H8–H3' distances than the C2' endo conformation, and in particular, the shortest H8–H3' distance in the C3' endo conformation is close to the van der Waals contact; thus, when J(H1'–H2') decreases, the contribution of the H3' proton to the dipolar relaxation of the H8 proton and the corresponding ROESY cross peak increases.

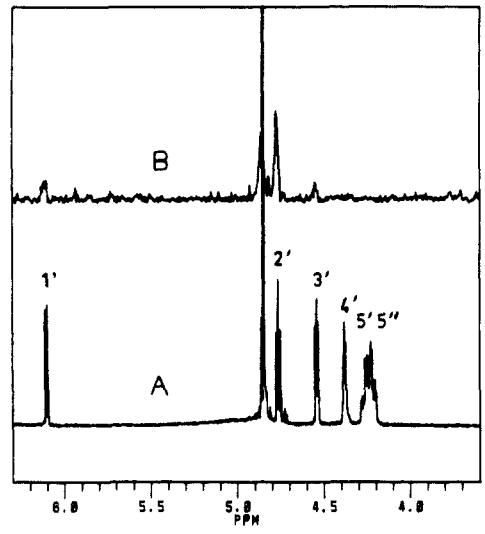

Figure 2. 500-MHz 1D spectrum (A) and corresponding 2D ROESY matrix column at the H8 frequency (B) of ATP (5 mM) ribose protons at 15 °C in the presence of Mg ions (10 mM).

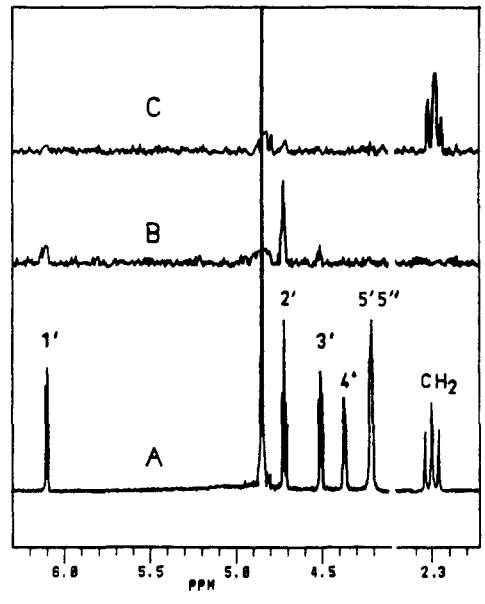

Figure 3. 500-MHz 1D spectrum (A) and corresponding 2D ROESY matrix columns at the H8 frequency (B) and at the H2 frequency (C) of AMPPCP (5 mM) ribose protons at 15 °C in the presence of Mg ions (10 mM).

Lastly, one can notice that the H5',H5" protons give weak but not negligible cross peaks with the H8 proton (Figure 1B,D) in accordance with a predominance of a *gg* conformation of the ribose exocyclic group, also revealed by the small J(H4'–H5") and J(H4'–H5") values.

(2) ATP and AMPPCP Conformations. Figures 2 and 3 (A, B) show the 1D spectrum and the corresponding ROESY matrix column at the H8 frequency of ATP and AMPPCP sugar protons in the presence of Mg ions. Apart from the magnetic nonequivalence of the H5' and H5" resonances, the chemical shifts, J values, and ROESY spectra of ATP and AMPPCP in the absence of ions are similar to those recorded after addition of Mg ions and are not shown.

ATP and AMPPCP exhibit similar ROESY H8 proton–ribose proton connectivities: the three ribose protons, H1', H2', and H3', contribute to the dipolar relaxation of the H8 proton and the volume ratios of the corresponding cross peaks are 20:65:15. As mentioned above, addition of Mg ions does not affect the ROESY spectra of ATP and AMPPCP.

