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Acidity of Secondary Hydroxyls in ATP and Adenosine Analogues and the Question of a 2′,3′-Hydrogen Bond in Ribonucleosides

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Incorporation of modifications in mono-, di-, and oligonucleotides has found increasing use as tools in the investigation of enzyme mechanisms.[1−3] Special attention has been given to modifications in the ribose moiety (e.g., replacement of the 3′- and, in particular, the 2′-hydroxyl by hydrogen, amino, fluoro, thio, or methoxy functionalities.[2,3] If the influence from a modification is to be understood in detail, information on the properties of the modified nucleosides is essential. One important property is the acidity of hydroxyls in the sugar moiety. The pK_a value for ionization of one of the secondary hydroxyls of adenosine, under a couple of different experimental conditions, is reported to be 12.78 [4] and 12.17 (in D_2O).[5] Of particular value for reactions with the 3′-oxyanion as a leaving group, is the relative pK_a values for the 3′-hydroxyls in differently substituted nucleoside residues. To obtain relative pK_a values of the most commonly used analogues, we have, in the present study, determined the acidities of secondary hydroxyls in five ATP derivatives (**1a**−**e** in Chart 1) by ^{13}C NMR shift titrations. In addition, we have determined the relative acidities of adenosine, 2′-*O*-methyladenosine, and 3′-*O*-methyladenosine (**2a**−**c** in Chart 1) in three different solvents.

It has been suggested that a hydrogen bond between the 2′-hydroxyl and the 3′-oxygen would substantially influence the acidity of the 3′-hydroxyl and thus also the energetics of transesterification reactions involving a 3′-oxyanion leaving group.[6,7] To our knowledge, hydrogen bonding between the 2′-hydroxyl and the 3′-oxygen (or vice versa) in nucleosides has not been detected in water. Although it has been reported to be detected in organic aprotic solvents,[8] an important question to answer, however, is what the energy gain of such a hydrogen bond would be, especially when the 3′-oxygen carries a partial negative charge, as it would in a transition state with the 3′-oxyanion as the leaving group. A limiting case that should give the strongest hydrogen bond is when the 3-hydroxyl (or 2′-hydroxyl) is fully deprotonated so that the hydrogen bond is between an oxyanion and a hydroxyl group. Hence, the difference in pK_a values of secondary hydroxyls in nucleoside derivatives should reflect the possible energetic influence of a potential hydrogen bond. If this is significant, one would also expect a larger difference in acidity, between compounds that have or do not have H-bonding possibility, in less-polar solvents.

For determination of the relative pK_a values of 2′-analogues in H_2O, commercially available 5′-triphosphate derivatives **1** were chosen to ensure complete solubility of all of the analogues. For enhanced sensitivity in the determination of ^{13}C shifts of the C-3′-positions at different pH values, DEPT-90 or DEPT-135 sequences were used. The acidity of the secondary hydroxyls of the ATP analogues (Table 1) follows a trend that is related to the electron-withdrawing ability of the 2′-substituent. The ΔpK_a value between ATP and 2′-*O*-Me-ATP is less than one unit (0.8). With a hydroxyl being slightly more electron withdrawing than a methoxy and 2′-OH being probably slightly more acidic than 3′-OH, this indicates that either there is no hydrogen bond or the influence on the stability of the anion is insignificant. As a stronger support for this, there is a good linear correlation of pK_a values of **1a**−**e** to the group electronegativity[9] (Figure 1).

Chart 1. ATP and Adenosine Derivatives for which Acidity Constants were Determined

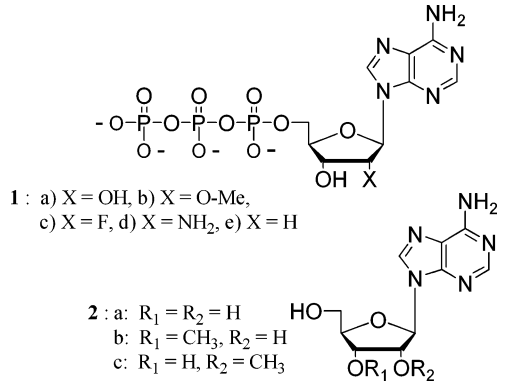

Table 1. Acidity Constants for Secondary Hydroxyls of ATP Analogues **1a**−**e** in H_2O Buffers (20 °C, I = 1.2 M)

compound	pK_a[a]
ATP (**1a**)	12.98 ± 0.04[b]
2′-*O*-methyl-ATP (**1b**)	13.80 ± 0.02[b]
2′-F-dATP (**1c**)	12.74 ± 0.02[b]
2′-H_2N-dATP (**1d**)	13.69 ± 0.02[b]
dATP (**1e**)	14.3 ± 0.13[b]

[a] The pK_a values were obtained by linear regression analysis of plots of $\log(\delta_H-\delta_{obs})/(\delta_{obs}-\delta_L)$ vs pH (where δ_H is the 3′-C ^{13}C chemical shift of the ionized form and δ_L is the 3′-C ^{13}C chemical shift of the protonated form).[10] [b] Errors reported are standard errors from the linear regression analysis. If we take into account that some measurement errors are present, we can estimate the errors to be somewhat higher (~±0.1 and up to ±0.2 for dATP).

