INHIBITION OF THE PROTEIN KINASE BY ADENINE COMPOUNDS: COMPETITIVE INHIBITION WITH RESPECT TO ATP

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SUMMARY

Rat liver adenosine 3',5'-monophosphate(cAMP)-dependent protein kinase was inhibited by $5x10^{-4}M$ of adenine, adenosine, ADP and AMP, when assayed in the presence of $2x10^{-5}M$ ATP. High concentrations of cAMP itself were inhibitory. The adenine compounds also inhibited the cAMP-independent form of the protein kinase. The inhibition of both cAMP-dependent and -independent forms of protein kinase was abolished by higher concentrations($5x10^{-4}M$) of ATP. It was concluded from the kinetic studies that the adenine compounds inhibited the protein kinase competitively with respect to ATP and they had no inhibitory effect on the activation step by cAMP.

INTRODUCTION

Ample evidence supports the theory that cAMP mediates the action of many hormones as an intracellular second messenger(1). It has also been proposed that all of the wide varieties of effects elicited by cAMP are mediated through stimulation of cAMP-dependent protein kinase(2 -5).

Yamamura et al.(6) and others(7, 8) have demonstrated that cAMP-dependent protein kinase consists of two proteins, the catalytic and regulatory proteins, which are bound together when the enzyme is inactive and cAMP activates the inert kinase by binding itself to the regulatory protein of the enzyme complex and by thus

dissociating the catalytic protein from the regulatory protein. The catalytic protein alone is fully active in the absence of cAMP.

Miyamoto et al.(9) reported that cAMP-dependent protein kinase from bovine brain was inhibited by adenine, adenosine, 2'-deoxyadenosine, AMP and ADP, and the activity was partially recovered by increasing the concentration of either cAMP or ATP. cAMP-dependent protein kinase from other bovine tissues was also inhibited by adenosine and AMP(10).

A question arises as to whether these adenine compounds inhibit the activation step by cAMP or the phosphorylation step by ATP or both. The experiments described in this paper demonstrate that the adenine compounds inhibit both cAMP-dependent and -independent forms of protein kinase from rat liver and the inhibition is competitive with respect to ATP.

MATERIALS AND METHODS

The 0 -50% ammonium sulfate fraction was prepared from livers of six male Sprague Dawley rats(230-340g) and dialyzed according to the method of Kumon et al.(11), and applied on a DEAE-cellulose column(2.6 x 45cm). After washing the column with 400 ml of the buffer consisting of 10 mM Tris-HCl, pH 7.5, 10% glycerol and 6 mM 2-mercaptoethanol(TMG buffer), the cAMP-dependent and -independent protein kinases were eluted separately by gradient elution (mixing chamber, 500 ml of TMG buffer, reservoir, 1500 ml of TMG buffer containing 0.4 M NaCl) at the flow rate of 1.7 ml/min. Protein kinase activity of each fraction(5 ml) was measured in the presence and absence of 2 x 10^{-6}M cAMP. Two active peaks were obtained in the presence of cAMP. The first peak(fraction No.71) was as active in the absence of cAMP as in its presence, and the second peak(fraction No.131) was much less active without cAMP. Fractions No.71-72 and fractions No.130-132 were separately pooled

and used as the cAMP-independent and -dependent protein kinases respectively.

