STABILIZATION OF STREPTOLYSIN S BY POTASSIUM IONS*

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Streptolysin S is a potent hemolytic toxin formed by strains of streptococci of a number of Lancefield groups, in growing cultures as well as in the resting state (1, 2). A variety of methods has been used for its preparation, and all of them yield an extremely labile product the instability of which has been the subject of comment by nearly all investigators who have studied the toxin. It has been observed that the hemolytic activity of fresh preparations falls off greatly within a few hours at room temperature (1). In another study (3), solutions of streptolysin S were found to lose activity quickly at all pH values tested between 3.0 and 8.0. So far as is known, the inactivation is irreversible.

The lability of streptolysin S (unlike streptolysin O) is not due to oxidation, since reducing agents fail to stabilize it (1), nor is it due to surface inactivation, for gelatin (3) and other proteins, including those of serum, also fail to protect the toxin. The experiments to be described show that potassium ions, and to a less extent, certain other cations, strikingly prevent the inactivation which occurs in the absence of these ions or in the presence of sodium ions.

Materials and Methods

Streptolysin S.—Streptolysin S was prepared by incubating washed streptococci in the presence of polynucleotide, maltose, and inorganic salts, according to the method described in a previous report (3). The streptolysin S used in this study was prepared at the Medical Research Division of Sharp and Dohme, Inc., through the courtesy of Dr. W. F. Verwey.

Inorganic Salts.—The inorganic salts were of reagent grade and were obtained from Merck and Co., Inc.

Estimation of Hemolytic Activity.—Titrations of hemolytic activity were carried out by incubating increasing dilutions of solutions to be assayed with a fixed amount of red blood cell suspension. Details of the method have been published previously (4). The unit of hemolysin is that amount of streptolysin S which lyses half the erythrocytes (human, 0.35 per cent by volume) contained in 2 cc. of phosphate-buffered saline, pH 7.0, in 30 minutes at 37°.

EXPERIMENTAL

Effect of Sodium and Potassium Ions.—

One-tenth cc. portions of streptolysin S previously dialyzed against distilled water, and containing approximately 10,000 units per cc., were mixed with 0.9 cc. portions of solutions of

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salts of appropriate concentrations. The mixtures were placed at 50°, chilled at the end of 30 minutes, and titrated for hemolytic activity. The stability of streptolysin S in the presence of various concentrations of KCl, NaCl, KH_2PO_4, and NaH_2PO_4 is shown in Table I.

It is evident that in the complete absence of salt the activity of the toxin fell from 1000 units per cc. to less than 50. When, however, either KCl or KH_2PO_4 were present in relatively high concentration, there occurred little or no loss of

TABLE I

Protection of Streptolysin S by K^+ and Lack of Protection by Na^+

Concentration of Salt	Hemolytic units per cc. after 30 min. at 50° in the presence of			
	KCl	NaCl	KH_2PO_4	NaH_2PO_4
M/1	1000	<100	1000	80
M/5	930	<50	1000	<50
M/20	900	<50	570	<50
M/100	670	<50	430	<50
M/500	270	—	400	—
M/2000	130	—	250	—
None	<50			

Control solution kept at 3°: 1000 hemolytic units per cc.
—, not tested.

TABLE II

Effect of Li^+, NH_4^+, Mg^{++}, Ca^{++}, and Ba^{++} on Stability of Streptolysin S

Concentration of salt	Hemolytic units per cc. after 30 min. at 50° in the presence of				
	LiCl	NH_4Cl	$MgCl_2$	$CaCl_2$	$BaCl_2$
M/1	<50	1100	500	<50	95
M/5	<50	600	110	<50	350
M/20	<50	175	<50	<50	400
M/100	<50	80	<50	<50	400
None	<50				

Control solution kept at 3°: 1000 hemolytic units per cc.

activity. In the same concentrations, the corresponding sodium salts failed to prevent inactivation. It is notable that as little as M/2000 potassium ion afforded partial protection.

