EFFECT OF ADENINE NUCLEOTIDES ON THE ACTIVATING FUNCTION OF STREPTOKINASE

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Among factors regulating the reactions of hemostasis an important role is played by adenine nucleotides. ADP, for example, is known to be an inducer of platelet aggregation, whereas ATP and 3',5'-AMP (cAMP) inhibit this process [11]. The possibility that adeninine nucleotides may also control reactions of fibrinolysis has virtually not been studied, although activation of certain processes of proteolysis by ATP has been described [1].

Accordingly the writers suggested that adenine nucleotides may have a direct influence on the activating function of streptokinase (SK), a protein activator of human plasminogen, synthesized by β-hemolytic streptococci.

EXPERIMENTAL METHOD

Experiments were carried out on gel-chromatographically (on Sephadex G-100) and electrophoretically (in polyacrylamide gel with the addition of sodium dodecylsulfate [9]) homogenous samples of SK with specific activity of 100,000-150,000 IU/mg protein, isolated directly from the culture fluid of a β-hemolytic streptococcus of strain H46A, by adsorption on silica with elution by 0.1M sodium carbonate solution [10], followed by ion-exchange chromatography on DEAE-cellulose in the chloride form, in 0.05 M Tris-HCl buffer, pH 7.4 [3], with elution by 0.3 M NaCl solution, and precipitation with ethanol at pH 5.0 and NaCl in a final concentration of 10% at pH 2.0. SK activity was determined by measuring lysis of fibrin plates [5] or fibrin clots [14], and protein was determined by Lowry's method [13] or by measuring absorption at 280 nm, assuming that for SK $A_{280}^{1\%} = 9$ [12].

The activating function of SK was estimated by lysis of fibrin plates containing human plasminogen [15]. The plates were prepared on a strictly horizontal surface by mixing (per plate) 9 ml of a solution of human fibrinogen containing plasminogen (3 mg protein/ml) and 0.2 ml of a solution of thrombin (100 U/ml). Proteins were dissolved in isotonic NaCl solution and SK in 0.2 M sodium acetate–HCl buffer solution, pH 3.0, or in 0.2 M phosphate buffer, pH 7.0, or 0.1 M glycine-NaOH buffer solution, pH 9.5. The solution of SK (1500 IU) or a mixture of it with the test substances, in a volume of 30 µl, was applied to the surface of the plates or introduced into wells cut out of them. The fibrin plates were incubated on a strictly horizontal surface at 37°C for 20 h and area of the zones of lysis was measured. Variations of activity in parallel determinations did not exceed 7%. All the investigations were conducted at least four times and the results were subjected to statistical analysis [8].

The following reagents were used: ADP-Na_3 from Fluka (Switzerland), sodium pyrophosphate from Merck (West Germany), D-ribose from Loba-Chemie (Austria), DEAE-cellulose, ATP-Na_2, AMP-Na, 2',3'-AMP, cAMP, UTP-Na, GTP-Na, CTP-Na, and ribose-5-phosphate (all from Reanal, Hungary), human fibrinogen containing plasminogen, and human thrombin, and urokinase (Urokinin) were of Soviet origin, as were the other reagents, which were additionally purified.

EXPERIMENTAL RESULTS

Addition of ATP to the solution of SK at pH 7.0 inhibited SK-initiated fibrinolysis if the nucleotide was present in a concentration of 10^{-1} M (Fig. 1). ADP and AMP, in a concentration of 10^{-4}-10^{-1} M, did not affect this process. The action of ATP thus revealed

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TABLE 1. Effect of Addition of Nucleotides and Their Components in a Final Concentration of 10^{-1} M to a Solution of SK on Its Induced Fibrinolysis (M ± m, n = 6)

Experimental conditions	pH	Area of zones of lysis, mm²	% of control	Experimental conditions	pH	Area of zones of lysis, mm²	% of control
SK (control)	7,0	411±14	100	SK + GTP	7,0	386±25	94
	3,0	390±10	100		3,0	0*	0
	9,5	399±19	100		9,5	455±18	114
SK + ATP	7,0	206±5*	50	SK + CTP	7,0	362±19	88
	3,0	0*	0		3,0	0*	0
	9,5	263±5*	66		9,5	402±12	101
SK + ADP	7,0	370±17	90	SK + sodium pyrophosphate	7,0	382±19	93
	3,0	0*	0		3,0	362±11	93
SK + AMP	7,0	432±10	105	SK + ribose-5-phosphate	7,0	362±18	88
	3,0	0*	0		3,0	0*	0
SK + cAMP	7,0	247±20	60		9,5	200±10*	50
SK + UTP	7,0	370±15	90	SK + ribose	7,0	363±21	88
	3,0	82±4*	21				
	9,5	387±12	97		3,0	363±18	93

Legend. Asterisk indicates that changes are statistically significant ($p \leq 0.05$).