Protein kinase activity was assayed by measuring the phosphorylation of histone according to the methods by Walsh et al.(2) and Kuo and Greengard(12) with partial modification. The incubation mixture(0.1 ml) contained; potassium phosphate buffer, pH 6.5, 10^{-2}M; theophylline, 5 x 10^{-3}M; NaF, 10^{-2}M; histone(type 2, from calf thymus), 1 mg/ml; γ-^{32}P-ATP(100 cpm/pmole), 2 x 10^{-5}M; magnesium acetate, 10^{-2}M; cAMP, 2 x 10^{-6}M when added; and an enzyme solution. After preincubation for 2 min at 30°C without γ-^{32}P-ATP and magnesium acetate, the reaction was started by adding these two components and the incubation was continued for 5 min at 30°C. The reaction was terminated by addition of 1.5 ml of 6.6% trichloroacetic acid. Immediately before the addition of trichloroacetic acid, 0.2 ml of 6.25 mg/ml bovine serum albumin was added. The mixture was allowed to stand at 0°C for 5 min, centrifuged, and the supernatant was diacarded. The precipitate was dissolved in 0.1 ml of 1 N NaOH, and the protein was reprecipitated with 1.5 ml of 6.6% trichloroacetic acid, centrifuged and the supernatant discarded. This procedure was repeated two more times. The precipitate was dissolved in 0.2 ml of 23 M formic acid and the radioactivity was counted in a Horiba liquid scintillation spectrometer. The protein kinase activity is expressed as pmoles of ^{32}P transfered from γ-^{32}P-ATP to histone in the above assay system.

γ-^{32}P-ATP was purchased from the Radiochemical Centre, Amersham, and histone(type 2, from calf thymus) from Sigma Chemical Co.

RESULTS AND DISCUSSION

Inhibition of the cAMP-dependent protein kinase by four adenine compounds(5 x 10^{-4}M) in the presence of 2 x 10^{-5}M ATP was

examined at various concentrations of cAMP (Fig.1). The order of their inhibitory potencies(ADP > adenosine > adenine > AMP) was the same as the result by Miyamoto et al.(9). This inhibition was partially, but not completely, overcome by increasing the cAMP concentration up to 2 x 10^{-6}M. When the cAMP concentration exceeded this concentration, cAMP itself became inhibitory. Miyamoto et al. also reported that high concentrations of cAMP inhibited the cAMP-dependent protein kinase(9).

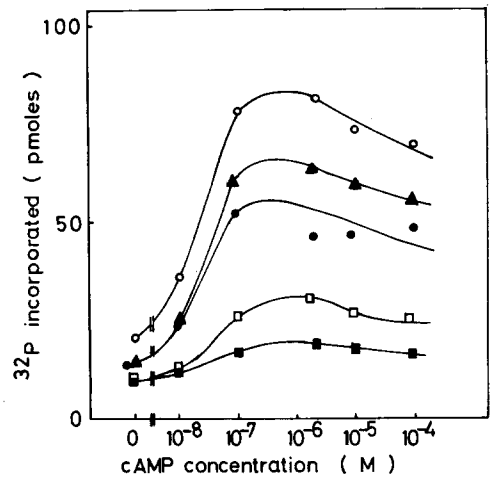

Fig. 1. Inhibition of the cAMP-dependent protein kinase by adenine compounds. Incubation conditions were as described in the text, except for the addition of adenine compounds(5 x 10^{-4}M) and the variation in cAMP concentration as indicated. ○ , control; ▲ , AMP; ● , adenine; □ ,adenosine and ■ ,ADP.

Effects of the adenine compounds on the cAMP-independent form of the protein kinase were examined at various concentrations of ATP. Fig.2 shows that the adenine compounds, including cAMP, inhibit the cAMP-independent enzyme and this inhibition is reversed in a competitive manner by raising the ATP concentration. Ki values of ADP, adenosine, cAMP, adenine and AMP were estimated from the Lineweaver-Burk plots as 1.1 x 10^{-4}, 1.4 x 10^{-4}, 3.3 x 10^{-4}, 5.0 x 10^{-4} and 1.5 x 10^{-3}M respectively, while Km of ATP for the kinase reaction was calculated to be 2.5 x 10^{-5}M.

