Inhibition of cAMP-Phosphodiesterase by Molybdate

Beatus Ofenloch-Hähnle and Karl Eisele

Physiologisch-Chemisches Institut der Universität Tübingen, Hoppe-Seyler-Straße 4, D-7400 Tübingen, Bundesrepublik Deutschland

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Inhibition of 3′:5′-cyclic-AMP-5′-nucleotidohydrolase (EC 3.1.4.17) type II (cAMP-phosphodiesterase) by sodium molybdate was studied: While determination of inorganic phosphate and 5′-ribonucleotide phosphohydrolase (5′-nucleotidase) (EC 3.1.3.5) activity was not disturbed by sodium molybdate in concentrations up to 10 mM, cAMP-phosphodiesterase was inhibited by millimolar concentrations of molybdate. The half maximal effect was observed at about 2 mM sodium molybdate (0.75 mM cAMP in the assay).

Introduction

Molybdate is known as a potent inhibitor of several phosphoprotein phosphatases [1–3]. On the other hand the activation of adenylate cyclase from several tissues [4, 5] and cGMP-phosphodiesterase of retinal rod outer segment by molybdate has been described [6]. Beyond that molybdate is an important *in vitro*-effector of steroid receptors, but this action is in most cases not due to enzyme inhibition or activation but to a direct binding to the receptor [7, 8]. Investigations of our group concerning the influence of cAMP on androgen receptor showed that molybdate was necessary for cAMP-action in our system (cytosol of murine skeletal muscle) [9]. We questioned if these effects of molybdate were due to an interaction with cAMP metabolizing enzymes or the receptor itself. Therefore we started to examine, if molybdate inhibits 3′:5′-cyclic-AMP-nucleotidohydrolase.

Materials and Methods

Chemicals and biochemicals

3′:5′-cyclic-AMP-5′-nucleotidohydrolase (EC 3.1.4.17) type II (cAMP-phosphodiesterase) from bovine heart, activator independent (0.064 units/mg protein), 5′-ribonucleotide phosphohydrolase (EC 3.1.3.5) (5′-nucleotidase) from Crotalus atrox venom, 5′-AMP, p-methylaminophenol (Elon) and sodium molybdate were obtained from Sigma Chemical Co., Munich, F.R.G.

3′:5′-cAMP (free acid) was purchased from Boehringer, Mannheim, F.R.G. All other chemicals were of analytical grade and obtained from E. Merck, Darmstadt, F.R.G.

Tris-buffer: 40 mM Tris, 100 mM KCl, 10 mM MgCl, adjusted to pH 8.0 at 22 °C.

Determination of inorganic phosphate

Inorganic phosphate was determined according to Le Bel *et al.* [10]. Solutions used:

A : 0.25% (w/v) $CuSO_4 \times 5H_2O$, 4.6% (w/v) sodium acetate $\times 3H_2O$ in 2 M acetic acid, adjusted to pH 4.0 at 22 °C;

B : 5% (w/v) ammoniumheptamolybdate in H_2O;

C : 2% (w/v) p-methylaminophenol, 5% (w/v) sodium sulfite in H_2O.

1 ml of solution A was mixed to 0.25 ml solution B. 0.5 ml sample or phosphate standard was added and vortexed. Finally 0.25 ml solution C was added. After 10 min extinction was determined at 865 nm.

Determination of inorganic phosphate in samples containing sodium molybdate

0.5 ml Tris-buffer containing 5–100 nmol potassium phosphate and 0, 5, 10 or 20 mM sodium molybdate was incubated for 30 min at 37 °C. Thereafter 0.05 ml 55% trichloroacetic acid (TCA) was added and centrifuged for 5 min at $10{,}000 \times g$. Phosphate content of the supernatant was determined as described above.

Determination of 5′nucleotidase activity

The activity of 5′-nucleotidase was determined according to [11] in presence of varying sodium molybdate concentrations (0, 5 and 10 mM). The test mixture consisted of 1 mM 5′-AMP in 40 mM Tris-buffer (pH 8.0) containing 100 mM KCl, 10 mM MgCl, 10 mU 5′-nucleotidase/ml and the molybdate concentrations mentioned above. The reaction was carried out at 37 °C and started by addition of the enzyme. Immediately after start of the reaction (0 min) and after 5, 10 and 20 min an aliquot (0.5 ml) of the test mixture was taken and the reaction stopped by addition of 0.05 ml 55% TCA. After centrifugation for 5 min at $10{,}000 \times g$ the phosphate content in an aliquot (0.5 ml) of the supernatant was determined.

Reprint requests to Prof. Dr. Karl Eisele.

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The rate-curves were linear and their slope was taken as the velocity of the reaction.

