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NOTES

Streptolysin O: Activation by Thiols

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Various straight-chain thiols produce approximately the same maximal activation of streptolysin O.

The hemolytic and other biological activities of streptolysin O are dependent upon the presence of reducing agents of which thiols are the most effective. Herbert and Todd (2) found that cysteine, 2-mercaptopropionic acid, *o*-thiolbenzoic acid, reduced glutathione, H_2S, $Na_2S_2O_4$, and Na_2SO_3 all activated streptolysin O to the same extent. In contrast, Rahman et al. (4) recently reported substantial differences in the degree of activation produced by a series of straight-chain thiols. Cysteine, in particular, effected only 28% as much activation as 2,3-dimercaptopropanol, and glutathione effected only 12% as much. Because these results disagree with the older findings and because cysteine is commonly used as an activator in studies involving streptolysin O, we reinvestigated the efficacy of cysteine in relation to that of a number of other SH-compounds.

Streptolysin O was a relatively crude preparation obtained as described earlier (3) from supernatant fluids of cultures of the C203S strain of group A streptococcus. It was stored in the cold, under 80% saturated ammonium sulfate, and portions were diluted in nine volumes of 0.067 M sodium phosphate, *p*H 7.4, just prior to use. It was largely in the inactive or oxidized form as indicated by the fact that its hemolytic activities, without and with activation by cysteine, were in the ratio of about 1 to 10. Hemolytic activity was assayed by finding the highest dilution that liberated, in 30 min at 37 C, half the hemoglobin from a suspension of washed rabbit erythrocytes contained in a volume of 2 ml. The reaction mixture contained 0.05% bovine serum albumin in 0.077 M NaCl buffered, at *p*H 7.0, with 0.067 M sodium phosphate, as described elsewhere (1). Unless otherwise stated, for activation, an equal volume of activating solution was added to streptolysin O solution in the presence of 0.067 M sodium phosphate, *p*H 7.4, and the mixture was allowed to stand at about 22 C for 10 min.

TABLE 1. *Activity of streptolysin O[a] after reacting with various thiols*

Activator	Concn of thiol in activating mixture				
	0.0042 M	0.0069 M	0.0115 M	0.0192 M	0.0322 M
	HU/ml	*HU/ml*	*HU/ml*	*HU/ml*	*HU/ml*
Reduced glutathione	9,200	9,500	9,500	8,750	8,300
Sodium thioglycolate	4,000	5,000	7,500	9,500	11,300
2-Mercaptoethanol	7,500	8,750	10,000	10,000	14,500
Dithiothreitol	10,600	11,300	14,500	14,500	14,500
Cysteine	8,750	10,000	14,500	16,500	11,300
2,3-Dimercaptopropanol	12,000	12,500	15,000	16,000	16,500

[a] Unactivated streptolysin O contained 1450 hemolytic units (HU) per ml.

Cysteine, 2-mercaptoethanol, dithiothreitol, and 2,3-dimercaptopropanol produced approximately equal maximal activity (Table 1). Under the same conditions, sodium thioglycolate and glutathione yielded less activity, but the data of Table 2 show that these two compounds are relatively sluggish activators which, if given sufficient time, yield nearly the same activity as the others. Table 1 shows that the degree of concentration depend-

TABLE 2. *Activation of streptolysin O as a function of time*

Time	Activator (0.019 M)		
	Cysteine	Reduced glutathione	Sodium thioglycolate
min	*HU*[a]*/ml*	*HU/ml*	*HU/ml*
0	1,450		
0.5	6,300	4,800	3,500
1	7,100	4,860	
2	10,000	5,500	6,600
4	11,500	7,300	
8	13,700	7,100	
12	15,000	9,200	8,900
16	16,700	10,000	
20	16,700	11,700	12,700
30		11,100	
60		15,600	12,700

[a] Hemolytic units.

ence varies considerably among the compounds tested, and is greatest for the two monothiols of lowest molecular weight, sodium thioglycolate and 2-mercaptoethanol. The general inference that can be drawn is, however, that at appropriate time and concentration, all thiols examined produce about the same maximal activation. The reason for the discrepancies between the present results and those of Rahman et al. (4) is not known, but the absence of information as to the *p*H of the activating mixtures used in the earlier study may be relevant.

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