It is now interesting to compare these spectra with that of ADP recorded in the absence of Mg ions since, in these conditions, the three nucleotides exhibit the same sugar conformation and

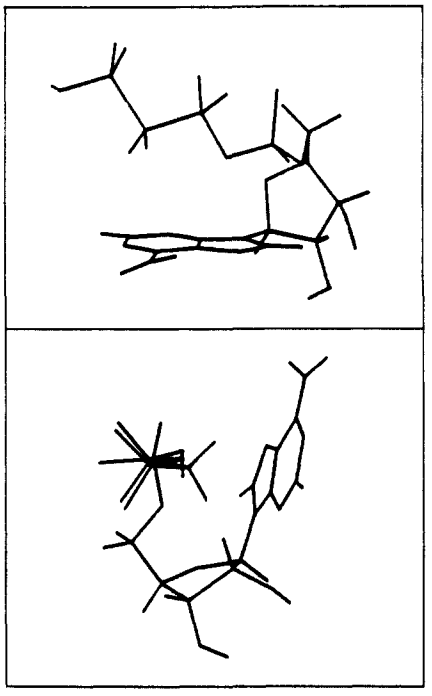

Figure 4. Two views of an AMPPCP conformation model with a high anti conformation of the base and a phosphate–base stacking leading to a close proximity between H2 and the methylene protons.

therefore any difference observed between the ROESY spectra is necessarily due to a change of the base conformation. Indeed, such differences exist: the distribution of the ROE intensities (volumes) between the H1′, H2′, and H3′ protons is 25:50:25 for ADP and 20:65:15 for ATP and AMPPCP. Hence, in the case of the two nucleoside triphosphates, the contribution of the H2′ proton is increased. The fact that this increase is accompanied by a decrease of the H1′ contribution on one hand and, to a larger extent, of the H3′ contribution on the other hand, indicates that, whereas the syn–anti equilibrium still occurs, a conformational region where the H8 base proton is close to the H2′ ribose proton is predominant. Such a conformational region, sometimes so-called high anti, is located around the limit between the syn and anti domains and corresponds to χ values[13] close to −90°. This conclusion is also supported by the following point: in contrast to the ADP spectrum, no H8–H5′ or H8–H5″ cross peak is observed in the ATP and AMPPCP ROESY spectra.

Another result is that, in the case of AMPPCP, the ROESY matrix column at the frequency of the H2 base proton (Figure 3C) contains an intense cross peak corresponding to the CH_2 protons of the phosphate backbone. Assuming that this effect is not due to an intermolecular interaction, as it will be discussed below, this connectivity indicates the existence of a intramolecular stacking between the phosphate backbone and the adenine ring, at least a large proportion of the time. Indeed, it is easy to find nucleotide conformations in the conformational region defined above, where H8 is close to H2′ and H2 is close to the phosphate groups (see Figure 4). The presence of internucleotide base–phosphate stacking in nucleic acids has been reported by Saenger.[13] It has to be pointed out that the predominance of the *gg* conformation for the exocyclic group of AMPPCP is in agreement with a base–phosphate stacking since the *gg* conformation about the C4′–C5′ bond highly favors the folding of the phosphate chain toward the base.

As far as the cross-peak intensity is concerned, it is noticeable that, whereas the experimental conditions are the same for all the studied nucleotides, the sum of the cross-peak volumes relative to ADP is twice as large as those of ATP or AMPPCP. Such an effect will be discussed below.

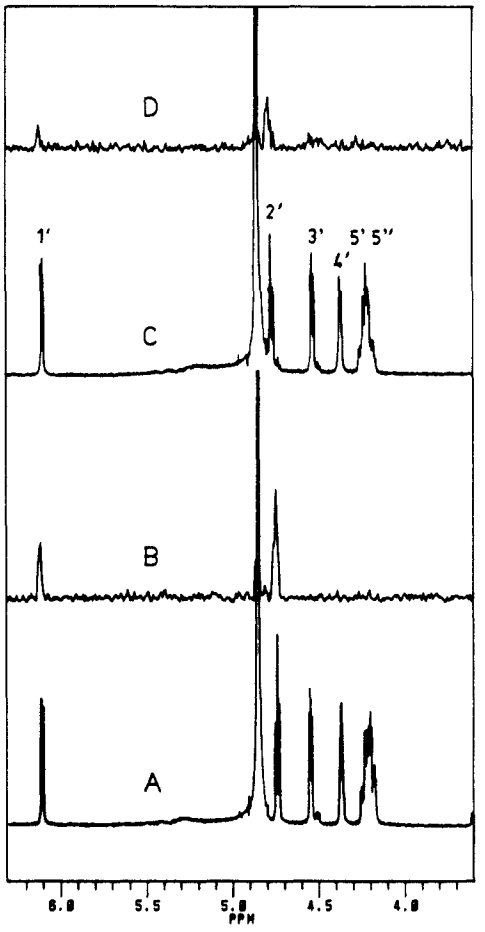

Figure 5. 500-MHz 1D spectrum and corresponding 2D ROESY matrix column at the H8 frequency of AMPPNP (5 mM) ribose protons at 15 °C in the absence (A, B) and presence of Mg ions (10 mM) (C, D).