The conclusion must be that, in water, there is either no hydrogen bond between the 2′- and 3′-oxygen in the monoanion or that this does not contribute toward the stability of the anion. An intramolecular hydrogen bond would be expected to be more stable in a less-polar solvent. To investigate if we could detect any influence of a hydrogen bond on the stability of the adenosine oxyanion, we determined the acidity constants for adenosine, 2′-*O*-methyladenosine, and 3′-*O*-methyladenosine in H_2O, methanol, and dimethyl sulfoxide (Table 2). Dimethyl sulfoxide (DMSO) was chosen because it has been considered as a mimic of a desolvated enzyme active site,[11] and it has been shown that intramolecular hydrogen bonds between an oxyanion acceptor and an OH donor are significantly stabilized in DMSO compared to in water or methanol.[11,12]

The differences in relative acidities of **2a**−**c** are quite similar in all of the solvents and even slightly less in the less-polar DMSO. We can conclude from this that a potential hydrogen bond between

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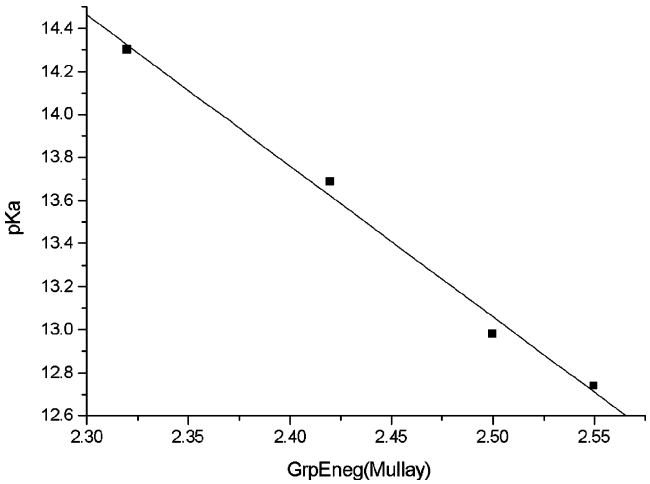

Figure 1. Plot of group electronegativities (where the values used are those for $HOCH_2$, FCH_2, H_2NCH_2, and CH_3 groups) vs pK_a for ATP analogues **1a**,**c**−**e**. Mullay's improved group electronegativities[9] were used (the value for **1b** is not available). A plot against Inamoto's ι values[13] also gives a good correlation, even slightly better (data not shown).

Table 2. Acidity Constants (p$K_{a_{solvent}}$)[a] for Secondary Hydroxyls of Adenosines **2a**−**c** in Different Solvents at 20 °C

solvent	adenosine	2′-*O*-methyladenosine	3′-*O*-methyladenosine
H_2O	12.79 ± 0.05[b]	13.53 ± 0.07	13.69 ± 0.09
MeOH	16.1 ± 0.09	16.8 ± 0.04	16.4 ± 0.09
DMSO	27.18 ± 0.06	27.44 ± 0.01	27.57 ± 0.02

[a] The pK_a values were obtained by linear regression analysis of plots of $\log(\delta_H-\delta_{obs})/(\delta_{obs}-\delta_L)$ vs pH (where δ_H is the 3′-C ^{13}C chemical shift of the ionized form and δ_L is the 3′-C ^{13}C chemical shift of the protonated form).[5,10] [b] Errors reported are standard errors from the linear regression analysis. If we take into account that some measurement errors are present, we can estimate the errors to be somewhat higher (∼±0.1).

a 2′-hydroxyl and a 3′-oxyanion cannot be of significant importance for the stability of the anion. It is also difficult to see how such a hydrogen bond could significantly contribute toward the stability of a transition state where the 3′-oxygen is only partially charged. We cannot completely exclude the possibility that an intramolecular hydrogen bond does exist in the anion, but its energy contribution must then be small as the pK_a values are hardly affected. If a hydrogen bond between the 2′-OH and the 3′-oxyanion does exist, the reason for the overall energetic invisibility for the absence of a contribution to the energy of the anion could possibly be that this is counteracted by another event (e.g., loss in conformational energy). Even so, it would still be a very weak H-bond that probably makes a minor energy contribution even in DMSO.

Stabilization of a transition state in phosphate transfer through a hydrogen bond from the 2′-OH to a 3′-oxyanion leaving group is then likely to be very modest or insignificant. We would like to suggest that significantly higher rates in such reactions in the presence of a vicinal 2′-hydroxyl may be best explained by other interactions, in some cases perhaps through hydrogen bonding between the 2′-OH and a phosphoryl oxygen.