Determination of cAMP-phosphodiesterase activity

The activity of cAMP-phosphodiesterase was determined by converting its reaction product 5′-AMP into adenosine and inorganic phosphate and then measuring the inorganic phosphate release [12]. The test mixture consisted of 0.75 mM cAMP in Tris-buffer containing 2.5 mU cAMP-phosphodiesterase/ml, 175 mU 5′-nucleotidase/ml and varying molybdate concentrations (0, 0.5, 2, 5, 10 mM). The test was carried out at 37 °C and started by addition of the cAMP-phosphodiesterase. Immediately after start of the reaction (0 min) and after 10, 20, 30, 40 and 60 min an aliquot (0.5 ml) of the test mixture was taken, the reaction stopped by addition of 0.05 ml 55% TCA and the samples centrifuged for 5 min at $10{,}000 \times g$. 0.5 ml of the supernatant was used for determination of inorganic phosphate. The rate curves were linear and their slope was taken as activity of the cAMP-phosphodiesterase.

Results and Discussion

Before performing the tests concerning the inhibition of cAMP-phosphodiesterase it was necessary to find out, whether the determination of inorganic phosphate was disturbed by the sodium molybdate added to the test. Fig. 1 shows that molybdate concentrations up to 10 mM did not disturbe the determination of inorganic phosphate, while 20 mM molybdate affected the test significantly. Therefore we used in our inhibition experiments concentrations of molybdate only up to 10 mM. Since 5′-nucleotidase is needed as a helper enzyme to determine phosphodiesterase activity, we examined, if molybdate inhibits the 5′-nucleotidase. In our experiments we found no significant inhibition of this enzyme by molybdate concentrations up to 10 mM. cAMP-phosphodiesterase, however, was inhibited by molybdate, as shown in Fig. 2. The half maximal effect was observed at about 2 mM molybdate. Therefore

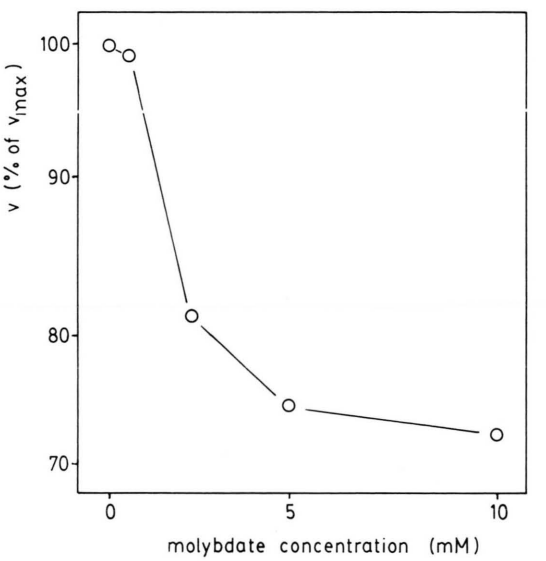

Fig. 2. Inhibition of cAMP-phosphodiesterase by molybdate. The activity of cAMP-phosphodiesterase was determined in presence of varying molybdate concentrations in the test as described in Materials and Methods.

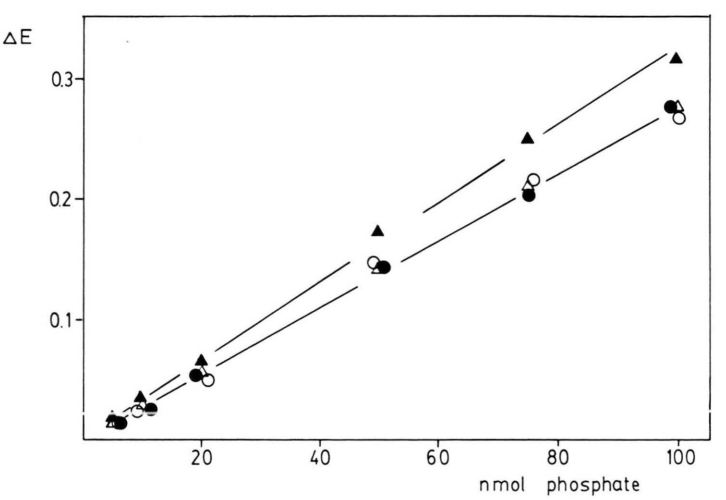

Fig. 1. Determination of inorganic phosphate in samples containing sodium molybdate. 0.5 ml Tris-buffer containing 5–100 nmol potassium phosphate and 0 (○), 5 (●), 10 (△), 20 (▲) mM sodium molybdate was incubated for 30 min at 37 °C. Thereafter 0.05 ml 55% TCA was added and centrifuged for 5 min at $10{,}000 \times g$. Inorganic phosphate was then determined as described in Materials and Methods.

molybdate inhibits cAMP-phosphodiesterases less effectively than phosphoprotein phosphatases, which are inhibited by μmolar concentrations of molybdate [1, 2]. Further investigation has to show, what are the reasons for the contrary effects of molybdate (inhibiting in case of phosphatases and cAMP-phosphodiesterase and activating in case of adenylate cyclase and cGMP-phosphodiesterase) on enzymes involved in phosphoryl-metabolism. The inhibition of cAMP-phosphodiesterase by molybdate described in this paper has to be especially considered in investigations concerning steroid hormone receptors, where molybdate is often used as effector in concentrations of about 10 mM.

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