(3) AMPPNP Conformation. Figure 5 shows the 1D spectrum and the ROESY matrix column at the H8 frequency of AMPPNP sugar protons in the absence (Figure 5A,B) and presence of Mg ions (Figure 5C,D). In contrast to ROESY spectra shown above, no H8–H3′ cross peak (in the absence of Mg) or a very weak one (in the presence of Mg) is observed in the AMPPNP spectrum. H8–H1′ and H8–H2′ connectivities are still detected with a volume ratio of 30:70. Once again, it has to be pointed out that, as mentioned in the previous section, the sugar conformation of AMPPNP and MgAMPPNP is similar to that of ADP, ATP, MgATP, AMPPCP, and MgAMPPCP. Hence, the differences observed between the corresponding ROESY spectra are due to changes of the base conformation. By comparing all these ROESY spectra, it is clear that the presence of a third phosphate group is accompanied by a decrease of the H3′ contribution (to the H8 dipolar relaxation) in favor of an increase of the H2′ contribution: the H3′ contribution is 25% for ADP, 15% for ATP an AMPPCP, and becomes negligible for AMPPNP. In parallel, the H2′ contribution increases from 50% (ADP) to 65% (ATP, AMPPCP) and 70% (AMPPNP). Moreover, the H8–H5′ and H8–H5″ cross peaks detected in the ADP ROESY spectrum are absent in the AMPPNP spectrum as well as in those relative to ATP and AMPPCP. Indeed, the ROESY spectrum of AMPPNP is consistent with the predominance of a base conformation similar to that described for AMPPCP and ATP but with lower χ values, in order to take into account the large H1′–H8 cross peak.

In contrast to ATP and AMPPCP, addition of Mg ions modifies the ROESY spectrum of AMPPNP (Figure 5D). The H8–H1′ and H8–H2′ connectivities of the AMPPNP ROESY spectrum as well as their relative intensities are conserved but the sum of the absolute intensities, which, in the absence of Mg ions, is close

(13) χ value is the value of torsion angle O4′–C1′–N9–C4, as defined by Saenger for purine nucleosides. This value describes the orientation of the base around the glycosidic bond. Anti and syn conformational ranges are defined for χ values centered, respectively, around 180° and 0°: Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984; pp 338–340.

Table I. Transverse Relaxation Times T_2 at 300 MHz of the H8 Proton of ADP, ATP, AMPPCP, and AMPPNP Measured at 15 °C[a]

	T_2, s	
	without Mg	with Mg
ADP	0.57 (1.04)	0.56 (1.06)
ATP	0.58 (1.04)	0.53 (0.96)
AMPPCP	0.58 (1.12)	0.49 (1.04)
AMPPNP	0.60 (1.03)	0.50 (1.04)

[a] Values in parentheses correspond to the T_2(300 MHz)/T_2(90 MHz) ratios.

to that of ATP or AMPPCP, is reduced by a factor of 2 in the presence of Mg ions.

We can now wonder if all the conformational properties observed above are due to intramolecular forces or are induced by self-association processes. The aggregation effects[14] are well-known but they are a priori negligible at the 5 mM concentration used in our experiments: for the described nucleotides, indeed no concentration dependence of chemical shifts could be detected between 0.5 and 5 mM. Nevertheless, in order to estimate the possible role of such intermolecular effects, we have measured the proton transverse relaxation times—an efficient probe of self-association—of the four studied nucleotides under the same experimental conditions (5 mM, 15 °C) as those used for the above ROESY experiments. In addition, concentration dependence 2D NMR experiments performed on AMPPCP solutions are described.

