Effect of Hydration and Ribose Ring Puckering on the Enthalpy of Hydrolysis of Cyclic Adenosine 3',5'-Monophosphate. A Quantum-Chemical Study

Miek M. E. Scheffers-Sap* and Henk M. Buck

Contribution from the Department of Organic Chemistry, Eindhoven University of Technology, Eindhoven, The Netherlands. *Received March 17, 1980*

Abstract: Three main hydration sites of a ribose ring, as a model for 3'-AMP and 5'-AMP, have been studied with the ab initio method, using an STO-3G basis set, in simulation of the proposed regiospecific solvation of O(1') and O(5') in 5'-AMP and 3'-AMP. This solvation, which does not occur in c-AMP, contributes to the large exothermic net solvation enthalpy of hydrolysis of c-AMP. It is concluded that the bridge position between O(1') and O(5') is an important hydration site. Formation of a five- as well as a seven-membered ring with one molecule of water between O(1') and O(5') is possible. In addition to this effect the conformational difference of the ribose ring in c-AMP, on the one hand, and in 5'-AMP and 3'-AMP, on the other hand, has been taken into account. It is clearly demonstrated that this difference also contributes to the overall enthalpy of hydrolysis.

Introduction

The coenzyme cyclic adenosine 3',5'-monophosphate[1] (Figure 1), which acts as a "second messenger", has been recognized in the past years[2] as a key substance in the regulation of many metabolic processes. Its level of concentration is controlled by the enzyme adenylate cyclase which catalyzes the conversion of ATP[1] into c-AMP. The conversion of c-AMP into adenosine 5'-monophosphate[1] takes place via a phosphodiesterase. Most phosphodiesterases degrade c-AMP solely into 5'-AMP. The hydrolysis of c-AMP by the *Enterobacter aerogenes* phosphohydrolase, as studied by Westheimer et al.,[3] delivers a mixture of 5'-AMP and adenosine 3'-monophoshate.[1] The hydrolysis to either 3'-AMP or 5'-AMP involves a large exothermic Gibbs free energy (-8.9 kcal/mol) and enthalpy[3] (-11.1 kcal/mol). Although Westheimer et al.[3] suggested that solvation might give an important contribution to the large exothermic enthalpy, no further explanation has been offered hitherto for this peculiar phenomenon. In a previous paper[4] the solvent effect on the enthalpy of hydrolysis has been studied with the traditional "continuum" model,[5] which tries to account for the bulk effect of the surrounding medium. In this model the solute molecule is supposed to be enclosed in a sphere that is embedded in the solvent. Molecular orbital calculations were performed, using the Extended Hückel method for c-AMP and other cyclic and acyclic phosphate diesters (i.e., ethylene, trimethylene, diethyl and dimethyl phosphate) and their products of hydrolysis. It was found that the net solvation enthalpy of the hydrolysis of c-AMP is 2-3 kcal/mol more exothermic than that of the other phosphate diesters. *We also suggested that to a great extent this difference in net solvation enthalpy is caused by a regiospecific hydration between O(1') and O(5') in 5'-AMP as well as in 3'-AMP.* In c-AMP this specific interaction cannot be present because of the large distance between O(1') and O(5'). This specific hydration is also impossible in the other phosphate diesters (vide supra) and in their products of hydrolysis. Our proposed model for regiospecific hydration seems to be strongly supported by *^{13}C NMR spin-lattice relaxation times* (T_1's). T_1 data make it possible to analyze intermolecular effects, e.g., hydrogen bonding.[6] Comparison of NT_1 values (N = number of directly attached protons to the carbon atom) indicates the presence of anisotropy of segmental motion in a molecule. As an example, Czarniecki and Thornton[6] compared the NT_1 values of the exocyclic carbon atom of galactose and glucose derivatives of ganglioside head groups with each other and with the ring carbon atoms. They found that the exocyclic carbon atom of the glucose compound is isotropic with respect to the ring carbon atoms and explained this phenomenon by intermolecular hydrogen bonding via one molecule of water between the exocyclic CH_2OH group and the pyranose ring oxygen atom. For 5'-AMP the NT_1 values of the ribose carbon atoms, as determined by Norton and Allerhand,[7] are between 0.18 and 0.20 s, whereas the value of the exocyclic C(5') atom is 0.20 s. This implies that the degree of rotational freedom for C(5') is as large as that of the ring carbon atoms. This isotropy underlines our proposed model for the occurrence of regiospecific hydration between O(1') and O(5') in 5'-AMP. *X-ray crystallographic data*[8] of the aminoglycosyl antibiotic puromycin dihydrochloride pentahydrate, which is a modification of 3'-AMP, wherein the phosphate moiety is replaced by a *p*-methoxy-L-phenylalanylamino group, unambiguously demonstrate that hydrogen bonding *does occur* between O(1') and O(5'). Although in this compound a phosphate group is replaced by a large amino acid group, the conformation of the 3'-aminoacyl nucleotide is still the preferred conformation of acyclic mononucleotides.[8] It seems that puromycin provides a good conformational model for this compound.

