Zusammenfassung. Zwischen der Tetramer- und der Dimer-Form der Erythrocyten-Katalase besteht ein Gleichgewicht. Dieses lässt sich in vitro durch Variation der Harnstoffkonzentration beliebig verschieben. Das dabei entstehende Dimer zeigt Peroxidase-, nicht aber Katalase-Aktivität. Bei der Reassoziation, deren Geschwindigkeit sich durch andere Proteine beeinflussen lässt, entsteht ein Produkt, das vom nativen Enzym nicht unterscheidbar ist.

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Effect of Nucleoside Di- and Triphosphates and $MgCl_2$ on the Activity of 5′-Nucleotidase from Bull Seminal Plasma[1]

In recent years considerable attention has been given to the control of the activity of 5′-nucleotidase (EC 3. 1. 3. 5.). The enzyme, partially purified from mammalian, avian and bacterial sources, has been found to be inhibited by nucleoside di- and triphosphates[2-9]. Such inhibition may constitute a control of the 5′-nucleotidase activity to prevent unregulated catabolism of 5′-mononucleotides required for nucleic acid and coenzyme synthesis[10]. Furthermore, a role of Mg^{++} ions in overcoming this inhibition has been suggested by Sullivan and Alpers[6] and by Magni et al.[11] for the rat heart muscle and guinea-pig skeletal muscle 5′-nucleotidase respectively.

The occurrence of a 5′-nucleotidase in bull seminal plasma has long been known[12], and the fundamental kinetic parameters have been reported by Heppel and Hilmoe[13] and Levin and Bodansky[14]. However, the regulation of this enzymic activity had never been reported.

The present comunication describes the inhibition exerted by nucleoside di- and triphosphates on bull seminal plasma 5′-nucleotidase and the role of magnesium ions in overcoming the inhibition imposed by ATP and ADP. The experiments have been conducted at pH 7.2, where the activity does not show any magnesium dependence.

Methods. The 5′-nucleotidase reaction was carried out spectrophotometrically at 25 °C as previously described by Ipata[15], with 5′-AMP as substrate, in the presence of adenosine deaminase excess. The standard reaction mixture, in a final volume of 2.0 ml, contained 75 m*M* *Tris*-HCl pH 7.2, 0.1 unit of commercial adenosine deaminase (Boehringer) and 40 μM 5′-AMP. Linearity of the reaction rate was maintained up to at least 50 µg of protein per reaction mixture. One enzyme unit equals an activity equivalent to a decrease of 0.001 absorbance unit per min. The protein concentration was determined by the biuret method of Gornall et al.[16].

The 5′-nucleotidase was partially purified from 11 ml batches of bull seminal plasma according to Levin and Bodansky[14] through precipitation with protamine sulfate, precipitation with ammonium sulfate between 40 and 60% saturation and heat treatment. Further purification was achieved as follows: the heat treated fraction was centrifuged in the cold to remove any precipitated material and the supernatant fluid was dialyzed overnight against 0.05 *M* *Tris*-HCl buffer pH 8.2. The dialyzed material (10 ml containing 28 mg protein/ml) was adsorbed on a DEAE cellulose (Whatman DE 32) column (1.5 × 10 cm). After washing the column with *Tris*-HCl 0.05 *M*, pH 8.2, the elution was carried out with a linear gradient *Tris*-HCl 0.05 *M*, pH 8.2 (250 ml) and 0.5 *M* NaCl in the same buffer (250 ml). The enzyme activity was eluted around a concentration of 0.2 *M* NaCl. The active fractions were pooled and brought to 80% saturation with ammonium sulfate. The precipitate, dissolved in a minimal amount of water, was gel-filtered at 4 °C through a 1.8 × 60 cm Sephadex G-100 column, equilibrated with *Tris*-HCl buffer 0.05 *M*, pH 7.2. The 5′-nucleotidase was recoverde between the 30th and the 50th ml of the eluate as a sharp

Nucleotide concentrations required for 50% inhibition of bull seminal plasma 5′-nucleotidase and K_i values of inhibitory nucleotides

Nucleotide	Concentration[a] (μM)	K_i (μM)
ITP	50	25.5
CTP	15	5.1
GTP	8.0	1.73
ATP	2.5	0.6
UTP	2.3	0.45
IDP	11	4.55
CDP	3.5	1.55
GDP	2.8	0.32
ADP	2.2	0.30
UDP	0.9	0.29

[a] The values were obtained from inhibition curves, all showing hyperbolic shapes. The final 5′-AMP concentration was 40 μM.