III. Transverse Relaxation Time Measurements and Concentration Dependence of AMPPCP ROESY Experiments. Transverse relaxation times (T_2) of ADP, ATP, AMPPCP, and AMPPNP protons were measured at 90 and 300 MHz by using a spin-echo sequence. The T_2 values obtained at 300 MHz for the H8 base proton and the corresponding T_2(300 MHz)/T_2(90 MHz) ratios are listed in Table I. In the absence of Mg ions, the T_2 values of the four nucleotides are nearly identical: the mean value is 0.59 ± 0.02 s. Addition of Mg ions induces a slight decrease of the T_2 values; the maximum T_2 variation, observed for both AMPPCP and AMPPNP, is about 15%.

As far as the T_2(300 MHz)/T_2(90 MHz) ratios are concerned, one can notice that, whatever the nucleotide and the absence or presence of Mg ions, the observed values are close to 1: the T_2 values are frequency independent. According to a model of an isotropic motion characterized by a correlation time τ_c, this means that either the condition $\omega\tau_c \ll 1$ (1) or $\omega\tau_c \gg 1$ (2) is satisfied, where ω is the Larmor frequency. As expected, observation of positive proton–porton NOEs at 90 MHz (data not shown) validates relation 1.

The transverse relaxation time is known to be strongly sensitive to long correlation times τ_c and thus to aggregation. The mean T_2 value observed, which corresponds to a line-width value of 0.6 Hz, and the frequency independence of the T_2 values indicate that the proportion of aggregates is negligible. Moreover, whereas the ROESY spectra of the four nucleotides can be quite different, the corresponding H8 protons exhibit the same T_2 value (in the absence of Mg ions). These results show that, at the 5 mM concentration used in this study, the various conformations observed for the nucleotides are essentially governed by intramolecular interactions.

Nevertheless, proton chemical shift and T_2 could be insensitive to slight self-association effects. In particular, the base–phosphate stacking observed for AMPPCP could be due to intermolecular interactions and thus could indicate the presence of a small proportion of aggregates. Therefore, we recorded a ROESY spectrum of a 0.5 mM AMPPCP solution. This experiment was performed on a 600-MHz spectrometer for sensitivity reasons, and in order to respect the experimental conditions, a ROESY spectrum of a 5 mM AMPPCP solution was recorded on the same spectrometer. Moreover, these experiments were completed by

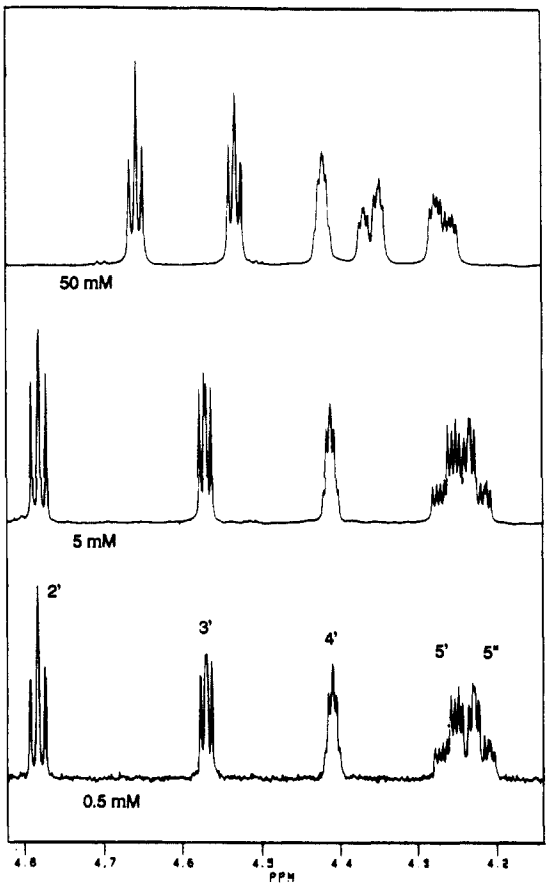

Figure 6. 600-MHz 1D spectra of AMPPCP ribose protons (H2', H3', H4', H5', and H5") at 15 °C in the absence of Mg ions, for various nucleotide concentrations.

