Vol. 201, No. 1, 1994 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 30, 1994 Pages 155-159

THE CLEAVAGE OF PHOSPHOENOLPYRUVATE BY VANADATE

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Received March 23, 1994

Vanadate rapidly promotes the cleavage of phosphoenolpyruvate with phosphate liberation. This was not observed when ATP, glucose-6-phosphate and acetyl phosphate were incubated with vanadate. ^{51}V NMR spectra shows that phosphoenolpyruvate and acetyl phosphate broadened and shifted upfield the monomeric vanadate signal at -561 ppm,indicative of vanadate/phosphate interactions. Comparatively, smaller changes were detected when glucose-6-phosphate was added to the vanadate solution. The shift behavior was not observed in the presence of ATP, ADP or pyruvate.

Vanadate is known to inhibit different enzymes involved in energy transduction (1). These include the E1-E2 transport ATPases and the myosin ATPase (2,3). For the membrane bound transport ATPases, the inhibition is promoted by the binding of vanadate to an aspartic residue located in the catalytic site of the enzyme. In the case of myosin, the inhibition is due to the formation of a complex between vanadate and ADP.

In aqueous solutions vanadate can form oligomers (4), it can condense with phosphate (5), and forms complexes with different organic molecules of biological interest (6-9).

Phosphoenolpyruvate is frequently used in studies with ATPases when regeneration of ATP is needed (10). In this system, the enzyme pyruvate kinase transfers the phosphoryl (metaphosphate) of phosphoenolpyruvate to ADP leading to the formation of ATP and pyruvate. During the course of experiments using transport ATPase, we observed that vanadate promotes the cleavage of phosphoenolpyruvate. This observation was further explored and the results obtained are described in this report.

MATERIALS AND METHODS

Sodium orthovanadate, phosphoenolpyruvate and acetyl phosphate were purchased from Sigma. All other reagents were of grade for biochemical analysis.

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0006-291X/94 $5.00

Monovanadate stock solution (0.1 M total vanadium) was prepared from sodium orthovanadate as previously described (11). The pH was adjusted to 7.0 with HCl 5 N and the solution kept in ice before use. During the pH adjustment, the monovanadate solution was heated until the yellow/orange color indicative of decavanadate species disappeared.

The cleavage of phosphoenolpyruvate was measured in a medium containing 50 mM Mops-Tris pH 6.0, 50 mM KCl, and 10 mM $MgCl_2$. Monovanadate concentrations up to 10 mM were added to the medium before the addition of phosphoenolpyruvate. The reaction was carried out at either 25 °C or 35 °C.

Inorganic phosphate was measured by the method of Fiske and Subarrow (12). Acetyl phosphate was measured by the Lipmann method (13). All the experiments were repeated at least 3 times.

^{51}V NMR spectroscopy measurements were performed in a Varian XL-200 spectrometer, installed at the University of Coimbra, using a 90° pulse Fourier transform technique and a repetition time equal to 0.098 s. All spectra were obtained in the unlocked mode at 52.6 MHz, after a pre-lock operation with a vanadate sample containing D_2O. Chemical shifts values are given with reference to $VOCl_3$ (0 ppm). In practice, all shifts were referred to tetrameric resonance (-578 ppm). A line broadening of 20 Hz was applied. Spectra of vanadate solutions were obtained immediately after vanadate dilutions in a medium containing 50 mM KCl, 10 mM $MgCl_2$, 50 mM Mops-Tris pH 6.0. For 1 mM vanadate solutions, the time to run the spectra was never longer than 16 minutes. For quantitative measurements, all the spectral parameters were kept constant. Halfline width values were determined using the spectrometer software.