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Supporting Information Available: S1: Index. S2: Materials and Methods. S3: linear regression analysis of plots of pH versus $\log(\delta_H-\delta_{obs})/(\delta_{obs}-\delta_L)$ based on the ^{13}C chemical shift (δ) changes with pH for ATP analogues **1a**−**e** in H_2O buffers. S4: linear regression analysis of plots of pH versus $\log(\delta_H-\delta_{obs})/(\delta_{obs}-\delta_L)$ based on the ^{13}C chemical shift (δ) changes with pH for adenosine analogues **2a**−**c** in H_2O buffers and methanol. S5: plots of ^{13}C chemical shift (δ) versus pH for adenosine analogues **2a**−**c** in DMSO (left panel), and linear regression analysis of plots of pH versus $\log(\delta_H-\delta_{obs})/(\delta_{obs}-\delta_L)$ based on the ^{13}C chemical shift (δ) changes with pH for adenosine analogues **2a**−**c** in DMSO (right panel). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Verma, S.; Eckstein, F. *Annu. Rev. Biochem.* **1998**, *67*, 99−134. (b) Verma, S.; Vaish, N. K.; Eckstein, F. In *Comprehensive Natural Products Chemistry*; Barton, D., Nakanishi, K., Eds.; Elsevier: New York, 1999; Vol. 6, pp 217−233. (c) Warashina, M.; Takagi, Y.; Stec, W. J.; Taira, K. *Curr. Opin. Biotechnol.* **2000**, *11*, 354−362.

(2) Herschlag, D.; Eckstein, F.; Cech, T. R. *Biochemistry* **1993**, *32*, 8299−8311. (b) Cech, T. R.; Herschlag, D. In *Nucleic Acids and Molecular Biology*; Eckstein, F., Lilley, D. M. J., Eds.; Springer-Verlag: New York, 1996; Vol. 10, pp 1−17. (c) Strobel, S. A. *Curr. Opin. Biotechnol.* **1999**, *9*, 346−352. (d) Yoshida, A.; Shan, S.; Herschlag, D.; Piccirilli, J. A. *Chem. Biol.* **2000**, *7*, 85−96. (e) Sjögren, A.-S.; Strömberg, R.; Sjöberg, B.-M. *Nucleic Acids Res.* **1997**, *25*, 3543−3549. (f) Gordon, P. M.; Fong, R.; Deb, S. K.; Li, N.-S.; Schwans, J. P.; Ye, J.-D.; Piccirilli, J. A. *Chem. Biol.* **2004**, *11*, 237−246.

(3) Herschlag, D.; Eckstein, F.; Cech, T. R. *Biochemistry* **1993**, *32*, 8312−8321.

(4) Johnson, R. W.; Marschner, T. M.; Oppenheimer N. J. *J. Am. Chem. Soc.* **1988**, *110*, 2257−2263.

(5) Velikyan, I.; Acharya, S.; Trifonova, A.; Foeldesi, A.; Chattopadhyaya, J. *J. Am. Chem. Soc.* **2001**, *123*, 2893−2894.

(6) Lyne, P. D.; Karplus, M. *J. Am. Chem. Soc.* **2000**, *122*, 166−167. (b) Roussev, C. D.; Ivanova, G. D.; Bratovanova, E. K.; Vassilev, N. G.; Petkov, D. D. *J. Am. Chem. Soc.* **1999**, *121*, 11267−11272.

(7) Strobel, S. A.; Ortoleva-Donnelly, L. *Chem. Biol.* **1999**, *6*, 153−165. (b) Gordon, P. M.; Sontheimer, E. J.; Piccirilli, J. A. *Biochemistry* **2000**, *39*, 12939−12952.

(8) Pitha, J. *Biochemistry* **1970**, *9*, 3678−3682. (b) Acharya, P.; Chattopadhyaya, J. *J. Org. Chem.* **2002**, *67*, 1852−1865.

(9) Mullay, J. *J. Am. Chem. Soc.* **1985**, *107*, 7271−7275.

(10) Darzynkiewicz, E.; Sierakowski, H.; Shugar, D. *Z. Naturforsch., C* **1975**, *30*, 565−570.

(11) Shan, S.; Herschlag, D. *J. Am. Chem. Soc.* **1996**, *118*, 5515−5518. (b) Shan, S.; Herschlag, D. *Proc. Natl. Acad Sci. U.S.A.* **1996**, *93*, 14474−14479.

(12) Schwartz, B.; Drueckhammer, D. G. *J. Am. Chem. Soc.* **1995**, *117*, 11902−11905.

(13) Inamoto, N.; Masuda, S. *Tetrahedron Lett*. **1977**, 3287. (b) Inamoto, N.; Masuda, S.; Tori, K.; Yoshimura, Y. *Tetrahedron Lett.* **1978**, 4547. (c) Inamoto, N.; Masuda, S. *Chem. Lett.* **1982**, *1003*, 1007.

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