Effect of Certain Other Cations.—In order to see whether the effect just described is specific for potassium, the chlorides of lithium, ammonium, magnesium, calcium, and barium were tested under conditions similar to those used in the previous experiment. The results are shown in Table II. Lithium and calcium ions failed to protect in all concentrations tested. Magnesium and barium ions gave partial protection while ammonium ion protected completely

in a concentration of M/1 and partially in lower concentrations. Hence, although certain cations other than potassium partially or completely prevent inactivation, it is clear that none of those tested is as effective as potassium.

Stabilization by Potassium Ion at Various pH Values.—The stabilizing effect of potassium salts was investigated further at various hydrogen ion concentrations.

One-tenth cc. amounts of streptolysin solution, diluted to 1 cc. in solutions of selected pH, were heated at 50° for 30, 60, and 120 minutes, both in the presence and absence of 0.1 M KCl. In the series of tubes containing KCl, the acetate buffers were prepared from acetic acid and KOH so that no Na^+, other than that contaminating the reagents, was present in these tubes. Similarly, the acetate buffers of the series from which KCl was absent were prepared with

TABLE III

Stabilization of Streptolysin S by K^+ at Various Hydrogen Ion Concentrations

Milieu	pH	Hemolytic units per cc. after heating at 50° in presence of M/10 KCl for			Hemolytic units per cc. after heating at 50° in absence of KCl for		
		30 min.	60 min.	120 min.	30 min.	60 min.	120 min.
HCl	1.1–1.6	<80	<80	<80	<80	<80	<80
HCl	2.1–2.5	900	575	300	<80	<80	<80
N/20 acetate	3.0	1300	1100	1000	<80	<80	<80
" "	4.0	1300	1200	1000	<80	<80	<80
" "	5.0	1300	1000	1000	80	<80	<80
" "	6.0	1300	1000	725	<80	<80	<80
" "	7.0	1300	800	670	<80	<80	<80
" "	8.0	1200	800	600	<80	<80	<80
" "	9.0	1000	800	550	<80	<80	<80

Control solution kept at 3°: 1300 hemolytic units per cc.

NaOH so that no K^+, except as contaminant, was present in them. The hemolytic activity remaining after exposure to 50° for various times up to 2 hours, at pH 1 to 9, is shown in Table III.

It can be seen that in the absence of K^+, inactivation to the extent of 90 per cent or more occurred at all pH values tested within 30 minutes. In the presence of K^+, however, little or no inactivation occurred in solutions over the range of pH 3.0 to 9.0 in 30 minutes. Between pH 3.0 and 5.0, little activity was lost even after 2 hours; it is evident that the toxin exhibited maximum stability in this pH range.

DISCUSSION

Although the foregoing results may be attributed to stabilization of streptolysin S by K^+, they can be explained, alternatively, by assuming that Na^+ and certain cations other than K^+ promote inactivation of the toxin. The latter ex-

planation does not appear to be correct, however, because salt-free solutions of streptolysin S exhibit lability comparable to that observed when Na^+ is present. It has been observed also that the addition of K^+ to streptolysin S solutions already containing Na^+ is accompanied by stabilization.

The "protective" role of K^+ can be questioned also on the ground that K^+ may function in the test system; *i.e.*, that it activates the toxin. Experimental results bearing on this point indicate that the addition of K^+ to inactivated streptolysin S fails to reactivate it, and that substitution of K^+ for Na^+ in the test hemolytic system does not affect the measurement of activity. Thus, in contrast to certain enzyme systems in which specific cations are known to activate as well as to stabilize, K^+ in the present system appears only to stabilize.

The finding that streptolysin S is protected against inactivation by K^+ should prove useful in the routine handling of the toxin and in its purification. It would be of interest to know whether other bacterial toxins and labile enzymes may not also be protected by potassium against thermal inactivation.

SUMMARY

Potassium ions, and to a less extent, ammonium, magnesium, and barium ions, protect streptolysin S against thermal inactivation.

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