RESULTS

Vanadate promotes the cleavage of phosphoenolpyruvate with liberation of inorganic phosphate into the medium. The amount of Pi released depends on the vanadate and the phosphoenolpyruvate concentrations used (Fig. 1). In the conditions of Fig. 1, the

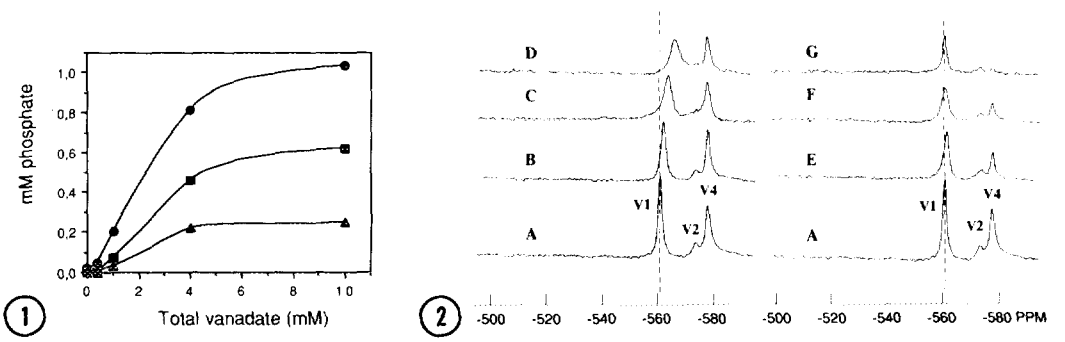

Figure 1. Phosphate liberation from phosphoenolpyruvate induced by vanadate. Effect of vanadate concentrations ranging 0 to 10 mM total vanadium on the phosphate production from 0.4 (▲), 1.0 (■) and 4.0 (●) mM phosphoenolpyruvate concentrations. Values were obtained after 30 minutes at 35 °C in a medium containing 50 mM Mops-Tris, pH 6.0, 50 mM KCl, and 10 mM $MgCl_2$.

Figure 2. 52.6 MHz ^{51}V NMR spectra of nominal 1 mM monovanadate solution in the absence (A) and in the presence of 10 mM glucose-6-phosphate (B), 10 mM phosphoenolpyruvate (C), 10 mM acetyl phosphate (D), 10 mM ATP (E), 10 mM ADP (F) and 10 mM pyruvate (G) at 22 °C in the following medium: 50 mM Mops-Tris, pH 6.0, 50 mM KCl, and 10 mM $MgCl_2$. Spectra obtained 16 minutes after mixing the reagents.

amount of Pi produced was the same after 1, 5, 10 or 30 minutes of incubation, indicating that this reaction reaches equilibrium in less than 1 minute after mixing the reagents. The Pi production was not significantly affected either by changing $MgCl_2$ concentrations added from 0 to 20 mM or by ranging the pH value of the medium from 6.0 to 8.0. Phosphate liberation was not observed when glucose-6-phosphate, ATP or acetyl phosphate were incubated with vanadate.

The interaction between vanadate and phosphoenolpyruvate was also studied by NMR spectroscopy. ^{51}V NMR spectra of 1 mM monovanadate solutions revealed the presence of monomeric (V1) at -561 ppm; dimeric (V2) at -574 ppm and tetrameric species (V4) at -578 ppm (Fig. 2). These three bands were already described in previous reports (14). We now show that when phosphoenolpyruvate (Fig. 2C) or acetyl phosphate (Fig. 2D) were added to the vanadate solution, the monomeric vanadate resonances shift upfield by 3 ppm and 5 ppm, respectively, and suffer a large broadening, whereas small effects were observed upon addition of glucose-6-phosphate (Fig. 2B). This behavior has been observed in studies of vanadate/phosphate interactions (5) and it is a consequence of a fast equilibrium between the vanadate and the acetyl phosphate/vanadate or the phosphoenolpyruvate/vanadate complexes resulting that the chemical shift of the observed signal is a weighted average of the signals from free vanadate and the vanadate complexes. Broadening of monomeric signal with a slight upfield shift was decteted with simultaneous decrease in intensity of all the vanadate resonances upon the addition of 10 mM ADP (Fig. 2F) or 10 mM ATP (Fig. 2E). The strong interaction with phosphoenolpyruvate suggests that the production of phosphate observed in Fig. 1 may be related to the formation of a complex between vanadate and the phosphate group of phosphoenolpyruvate.The effects described above can be better appreciated in Table 1. Comparatively to phosphoenolpyruvate, acetyl phosphate shifted upfield and broadened the monomeric resonance in a larger extension (Fig. 2D), but also decreases the intensities of the monomeric as well the tetrameric vanadate resonances probably due to a different type of interaction with vanadate. These observations have been confirmed in another set of experiments, when increasing amounts of phosphoenolpyruvate and acetyl phosphate were added to the vanadate solution. Changing the mode of coordination of vanadate, for instance to an octaedral structure, it is expected to slow down the equilibrium between the product and the free vanadate reflecting the effects observed in spectra.