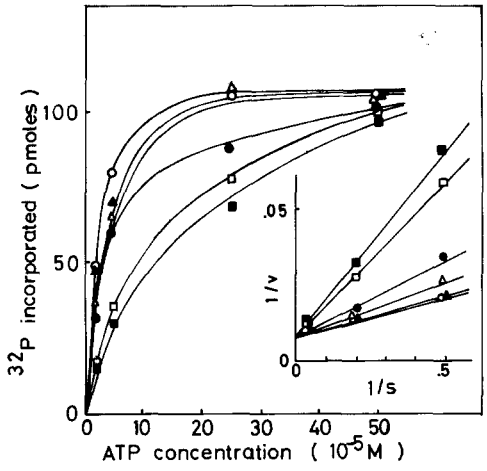

Fig. 2. Effect of ATP concentration on the cAMP-independent protein kinase in presence of the adenine compounds including cAMP. Incubation conditions were as described in the text, except for the addition of adenine compounds(5 x 10^{-4}M) and the variation in ATP concentration as indicated. ○ , control; ▲ , AMP; △ , cAMP; ● , adenine; □ , adenosine and ■ , ADP.

Fig.3 shows that when the ATP concentration was sufficiently high the adenine compounds did not influence the activation step by cAMP. The slight inhibition of a constant degree by ADP(5 x 10^{-4} M) at all concentrations of cAMP would be expected even at this concentration(5 x 10^{-4}M) of ATP because of the low Ki value of ADP for the kinase reaction.

From the above results it could be concluded that the adenine compounds do not inhibit the activation of the protein kinase by cAMP but inhibit the kinase reaction competitively with respect to ATP. The inhibitions of the protein kinase by adenine compounds observed by Miyamoto et al.(9) and Kuo et al.(10) are very likely to be an inhibition of this type, because these workers used low concentrations(5 x 10^{-6} and 10^{-6}M respectively) of ATP for the assay. Wastila et al. recently reported that 2.17 x 10^{-5}M ADP showed no inhibitory effect on cAMP-dependent skeletal muscle pro-

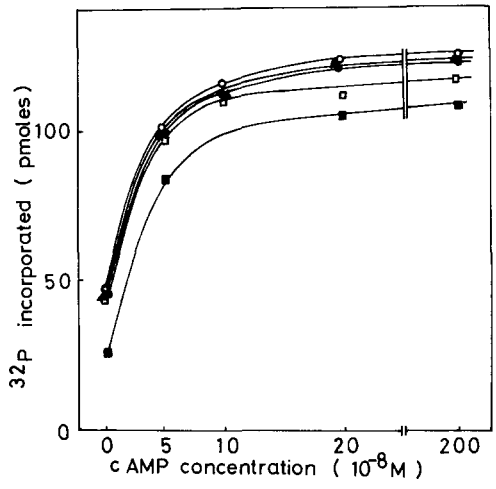

Fig. 3. Effect of cAMP concentration on the cAMP-dependent protein kinase in presence of the adenine compounds and a high concentration(5 x 10^{-4}M) of ATP. Incubation conditions were as described in the text, except for the addition of adenine compounds (5 x 10^{-4}M), ATP(5 x 10^{-4}M) and the variation in cAMP concentration as indicated. ○ , control; ▲ , AMP; ● , adenine; □ , adenosine and ■ , ADP.

tein kinase when they used a much higher concentration of ATP(approximately 1 x 10^{-3}M) (13).

Moriwaki and Foa reported that adenine compounds including ADP and adenosine inhibited the rat liver adenyl cyclase(14), and Gulyassy demonstrated that cyclic 3',5'-nucleotide phosphodiesterase of toad bladder epithelial cells was also inhibited by these adenine compounds(15). Thus all the enzyme systems controlling the intracellular level of cAMP are inhibited in vitro by the common adenine compounds. It could be speculated that the level of cAMP, hence the hormone actions, might be intricately regulated by the intracellular levels of the adenine compounds, especially by ADP in view of its low Ki value and relatively high intracellular levels, through their effects on the above three enzyme systems.

Adenosine has been implicated as an endogenous regulator of the colonary blood flow(16). Afonso and O'Brien reported that

the vasodilating effect of adenosine on the dog heart was counteracted by aminophylline(a phosphodiesterase inhibitor)(17). It seems possible that adenosine exerts its cardiac effect by inhibiting adenyl cyclase or protein kinase of the heart tissue and this inhibition is somewhat compensated by increased levels of cAMP.

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