In order to offer additional theoretical support for the proposed model of hydrogen bonding between O(1') and O(5'), we studied, using the ab initio SCF-LCAO method in a discrete treatment, polyhydration of favorable sites of a ribose ring as a simplified model for 5'-AMP and 3'-AMP. Westheimer et al.[3] have clearly demonstrated that the large exothermic enthalpy of hydrolysis of c-AMP cannot be ascribed to strain in the phosphate diester ring. No attention has been given hitherto to the conformational difference of the ribose ring in the various nucleotides. The ribose ring has a C(3')-endo conformation in 5'-AMP[9] and 3'-AMP,[10] whereas that of c-AMP exists in a C(4')-exo-C(3')-endo conformation.[11] *Therefore, we have also taken into consideration the distinction in ribose ring puckering by calculating the difference in enthalpy of the ribose ring in both conformations.*

(1) The abbreviations used are: c-AMP, cyclic adenosine 3',5'-monophosphate; 5'-AMP, adenosine 5'-monophosphate; 3'-AMP, adenosine 3'-monophosphate; ATP, adenosine triphosphate.
(2) Mahler, H. R.; Cordes, E. H. "Biological Chemistry"; Harper and Row: New York, 1971; pp 500-503.
(3) Gerlt, J. A.; Westheimer, F. H.; Sturtevant, J. M. *J. Biol. Chem.* **1975**, *250*, 5059-5067.
(4) Scheffers-Sap, M. M. E.; Buck, H. M. *Recl. Trav. Chim. Pays-Bas* **1978**, *97*, 252-256.
(5) Jano, I. *C. R. Acad. Sci.* **1965**, *t261*, 103-106.
(6) Czarniecki, M. F.; Thornton, E. R. *J. Am. Chem. Soc.*, **1977**, *99*, 8279-8282.
(7) Norton, R. S.; Allerhand, A. *J. Am. Chem. Soc.*, **1976**, *98*, 1007-1014.
(8) Sundaralingam, M.; Arora, S. K. *J. Mol. Biol.*, **1972**, *71*, 49-70.
(9) Kraut, J.; Jensen, L. H. *Acta Crystallogr.*, **1963**, *16*, 79-88.
(10) Sundaralingam, M. *Acta Crystallogr.*, **1966**, *21*, 495-506.
(11) Lespinasse, J. N.; Vasilescu, D. *Biopolymers*, **1974**, *13*, 63-75, and references cited herein.

0002-7863/80/1502-6422$01.00/0

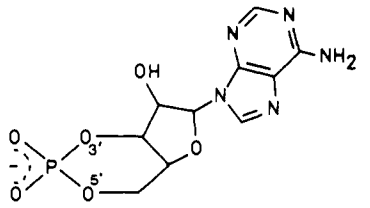


Figure 1. c-AMP.

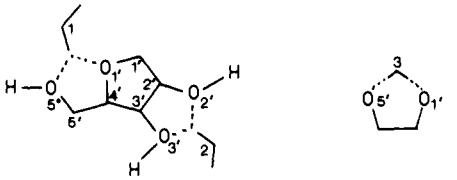


Figure 2. Ribose ring in C(3')-endo conformation with three main hydration sites.

