Vol. 108, No. 2, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 30, 1982 Pages 474-478

^{31}P- AND ^{13}C-NMR STUDY OF THE ATP(ADENOSINE TRIPHOSPHATE)-VANADYL COMPLEX

Hiromu Sakurai, Tetsuko Goda and Shigeru Shimomura

Faculty of Pharmaceutical Sciences, University of Tokushima,
Sho-machi 1, Tokushima 770, Japan

Received July 26, 1982

Summary: The state of existence of cytoplasmic vanadyl ion is known to be important: the vanadyl ion forms complexes with ATP and the vanadate form inhibits its (Na^++K^+)-ATPase. Therefore, the formation of complexes between ATP and vanadyl ion was investigated by ^{31}P- and ^{13}C-NMR spectrometry. From the present and previous results, it was concluded that three characteristic types of ATP-vanadyl complex are formed in aqueous solution: a blue 1:1 complex formed in the acidic pH region ATP has coordination sites between β- and γ-phosphates, a ribose 3'-protonated hydroxyl oxygen and adenine N-3 nitrogen and the vanadyl ion; a blue 1:1 complex formed in the neutral pH region contains coordination sites for vanadyl ion at β- and γ-phosphates and ribose 2'- and 3'-protonated hydroxyl oxygens; a green 2:1 complex at alkaline pH has binding sites between two sets of ribose 2'-protonated and 3'-deprotonated hydroxyl oxygens and vanadyl ion.

The discovery by Josephson and Cantley (1) that vanadate(+5 oxidation state) ion is a potent inhibitor of (Na^++K^+)-ATPase has aroused interest in the state of vanadium ion *in vivo*. Since glutathione is present in most tissues at a high concentration of about 3 mM, endogenous or injected vanadium ion has been thought to exist exclusively as a vanadyl(+4 oxidation state) ion (2,3). Thus Post et al. suggested that much of the cytoplasmic vanadium is present as a ATP-vanadyl complex (4). Macara et al. calculated the stability constant of this complex at pH 4.9 as K_{app}=2.18 (2). However, the characters of this complex have not been studied. In a previous paper we reported the existence of two types of ATP-vanadyl complex, a blue 1:1 complex and a green 2:1 complex, based on the results of potentiometric titration, and optical and EPR spectra (5). In further studies, we found by multi nuclei FT-NMR spectroscopy that there are three characteristic types of ATP-vanadyl complex formed in three pH regions. The NMR method has proved useful for obtaining unambiguous information on the metal-binding sites of ATP. In the present ^{31}P- and ^{13}C-NMR investigations, the

0006-291X/82/180474-05$01.00/0

coordination modes of these ATP-vanadyl complexes were determined: the blue 1:1 complex formed at acidic pH has coordination sites to vanadyl ion at two phosphates, a protonated hydroxyl oxygen of ribose and the N-3 nitrogen of an adenine moiety; the blue 1:1 complex formed at neutral pH has sites at two phosphates and two protonated hydroxyl oxygens of the ribose moiety; the green 2:1 complex has sites at protonated and deprotonated hydroxyl oxygen of two ribose moieties.

Materials and Methods

Reagent grade $VOSO_4 \cdot 6H_2O$ and $Na_2H_2ATP \cdot 3H_2O$ from Boehringer HmbH were used in all experiments. ^{31}P- and ^{13}C-NMR spectra were measured with a JEOL JNM-FX 200, Fourier transform NMR spectrometer operated at 80.76 and 50.10 MHz, respectively. In both NMR measurements, D_2O was used as a field frequency lock. The sample tubes used for ^{31}P- and ^{13}C-NMR measurements were 10mmø and 5mmø, respectively. The pH(pD) of the solution was measured with either a Horiba pH meter L-71S or an Ingold electrode connected to a Horiba pH meter F-7SS and all measurements were made at 22°C. ^{31}P- and ^{13}C-chemical shifts are calibrated with respect to external H_3PO_4 and trimethylsilane resonances, respectively. The reduction of signal intensity associated with line broadening, due to the presence of paramagnetic species, was estimated from the computed intensity %.