The ^{51}V NMR spectra of the vanadate solution in the presence of pyruvate shows that the monomeric resonance decreases without broadening whereas dimeric and tetrameric species almost disappear (Fig. 2G). In fact, several products of vanadate and pyruvate can be formed (not shown). In the ^{51}V NMR spectra, at least three main vanadate bond resonances were observed with chemical shifts assigned to octahedral and trigonal bipyramidal vanadate structure as observed for oxalate, succinate and lactate (15,16).

The condensation of vanadate with ADP results in a ATP analogue, ascribed to be involved in the inhibition of myosin ATPase activity (3). The condensation between

Table 1. Chemical shifts (δ), and line widths at half weight ($\Delta\nu_{1/2}$) of monomeric (V1) and tetrameric (V4) species of vanadate (1 mM total vanadium) in the absence and in the presence of severall compounds (10 mM) as indicated. Values in parentheses correspond to the increasing line width factor (f) at half peak weight. Abreviations: G6P, glucose-6-phosphate; PEP, phosphoenolpyruvate; ACP, acetyl phosphate; PYR, pyruvate.

	control	G6P	PEP	ACP	ATP	ADP	PYR
V1							
δ/ppm	-561	-562	-564	-566	-561	-561	-561
$\Delta\nu_{1/2}$/Hz	93	111	203	201	117	170	77
f	---	(1.2)	(2.2)	(2.2)	(1.3)	(1.8)	(0.8)
V4							
d/ppm	-578	-578	-578	-578	-578	-578	-578
$\Delta\nu_{1/2}$/Hz	114	100	126	132	89	104	---
f	---	(0.9)	(1.1)	(1.2)	(0.8)	(0.9)	---

vanadate and acetyl phosphate may be related to the inhibition of sarcoplasmic reticulum ATPase by vanadate. In fact, it has been recently reported that monomeric vanadate only binds to the sarcoplasmic reticulum ATPase after its phosphorylation (11). The catalytic site of this ATPase contains an aspartyl residue that is phosphorylated by ATP during the catalytic cycle forming an acyl phosphate residue (17).

DISCUSSION

Although acetyl phosphate interacts in a similar way as phosphoenolpyruvate with vanadate, no production of phosphate was observed as it occurs upon phosphoenolpyruvate interaction. Possibly, different vanadate structures may occur in the complexes formed between vanadate and phosphoenolpyruvate or acetyl phosphate. It has been described that vanadate complexes with ADP and ATP (18). The formation of vanadate complexes with ADP and ATP observed for concentrations above 25 mM gives products with ^{51}V chemical shifts at -530 ppm, assigned to vanadate trigonal bipyramidal structure, resulting from the binding of vanadate to C´2 and C´3 hydroxyl groups of the ribose ring of ADP and ATP. Weaker tetraedral species resulting from the interaction between vanadate and the terminal phosphate group also occurs, thus forming vanadate anhydrides. Pyruvate complexes with vanadate and gives products that can be observed in the ^{51}V NMR spectra, with chemical shifts assigned to vanadate octaedral and trigonal bipyramidal structures. Furthermore, the signal of tetrameric vanadate species almost vanishes in the presence of pyruvate. A possible mechanism of phosphoenolpyruvate cleavage includes the interaction of vanadate with the carboxyl group of phosphoenolpyruvate forming a complex with an octaedral or trigonal bipyramidal vanadate structure. However, our results point out to an interaction of vanadate with the

phosphate leading to the formation of a pyrophosphate analogue from phosphoenolpyruvate. This would be followed by the cleavage of the phosphovanadate complex leading to the production of phosphate. Another possible mechanism would be similar to phosphorolysis, except that the cleavage would be promoted by vanadate, instead of phosphate (19). In this case, the formation of pyruvate does not occur as a product since shift of monomeric species is observed and the tetrameric signal is not abolished. Therefore, it is suggested that, upon interaction of phosphoenolpyruvate with vanadate, vanadoenolpyruvate complex and phosphate are formed.

Acknowledgments: M. Aureliano is recipient of a JNICT grant (BD/1007/90-RM). J. Leta is recipient of a fellowship from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

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