Table I. Interaction Enthalpies and Hydrogen Bond Lengths for Hydration Sites of the Ribose Ring

hydration[a] site	ΔH_{hydr}, kcal/mol	hydrogen bond length,[b] Å
1	−8.2	1.74 (O(1'))
2	−7.7	1.76 (O(2'))
3	−7.9	1.83 (O(1'))

[a] Hydration sites as indicated in Figure 2. [b] In parentheses, the oxygen atom which is considered.

Method

Molecular orbital calculations have been performed with the ab initio SCF–LCAO procedure with a minimal STO-3G basis set, using the program GAUSSIAN 70.[12] In the calculations for the hydration, the ribose ring has the C(3')-endo conformation with a gauche–gauche orientation of the exocyclic CH_2OH group about the C(4')–C(5') bond. For nucleotides this is the preferred conformation. Input data have been taken from X-ray crystallographic data of 3'-AMP, as obtained by Sundaralingam.[10] The most important dihedral angles are: $\phi_{C(3')-O(3')} = 121.0°$, $\phi_{C(5')-O(5')} = 56.7°$, and $\phi_{C(2')-O(2')} = 111.2°$. The intermolecular hydrogen bonds are chosen so that they are in accordance with experimental data.[8,13] The difference in enthalpy between the two conformers (vide supra) is determined with tetrahydrofuran as model compound in both conformations. The geometrical data have been abstracted from X-ray crystallographic studies of 3'-AMP[10] and c-AMP.[11] While only 70 AO's can be used in the GAUSSIAN 70 program,[12] the number of water molecules which can be attached to the ribose ring is limited to four.

Results

Hydration. In the computations the ribose ring was fixed in the C(3')-endo conformation (vide supra). Interaction with one molecule of water with the ribose ring is considered in three possible ways, i.e., a five- and seven-membered ring via the bridge position between O(1') and O(5'), and a five-membered ring with O(2') and O(3') (Figure 2).

Interaction enthalpies are calculated by subtracting the sum of the enthalpies of the isolated compounds from the enthalpy of the adduct. In Table I the interaction enthalpies (ΔH_{hydr}) and the intermolecular hydrogen bond lengths are listed for monohydration of the ribose ring.

Furthermore, polyhydration is considered for the sites between O(1') and O(5'), *and* O(2') and O(3') as is shown in Figure 3. The calculated interaction enthalpies are listed in Table II.

Calculations[13] for the water dimer show that the linear structure is the most stable one, with a gain in binding enthalpy of 6.6 kcal/mol. We have also considered the binding enthalpies of linear systems $(H_2O)_n$ (Table III). The results of the net solvation enthalpy for c-AMP vs. other phosphate diesters are evaluated under polyhydration (vide infra).

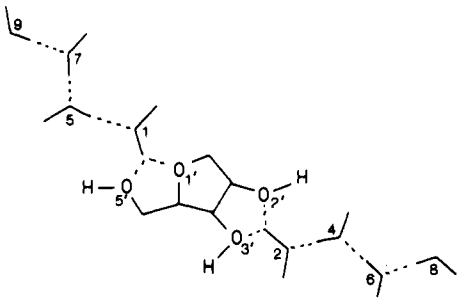
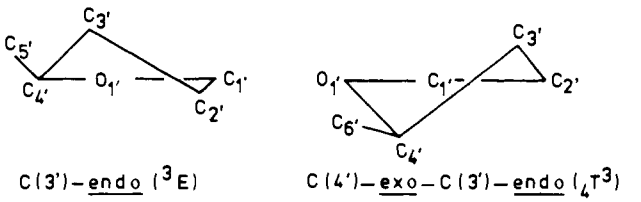


Figure 3. Polyhydration for the sites between O(1') and O(5') *and* O(2') and O(3')



C(3')–endo (^{3}E) C(4')–exo–C(3')–endo ($_4T^3$)

Figure 4. Conformations of the ribose ring.