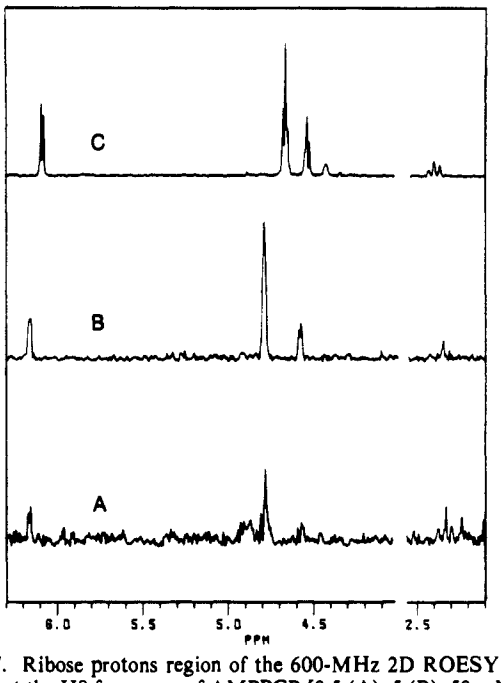

Figure 7. Ribose protons region of the 600-MHz 2D ROESY matrix columns at the H8 frequency of AMPPCP [0.5 (A), 5 (B), 50 mM (C)], at 15 °C in the absence of Mg ions.

a ROESY spectrum of a 50 mM AMPPCP solution: Figure 6 shows that, at this concentration, the 1D spectrum is drastically different (chemical shifts and line-width variations) from that of a 5 mM solution, which is identical with the 0.5 mM solution.

Figures 7 and 8 show the ROESY matrix column at the frequency of the H8 and H2 protons respectively for each AMPPCP concentration. The 0.5 and 5 mM ROESY spectra exhibit the same characteristic features: a high predominance of the H2'

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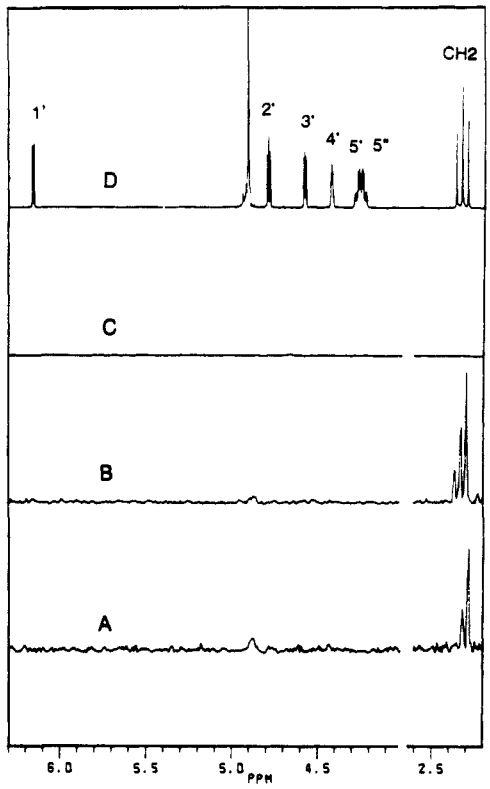

Figure 8. Ribose protons region of the 600-MHz 2D ROESY matrix columns at the H2 frequency of AMPPCP [0.5 (A), 5 (B), 50 mM (C)], and the corresponding 1D spectrum of AMPPCP [5 mM (D)], at 15 °C in the absence of Mg ions.

contribution and the presence of a CH_2–H2 connectivity. However, one can notice in the case of 0.5 mM spectrum a slight decrease of the H3′ contribution in favor of an increase of the H1′ contribution; because of the low signal-to-noise ratio of these two cross peaks, such as effect must be considered with precaution. At any rate, it is now clear that the phosphate–base stacking is due to intramolecular interactions. In contrast, the 50 mM ROESY spectrum reveals the effect of self-association: the H2–CH_2 contact is no longer detected (Figure 8C), and one can observe a H8–H4′ connectivity (highest field cross peak in Figure 7C), which is obviously due to intermolecular interactions.

Discussion

The present work, based on ROESY experiments, provides new data about the base conformation of nucleosides 5′-di- and 5′-triphosphates. Whereas ADP exhibits a conformational flexibility similar to that observed for the nucleoside 5′-monophosphates, one particular conformational region is predominant for the nucleoside 5′-triphosphates. This region is located around the limit between the syn and anti domains. In the case of AMPPCP, such a base orientation is accompanied by a base–phosphate stacking. ROESY spectra recorded at different nucleotide concentrations and transverse relaxation time measurements at two frequencies demonstrate that, at the 5 mM concentration used in our experiments, the conformations of the four studied nucleotides are essentially due to intramolecular interactions.