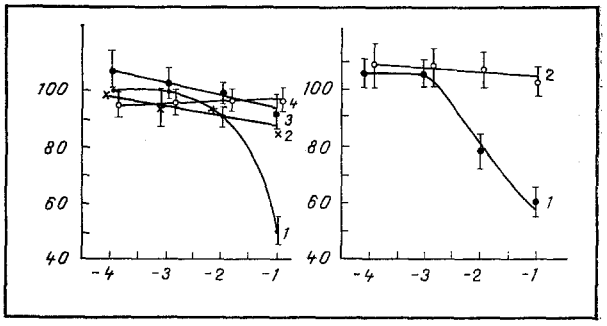

Fig. 1 Fig. 2

Fig. 1. Effect of addition of ATP (1), ADP (2), AMP (3), or GTP (4) on streptokinase-induced fibrinolysis (in % of control, taken as 100). Here and in Fig. 2: abscissa, concentration (log M).

Fig. 2. Changes in activating function of streptokinase (based on lysis of fibrin gels, in % of control, taken as 100) after addition of a solution of 3',5'-AMP (1) or 2',3'-AMP (2) to it.

could be due to the presence of an additional phosphate group and to differences in ionization of the hydroxyl groups of the phosphate residues. Nucleotide phosphates also are known to act on lipid peroxidation, an effect that is attributed to the presence of contaminating Fe ions [2]. However, the present writers showed previously [6] that Fe ions inhibit SK-induced fibrinolysis sharply only in a concentration of 10^{-1} M. Other nucleotide triphosphates (GTP, UTP, CTP), in a final concentration of 10^{-4}-10^{-1} M, at pH 7.0, likewise did not affect SK-induced fibrinolysis (Fig. 1; Table 1). Addition of inorganic pyrophosphate to the solution of SK in a concentration of 10^{-1} M had virtually no effect (Table 1). This suggests that inhibition of the activating function of SK by ATP cannot be explained either by the presence of the additional pyrophosphate bond in the nucleotide triphosphate or by differences in the degree of ionization (the secondary ionization constants of the phosphate groups of ATP, GTP, UTP, and CTP are very close [7]), or by the possible presence of iron as an impurity.

Moreover, if cAMP, but not 2',3'-AMP, were added to the solution of SK, inhibition of the activating function of SK was observed (Fig. 2). This suggests that ATP and cAMP, because of their conformational differences from the other nucleotides tested, may perhaps interact with components of the SK–plasminogen system.

The specific character of the inhibitory action of ATP on SK-induced fibrinolysis was virtually unaltered at pH 9.5, but disappeared at pH 3.0 (Table 1). In an acid medium nearly all the nucleotides used, in a final concentration of 10^{-1} M, completely suppressed

SK-induced fibrinolysis. The investigations also showed that the activating function of SK was completely suppressed at pH 3.0 by ribose-5-phosphate (but not by D-ribose) in a final concentration of 10^{-1} M (Table 1). It can be tentatively suggested that inhibition of the activating function of SK by nucleotides in an acid medium is due to the effect of the ribose-5-phosphate residue and also, perhaps, to the special state of the phosphoester bond.

It can be concluded from these results that ATP and cAMP can act directly on the activating function of SK in a neutral medium, inhibiting it. The effect described differs significantly from known examples of the effect of nucleotides on proteolysis. For instance, the proteolytic action of cathepsin D is appreciably potentiated by ATP and by other nucleotide triphosphates, and also by pyrophosphate, but is unchanged in the presence of cAMP [15]. This effect of ATP which we found was exhibited only in concentrations which are at least an order of magnitude higher than those known for animal tissues [4]. However, after injection of SK preparations into the blood stream, its concentration does not exceed 500 IU/ml, which is 2 orders of magnitude below that used in the present experiments. This suggests that the ATP level can influence SK activity in the blood stream and also, perhaps, in the cells of the producer. Moreover, if ATP and cAMP can interact with components of the SK-plasminogen system, when plasminogen is activated, transformation of some ATP or cAMP molecules may evidently take place.

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