Table II. Polyhydration of Ribose Ring with *n* Water Molecules

no.[a] of water molecules (*n*)	hydration site	interaction enthalpy (ribose ring–nH_2O), kcal/mol
2	1, 2	−16.2
3	1, 2, 4	−23.9
3	1, 2, 5	−24.3
4	1, 2, 4, 6	−31.0
4	1, 2, 5, 7	−32.0
4	2, 4, 6, 8	−29.1
4	1, 5, 7, 9	−29.9

[a] Hydration sites as indicated in Figure 3.

Table III. Binding Enthalpies in the Linear Systems $(H_2O)_n$

system[a]	interaction enthalpies, kcal/mol
$H_2O–H_2O–H_2O\}H_2O$	−6.9
$H_2O–H_2O\}H_2O$	−6.7
$H_2O\}H_2O$	−6.6

[a] } indicates the subsystem considered.

Ribose Ring Puckering. Calculation of the enthalpies of a free tetrahydrofuran ring in the C(4')-exo–C(3')-endo and C(3')-endo conformation (Figure 4) shows that the difference is 2.2 kcal/mol in stabilizing the latter conformation.

Discussion

Ribose Ring Puckering. Examination of Dreiding models shows that the C(4')-exo–C(3')-endo conformer is indeed more strained than the C(3')-endo conformer, which will contribute to the exotherm effect upon hydrolysis of c-AMP into 5'-AMP and 3'-AMP. Comparison of the measured enthalpies of hydrolysis of c-AMP and some cyclic phosphate diesters (e.g., trimethylene phosphate), as determined by Westheimer et al.,[3] demonstrates that the hydrolysis of c-AMP is about 8 kcal/mol more exothermic. So the loss of strain in the ribose ring by the conversion of c-AMP into 5'-AMP ($\Delta H^{\text{ribose ring puckering}}_{\text{c-AMP} \rightarrow \text{5'-AMP}} = -2.2$ kcal /mol) is partially responsible for the difference in the net enthalpy of hydrolysis.

Monohydration. The calculated interaction enthalpies for hydrogen bonding show that the considered three sites can act as hydration sites (vide supra), with interaction enthalpies more negative than the corresponding water–water interaction. Al-

(12) GAUSSIAN 70, Program 236, Quantum Chemistry Program Exchange, Indiana University.
(13) Berthod, H.; Pullman, A. *Theoret. Chim. Acta (Berlin)* **1978**, *47*, 59–66.

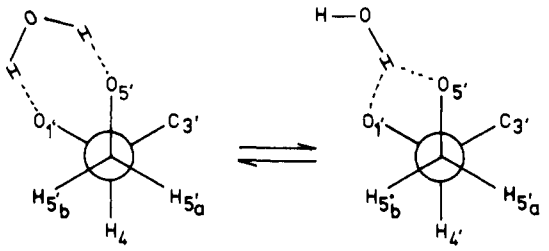


Figure 5. Transformation of intermolecular hydrogen bonding in 5'-AMP (*g,g*).

though we proposed in our previous paper[4] solely the occurrence of a seven-membered ring, the relatively small difference of the interaction enthalpies for the formation of a seven- and five-membered ring, 0.3 kcal/mol (Table I), predicts a dynamic interchange (Figure 5). The occurrence of the latter opens the potentiality for a "through-water" interaction of 5'-AMP and 3'-AMP with enzymic sites.

Polyhydration. The results in Table II show that upon polyhydration the positions between O(1') and O(5') as well as those between O(2') and O(3'), as illustrated in Figure 3, remain important sites, whereas the corresponding location between O(1') and O(5') in c-AMP cannot be hydrated in consequence of the large distance between the two oxygen atoms. Upon polyhydration of site O(1')–O(5') (with water molecule 1, 5, 7, 9) an interaction enthalpy of −29.9 kcal/mol is obtained. When this value is compared with the enthalpy which is necessary to release four water molecules from bulk water (4 × 6.9 = 27.6 kcal/mol (Table III)), it is clear that upon polyhydration a net enthalpy of hydration of about 2 kcal/mol remains. The same conclusion can also be drawn if less than four water molecules are involved in hydration. So the ab initio results give support to our previous suggestion that the difference in net solvation enthalpy (2–3 kcal/mol[4]) for the hydrolysis of c-AMP with respect to the other phosphate diesters can be mainly attributed to the fact that the location between O(1') and O(5') acts as an important hydration site.