Results and Discussion

From the ^{31}P-NMR of ATP over a wide pH range, the three resonances were assigned to α-, β- and γ-phosphorus atoms of ATP using inorganic H_3PO_4 as an external reference, based on the assignments of the resonances (6). Owing to the presence of phosphorus-phosphorus nucleus coupling, the signals of α- and γ-phosphorus and β-phosphorus appeared as doublet and triplet peaks, respectively. The reductions of the signal intensities associated with signal broadening were estimated from measurements on the highest signal. Observed reductions of signal intensities of phosphorus nuclei in ATP in the presence of paramagnetic vanadyl ion are shown in Fig. 1. It is clear that at pH 3.0 and 7.0, vanadyl ion interacts with β- and γ-phosphate groups. However, at pH 12.4 no interaction of phosphate with vanadyl ion was observed, being consistent with the conclusion in our previous report (5). ^{13}C-NMR spectra provided information on the coordination sites of ATP with vanadyl ion. The chemical shifts of ten carbon resonances of ATP were assigned with tetramethylsilane as an external reference, based on the assignments by Dorman and Roberts (7). Although the features of the signal reductions associated with line broadenings of carbon resonances in

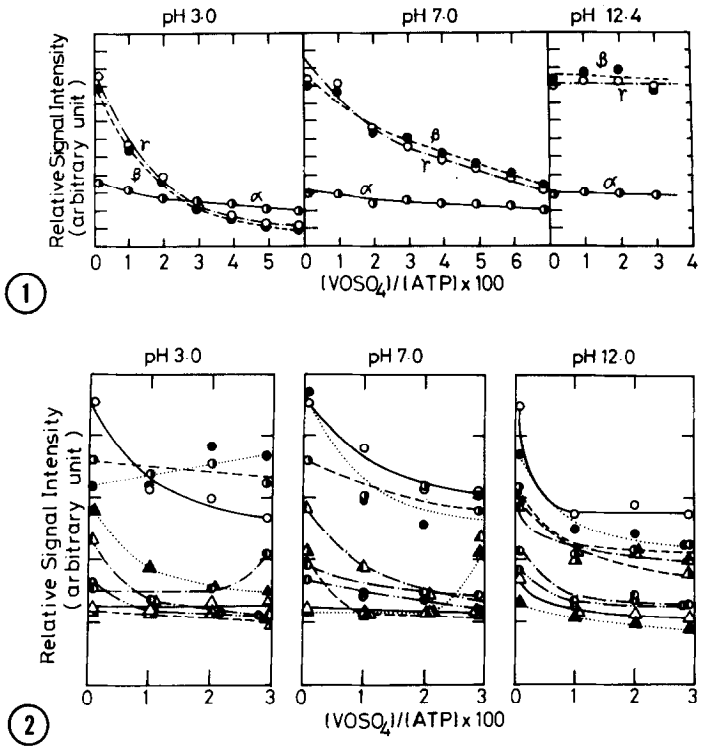

Figure 1. ^{31}P-NMR Titration of ATP with Vanadyl Sulfate.
ATP, 0.11 M (3ml in D_2O) was titrated with 0.1 M $VOSO_4$ and the pH of the solution was adjusted with 1 M NaOH.

Figure 2. ^{13}C-NMR Titration of ATP with Vanadyl Sulfate.
ATP dissolved in D_2O was titrated with 0.1 M $VOSO_4$ and the pH of the solution was adjusted with 1 M NaOH. Initial concentrations of ATP and solution volumes were as follows: 0.51 M, 0.8 ml for pH 3.0 solution; 0.83 M, 0.5 ml for pH 7.0 solution; 1.03 M, 0.4 ml for pH 12.0 solution. Position of carbon atom : 1', --●-- ; 2', ···●··· ; 3', —○— ; 4', —●— ; 5', —●—; 2, -▲- ; 4, ···▲··· ; 6, —△— ; 8, -▲-.

ATP in the presence of vanadyl ion were relatively complicated (Fig. 2), the following points were clear, (i) at pH 3.0 the carbon signal intensities of C-3', C-4 and C-8 were decreased significantly, suggesting the presence of the coordinations of vanadyl ion with a ribose oxygen atom and adenine nitrogen at the N-3 or N-7 position, (ii) at pH 7.0 the signal intensities of C-3', C-2' and C-8 were reduced, suggesting the presence of the coordinations with either the two oxygen atoms of ribose or oxygen of ribose and adenine N-3 nitrogen, (iii) at pH 12.0 signals of C-2' and C-3' were reduced most, indicating the coordination with the two oxygen atoms of ribose, and (iv) the reduction of the signal of C-8 was not pH-dependent, suggesting little possibility of nitrogen coordination at the N-7 position. The main findings obtained in previous studies