The question is now to know whether a base–phosphate stacking also occurs for both ATP and AMPPNP. Depending on the average distance between the H2 proton and the phosphate groups, the base–phosphate stacking could be, a priori, characterized by proton–phosphorus NOE. Two-dimensional proton–phosphorus NOE experiments have already been performed on ATP[16] but did not reveal any connectivity between the H2 proton and the phosphorus nuclei. However, these experiments were carried out under experimental conditions (500 mM ATP) where the intermolecular interactions govern the nucleotide conformation.[14] Unfortunately, in the case of a 5 mM ATP solution, the dipolar relaxation of the β- and γ-phosphates is essentially due to the protons of the residual HDO molecules.

Nevertheless, the close correspondence between the data relative to ATP and AMPPCP (the same base conformation, the absence of H8–H5′, –H5″ connectivity, and the absence of a Mg effect on the sugar and base conformation) is in favor of base–phosphate stacking interactions in ATP. It is interesting to notice that, in contrast to the nucleoside 5′-mono- and diphosphates, an exocyclic chain containing three phosphate groups is able to entirely overlap the purine ring and one can imagine that such a base–phosphate stacking is a general feature of the purine nucleoside 5′-triphosphates, including AMPPNP. This can explain the main result provided by our ROESY experiments on nucleotides: in the presence of a third phosphate group, high anti conformations are predominant.

The presence of Mg ions does not modify this result. One major effect of the Mg ions on the sugar and base conformations is to shift the C3′ endo ↔ C2′ endo equilibrium of the ADP ribose ring. Another is to decrease the AMPPNP ROESY cross-peak intensities without changing the relative volume ratios (and thus the average conformation); such an effect could be related to a change of internal molecular dynamics, according to arguments presented in the following section.

A more quantitative use of our data could be considered, but necessitates information about the molecular motions of nucleotides. This will not however be attempted here: the following arguments indicate that the molecular dynamics of nucleoside triphosphates is too complex to be described by simple models. First, the sum of the 1D proton–proton NOEs measured at 90 MHz for the H8 proton is about 25%, in disagreement with the extreme narrowing limit $\omega\tau_c \ll 1$ satisfied by the T_2 values. Second, the 1D proton–proton NOEs measured at 500 MHz are negligible whereas the classical $\omega\tau_c$ dependence of NOE predicts large negative values at this frequency according to the data obtained at 90 MHz. It has to be pointed out that, even if the presence of EDTA used in all these experiments was not sufficient to avoid additional paramagnetic relaxation, the classical equations of relaxation parameters indicate that the T_2 values are as sensitive as the NOE values to paramagnetic effects and cannot explain our experimental data. Another point is the discrepancy observed on the sum ($\sum$) of the cross-peak intensities between the nucleotides whereas the T_2 values are frequency independent. For example, in the absence of Mg ions, the $\sum$ value relative to ADP is twice as large as that of ATP, AMPPCP, and AMPPNP.

These data show that the classical equations that describe the τ_c dependence of the relaxation parameters are no more appropriate. Indeed, Allerhand et al.[15] showed that the introduction of an internal molecular motion in addition to the overall isotropic rotational diffusion leads to dramatic changes in the relationships between relaxation parameters and correlation times. Our data indicate that, in the case of nucleoside 5′-triphosphates, the molecular dynamics cannot be simply described by an approximate model of isotropic motion governed by a single τ_c as in the case of nucleoside 5′-monophosphates.[9]

In conclusion, it is clear that the ROESY experiment is a powerful technique for studying nucleotide conformation and compensates for the absence of significant NOEs at high-frequency field. This technique provides new data on nucleosides 5′-di- and 5′-triphosphates and could easily be extended to a larger set of nucleotides of general biological interest. A useful application of ROESY technique could concern the study of enzyme–nucleotide complexes. According to the results described by Clore et al.[3,4] on the conformation of nucleotides bound to enzyme obtained by transferred NOE experiments, it appears interesting to investigate the efficiency of ROESY experiments in this research field.