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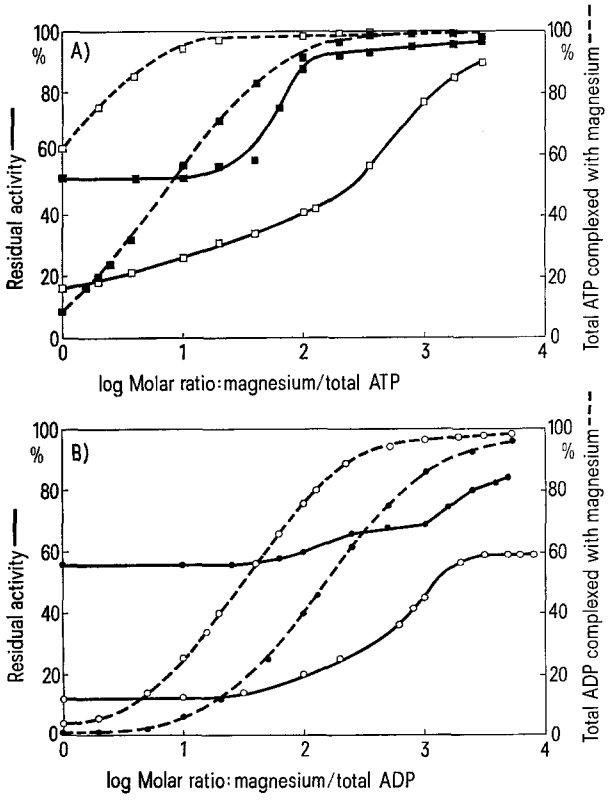
symmetrical peak. The final preparation, having 44,000 units per mg protein, represented a 44-fold purification and did not catalyze detectable splitting of inorganic phosphate from *p*-nitrophenylphosphate or 3'-AMP.

Results. Inhibition by nucleotides. The nucleotide concentrations required for 50% inhibition, at 40 μM 5'-AMP, are listed in the Table. It can be seen that the nucleoside diphosphates are more powerful inhibitors than the respective triphosphates. For both nucleoside di- and triphosphates, the inhibitory power follows the order: U > A > G > C > I. The inhibition is of the competitive type with respect to 5'-AMP. The K_i values of all inhibitory nucleotides are also reported in the Table.

Magnesium effect. The effect of increasing magnesium ions concentration on the inhibition exerted by two different levels of ATP and ADP is shown in Figures A and B, respectively. It can be seen that, in the presence of 2.5 μM ATP or 2 μM ADP giving 52% and 56% residual activity respectively, the addition of 5 mM $MgCl_2$, a 'physiological' concentration[17], reversed the inhibition to 97.5% and 82.5% residual activity. In the presence of 30 μM ATP or 10 μM ADP giving 16% and 12% residual activity respectively, the addition of 5 mM $MgCl_2$ reversed the inhibition to 47% and 35% residual activity. Considerably higher magnesium ions concentrations were required to observe significant reversal of the inhibition exerted by 30 μM ATP or 2 μM ADP. Furthermore, there was partial reversal of the inhibition exerted by 10 μM ADP, even at 100 mM $MgCl_2$, a 10,000 fold excess.



Effect of magnesium on the inhibition exerted by ATP and by ADP on 5'-nucleotidase. A) 2.5 μM ATP (-■-■-■) and 30 μM ATP (-□-□-□-). Calculated ATP-magnesium complex/total ATP × 100 at 2.5 μM ATP (-■--■--■-) and 30 μM ATP (-□--□--□-). B) 2 μM ADP (-●-●-●-) and 10 μM ADP (-○-○-○-). Calculated ADP-magnesium complex/total ADP × 100 at 2 μM ADP (-●--●--●-) and 10 μM ADP (-○--○--○-).

Conclusions. As already reported for 5'-nucleotidases from various sources[2,6,9-11] 5'-nucleotidase from bull semen is strongly inhibited by nucleoside di- and triphosphates.

The results, here obtained, show that magnesium ions are able, to some extent, to relieve such inhibition; this fact was also observed for other 5'-nucleotidases from rat heart[6], *B. subtilis*[9] and guinea-pig skeletal muscle[11]. SULLIVAN and ALPERS[6] have pointed out that the mechanism by which the inhibition exerted by ADP and ATP is relieved by magnesium, is complex in nature. The assumption made by the present authors, that the ADP- or ATP-magnesium complex has less affinity for the enzyme than the free nucleotides, might hold also for bull seminal 5'-nucleotidase. The complex magnesium ADP or ATP is surely inhibitory in turn. This statement is made by considering that: a) the inhibition is never completely relieved: particularly in the presence of 10 μM ADP the residual activity is not higher than 60%, even when 97% of the nucleoside diphosphate exists as a magnesium complex. b) The inhibition imposed by ATP or ADP in the absence of Mg^{++} ions was markedly lower than that imposed in the presence of the cation, by corresponding *free* ATP and ADP concentration, calculated from the dissociation constants[18,19] of Mg^{++} nucleotide complexes (unpublished observation). It must be emphasized that in contrast with other 5'-nucleotidases[7,16], the bull seminal plasma enzyme does not require added magnesium for activity at pH 7.2 and that at this pH value the inhibition imposed by ADP and ATP is relieved to a considerable extent by 'physiological' concentration of magnesium ions. These considerations lead us to suggest that 5'-nucleotidase, occurring in bull seminal plasma and mainly produced by seminal vesicles[12], might be modulated in vivo by the levels of magnesium ions rather that by those of free nucleoside di- and triphosphates.

Our observations are in agreement with the reported effect of free and magnesium-complexed ATP and ADP on enzymes for which these nucleotides are substrates[20] or inhibitors[21], and suggest that the cellular concentration of magnesium might contribute to the control of metabolic fluxes depending on enzyme activities sensitive to APD and ADP concentrations.

Riassunto. La 5'-nucleotidasi del plasma seminale bovino é inibita competitivamente da nucleosidi di- e trifosfato. L'inibizione osservata in presenza di ADP o ATP é rimossa da ioni magnesio a concentrazioni fisiologiche. Questo risultato suggerisce che l'attività della 5'-nucleotidasi secreta dalle vescichette seminali sia, al pari di altre presenti in vari organi dei mammiferi, modulata da cationi bivalenti.

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