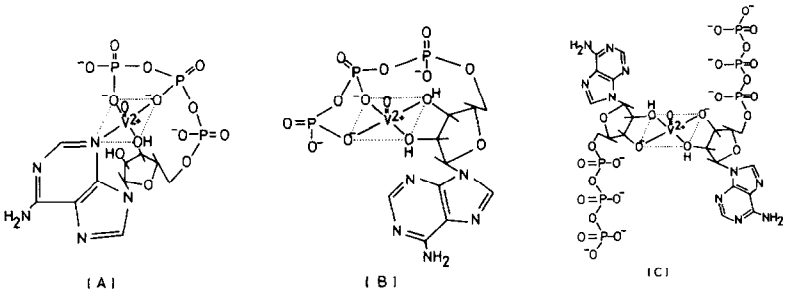

Figure 3. Possible Structures of Three Types of ATP-Vanadyl Complex. [A], Blue 1:1 ATP-vanadyl complex in acidic pH region; [B], Blue 1:1 ATP-vanadyl complex in neutral pH region; [C], Green 2:1 ATP-vanadyl complex in alkaline pH region.

on the ATP-vanadyl complex were that the blue 1:1 complex is formed with dissociation of two protons from hydroxyl groups of the triphosphate moiety at pH 3 and 7, and that the green 2:1 complex is formed with dissociation of a proton from a hydroxyl group of the ribose moiety (5).

On the basis of the present observations and these previous results (5), the three types of ATP-vanadyl complex formed are proposed to be as shown in Fig. 3. Coordination from adenine N-7 or N-3 nitrogen was suggested from the ^{13}C-NMR spectrum at pH 3.0, but our stereo-model ruled out the possibility of simultaneous coordination from C-3' oxygen and N-7 nitrogen. Therefore, it is proposed that ATP-vanadyl 1:1 blue complex [A] formed in the acidic pH region includes coordination to vanadyl ion through β- and γ-phosphate, a ribose 3'-protonated hydroxyl oxygen and an adenine N-3 nitrogen atom. The blue 1:1 complex [B] in the neutral pH region contains coordinations from β- and γ-phosphate and ribose 2'- and 3'-oxygen contributed from protonated hydroxyl groups. The green 2:1 complex [C] in the alkaline pH region includes two sets of oxygen coordinations contributed from a protonated and a deprotonated hydroxyl group of two ribose moieties.

In the previous paper (5) we concluded, without suggesting the detailed coordination sites in the triphosphate moiety, that only two types of ATP-vanadyl complex were formed in acidic-neutral and alkaline pH regions, respectively, based on the results of potentiometric titration, and optical and EPR spectra. However, from the present ^{31}P- and ^{13}C-NMR studies and previous results,

we were able to determine the detailed structures for the ATP-vanadyl complexes. The most important findings are that the coordination sites to vanadyl ion in the triphosphate group of ATP can be determined from the ^{31}P-NMR spectrum and that the ^{13}C-NMR spectrum suggests the contribution of a coordinating nitrogen atom in ATP. Thus by mere comparison of the results of the optical and EPR spectra and various parameters erroneous conclusions may be deduced about coordination sites of ligands, but ^{31}P- and ^{13}C-NMR spectrometries are very effective method for determining coordination sites in compounds containing organic phosphate.

The resent results on the three characteristic types of ATP-vanadyl complex provide useful information not only on the states of existence of endogenous vanadium ion, which may be trapped in ATP molecules, but also on the mechanism of enzymatic or non-enzymatic hydrolysis of ATP in the presence of vanadyl ion (8).

Acknowledgment

The authors thank Mr. K. Kida of this faculty for skillful technical assistance in measuring NMR spectra.

References

1. Josephson,L. and Cantley,L.C.Jr. (1977) Biochemistry 16, 4572-4578.
2. Macara,L.G., Kustin,K. and Cantley,L.C.Jr. (1980) Biochim.Biophys.Acta 629, 95-106.
3. Sakurai,H., Shimomura,S., Fukuzawa,K. and Ishizu,K. (1980) Biochem. Biophys.Res.Commun. 96, 293-298.
4. Post,R.L., Hunt,D.P., Walderhaug,M.O., Perkins,R.C., Park,J.H. and Beth, A.H. (1979) in : Na,K-ATPase Structure and Kinetics. (eds. Skou,J.C. and Nørby,J.G.) Academic Press, London, 389-410.
5. Sakurai,H., Goda,T., Shimomura,S. and Yoshimura,T. (1982) Biochem.Biophys. Res.Commun. 104, 1421-1426.
6. Cohn,M. and Hughes,T.R.Jr. (1960) J.Biol.Chem. 235, 3250-3253.
7. Dorman,D.E. and Roberts,J.D. (1970) Proc.Natl.Acad.Sci. 65, 19-26.
8. Imamura,T., Hinton,D.M., Belford,R.L., Gumport,R.I. and Haight,G.P.Jr. (1979) J.Inorg.Biochem. 11, 241-259.