Experimental Section

Chemicals. ADP, ATP, AMPPCP, and AMPPNP were purchased from Sigma. All other chemicals were of the highest purity commercially available. Deuterium oxide (99.93) was from S.M.M., C.E.A. Saclay, France.

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Samples Preparation. Nucleotides were extensively lyophilized and then solubilized at a final concentration of 5 mM either in a D_2O solution [NaCl 100 mM, phosphate buffer 10 mM (pD 7), EDTA 0.1 mM] or in the same solution supplemented with 10 mM $MgSO_4$. For nucleotide concentration dependence experiments, AMPPCP was first solubilized at a concentration of 50 mM and then diluted to final concentrations of 5 and 0.5 mM in the same buffer.

NMR Measurements. Proton spectra (90, 300, 500, and 600 MHz) were recorded on WH-90, MSL-300, WM-500, and AM-600 Bruker spectrometers, respectively. T_2 and ROESY sequences were, respectively, as follows: $\pi/2-(\tau-\pi-\tau)_n-t_2$ and $\pi/2-t_1-SL-t_2$ with a refocusing delay τ of 1 ms and a spin-lock SL time of 300 (WM-500) or 500 ms (AM-600). All the experiments were carried out at 15 °C in order to significantly separate the residual HDO peak and the signal of the H2′ ribose proton.

Acknowledgment. We thank Drs. P. Champeil and M. Garrigos for stimulating discussions.

An ab Initio Study of Model S_N2 Reactions with Inclusion of Electron Correlation Effects through Second-Order Møller–Plesset Perturbation Calculations

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Abstract: A systematic analysis of electron correlation effects in model S_N2 reactions shows large differences between the structures optimized at the Hartree–Fock (HF) and second-order Møller–Plesset (MP2) levels, but the relative energies critical to an understanding of gas-phase S_N2 reactions are essentially unaffected by the effect of electron correlation on the optimized structures. Integrated charges obtained from the topological definition of an atom in a molecule indicate that the HF method overestimates the ionic character at the transition state and leads to large negative charges on the nucleophiles and leaving groups. Also, the HF method underestimates the electron density at the bond critical point and overestimates the critical radius. The model systems studied are $N^- + CH_3X \rightarrow CH_3N + X^-$, where X = H, NH_2, OH, F, CCH, *C*N, *N*C, SH, and Cl for N = H and where X = H, NH_2, OH, F, *C*N, SH, and Cl for N = F, and also N = X = Cl. The 6-31G basis set supplemented with diffuse and polarization functions (standard notation 6-31++G**) was used for all atoms, except for the three methyl hydrogens for which the 6-31G basis set was used.

Introduction

Bimolecular nucleophilic substitution (S_N2) reactions at carbon are among the most extensively studied reactions of organic chemistry. While investigations of S_N2 reactions have played an important role in the development of fundamental ideas in physical organic chemistry, there are major conceptual problems associated with S_N2 reactions[1] and they continue to attract much attention from experimentalists and theoreticians.

Early theoretical studies of gas-phase S_N2 reactions[2] provided evidence of a double-well potential energy surface and led to the proposal of a three-step mechanism of gas-phase S_N2 reactions.[3] A systematic study[4] of S_N2 reactions at the HF/4-31G level showed that intrinsic barriers calculated from the Marcus equation agree remarkably well with those obtained by theoretical calculation. Moreover, it also showed that there is a correlation between the geometry at the transition state (TS) and the exothermicity of the reaction. However, the basis set did not include diffuse functions, which are known to be very important for a proper description of the electronic structures of anionic systems and for obtaining an accurate potential energy surface of a reaction involving anions.[5] Furthermore, electron correlation effects were not considered, and therefore there is a need to carry out higher level calculations.

Electron correlation effects on S_N2 reactions have been studied by Dedieu et al. (limited configuration interaction (CI) without the Davidson correction);[6] by Keil and Ahlrichs (coupled electron pair approximation (CEPA));[7] by Černušák, Urban, and coworkers (many-body perturbation theory at the fourth order (MBPT(4)));[8] by Havlas et al. (second-order Møller–Plesset perturbation theory (MP2) and multiconfiguration self-consistent field theory (MCSCF));[9] by Vetter and Zülicke (multireference configuration interaction (MRD-CI));[10] and by others.[11] Most

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