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The postulated acetyl esterase activity of streptokinase

It has been reported that streptokinase hydrolyzes β-naphthyl acetate[1]. We have found that while impure streptokinase hydrolyzes both *o*-nitrophenyl acetate and β-naphthyl acetate, two highly purified samples of streptokinase do not .

The crude streptokinase used was Varidase, Lot No. 1089-860A, and the pure streptokinase were Lots No. 7-1212-29A and 7-1212-33A (Lederle). Each vial was labelled by the manufacturer to contain 100 000 units of streptokinase. To each vial 4 ml of distilled water were added and aliquots of these solutions were used for testing. The solutions were refrigerated and not used after 1 week. Plasminogen was prepared from Fraction III of human blood plasma by a method previously described[2]. All the other reagents were commercially obtained.

All three lots of streptokinase were shown to activate human plasminogen (preparation R 9/17G) equally when judged by the rate of hydrolysis of 0.02 M *p*-toluenesulfonyl-L-arginine methyl ester in the presence of 5000 units of streptokinase per ml at pH 8 (0.25 M Tris) and 37°, determined by a modified Hestrin method[2]. These three lots of streptokinase were also tested for their ability to hydrolyze *o*-nitrophenyl acetate and β-naphthyl acetate.

A solution of *o*-nitrophenyl acetate in alcohol was added at zero time to a tube that finally contained 0.1 M phosphate buffer (pH 6.4), varying amounts of streptokinase, 1.2%ethanol and 1.8 mM *o*-nitrophenyl acetate, all at room temperature (22–25°). This was read in a Coleman Jr. spectrophotometer at 415 mμ against a buffer blank after 0, 15 and 30 min of reaction[3]. The values found in the absence of streptokinase (spontaneous hydrolysis of the substrate) were deducted from those found in the presence of streptokinase .Under these conditions, absorbancy increased linearly with time, and these values were converted to μmoles of *o*-nitrophenol formed per ml using a conversion factor determined by measuring the absorbancies of known solutions of *o*-nitrophenol under the same conditions as used in the tests.

It can been seen in Table I that while Varidase (crude streptokinase) hydrolyzed

TABLE I

THE ACTION OF STREPTOKINASE ON *o*-NITROPHENYL ACETATE

	μmoles of o-nitrophenyl acetate hydrolyzed/30 min/ml					
Units of streptokinase/ml	*0*	*2000*	*4000*	*6000*	*8000*	*10 000*
Varidase	(0.024)	0.096	0.191	0.298	0.377	0.451
Streptokinase No. 1212-29A	(0.021)	0.004	0.007	0.003	—	—
Streptokinase No. 1212-33A	(0.024)	0.004	0.004	0.006	—	—

o-nitrophenyl acetate at a rate proportional to the concentration of Varidase, neither of the two pure preparations of streptokinase hydrolyzed this substrate.

A solution of β-naphthyl acetate in alcohol was added at zero time to a tube that finally contained 0.25 M Tris (pH 8.0), varying concentrations of streptokinase, 2.5% ethanol and $375 \cdot 10^{-6}$ M β-naphthyl acetate, all at room temperature. This was read every 2 min for 10 min in a Beckman DU spectrophotometer at 328 mμ against a blank identical to the run but lacking the β-naphthyl acetate[1]. The values

obtained in the absence of streptokinase (spontaneous hydrolysis of the substrate) were deducted from those obtained in the presence of streptokinase. The absorbancy readings were converted to μmoles of β-naphthol formed per ml by a conversion factor obtained by measuring the absorbancy of known solutions of β-naphthol under the same conditions as used in the test. The hydrolysis of β-naphthyl acetate by crude streptokinase was linear with time under the conditions used, and as is shown in Table II, the rate of hydrolysis was proportional to the concentration of Varidase in the test. However, neither sample of pure streptokinase hydrolyzed this substrate.

TABLE II

THE ACTION OF STREPTOKINASE ON β-NAPHTHYL ACETATE

	μmoles of β-naphthyl acetate hydrolyzed/10 min/ml				
Units of streptokinase/ml	*0*	*1250*	*2500*	*3750*	*5000*
Varidase	(0.033)	0.046	0.088	0.127	0.170
Streptokinase No. 1212-29A	(0.031)	—	0.002	—	—
Streptokinase No. 1212-33A	(0.031)	—	0.000	—	—

It was found that the acetyl esterase activity of crude streptokinase could be destroyed without destroying significantly the ability to activate plasminogen. When Varidase was brought to 50% ethanol (v/v) a precipitate formed. This precipitate was immediately separated from the supernatant by centrifugation at 5°, and extracted with cold distilled water. The extract no longer hydrolyzed *o*-nitrophenyl acetate, but it activated plasminogen. The supernatant had neither the ability to activate plasminogen nor to hydrolyze *o*-nitrophenyl acetate.

From these results it is concluded that Varidase contains an acetyl esterase in addition to streptokinase but the highly purified preparations of streptokinase do not contain the acetyl esterase, and streptokinase does not possess the ability to hydrolyze esters of acetic acid.

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