The significance of hydrogen bonding as an explanation for the behavior of nucleotides has emerged more lately. Bolton and Kearns[14] offered a model for the intermolecular hydrogen bonding of the 2'-OH group of the ribose ring to the free phosphate oxygen atoms (with the O(2')–O(7) distance 3.6 Å). They conclude from ^{1}H NMR spectra of cyclic nucleotides in aqueous and mixed solvents that the 2'-OH proton is protected against exchange with bulk water. On the other hand, they could not find any crystallographic evidence for the proposed interaction. Berthod and Pullman[13] showed with ab initio calculations that hydrogen bonding is possible between the O(2') and O(3') atom in the free ribose ring via one molecule of water. Our results underline those of Berthod and Pullman for c-AMP and the products of hydrolysis, and furthermore crystallographic evidence is available.[13,16] The distance between the 2'-OH oxygen atom and the nearest phosphate oxygen atom in c-AMP is found to be 5.0 Å.[11] As a corollary to these data, there is unlikely to be an intermolecular hydrogen bonding between the 2'-OH group of the ribose ring and the free phosphate oxygen atom, but rather between the 2'-OH group and the O(3') phosphate ester oxygen atom. Very recently Bolton and James[15] discussed the local mobility of RNA and DNA also by intramolecular water bridges.

Our study clearly shows that the contribution to the large exothermic enthalpy of hydrolysis of c-AMP arises from solvation and more specifically from the regiospecific hydration in 5'-AMP and 3'-AMP and from the loss in strain of the ribose ring.

Acknowledgment. We gratefully acknowledge Unilever Research, Vlaardingen, The Netherlands, for the financial support of this investigation.

(14) Bolton, P. H.; Kearns, D. R. *J. Am. Chem. Soc.*, **1979**, *101*, 479–484.
(15) Bolton, P. H.; James, T. L. *J. Am. Chem. Soc.*, **1980**, *102*, 25–31.
(16) In the water dimers the proton donor OH bond was placed along the bissectrix of the water proton acceptor molecule with an O···H distance of 1.9 Å.

Substituent Effect on the Fifth Overtone of Aryl C–H Stretching Vibrations

Yoshihiro Mizugai and Mikio Katayama*

Contribution from the Department of Pure and Applied Sciences, College of General Education, The University of Tokyo, Komaba, Meguro-ku, Tokyo, 153, Japan. *Received March 17, 1980*

Abstract: The fifth overtones of aryl C–H stretching vibrations of more than 30 kinds of monosubstituted benzenes in the liquid state have been observed by a thermal-lens technique. The frequency shifts of the overtones from that of benzene, $\Delta\omega$, were found to be proportional to the σ_I values of the substituents, the inductive contribution of the Hammett σ, thus supplying information on the reactivity of the chemical bond. Our experimental results support that a local-mode model is much superior to a normal-mode model for the description of high overtones.

Introduction

The experimental study of highly excited molecular vibration in the ground electronic state provides valuable information on photodissociations, radiationless transitions of molecules, and chemical reactivity of the bond relevant to the observed vibrations. The first application of the thermal-lens technique on the investigation of highly excited C–H stretching vibration in liquids was made by Long, Swofford, and Albrecht[1,2] and the fifth overtones of aryl C–H vibrations of benzene, naphthalene, anthracene, toluene, xylene, and trimethylbenzene were observed. They made measurements of the absorptions in the spectral range of 15 800–17 400 cm^{-1} covered by the CW rhodamine 6G dye laser, and analyzed the spectra on the basis of a local-mode model. Henry and his collaborators have reported that the C–H stretching overtone spectra, $\Delta v_{C-H} = 3$–7 of alkanes and methyl-substituted benzenes observed by using a conventional absorption method,

(1) M. E. Long, R. L. Swofford, and A. C. Albrecht, *Science*, **191**, 183 (1976).
(2) R. L. Swofford, M. E. Long, and A. C. Albrecht, *J. Chem. Phys.*, *65*, 179 (1976).

0002-7863/80/1502-6424$01.00/0