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SHORT COMMUNICATION

CHARACTERIZATION OF STREPTOLYSIN S (SLS): STORAGE AND STABILITY

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ABSTRACT

The stability of streptolysin S during storage under different conditions has been studied. It is completely stable in 0.1% (w/v) bovine serum albumin supplemented with 20% (v/v) glycerol at -20°C for more than 6 months. The *in vitro* study suggests that removal of SLS from cellular environment subjects it to variety of conditions and processes that can lead to loss of activity.

Introduction

Streptococci are responsible for a large number of important diseases of man (Perker, 1978) and animal (Wilson and Salt, 1978). Streptolysin S (SLS) is the non-antigenic (non- immunogenic), oxygen-insensitive cytolysin which is produced by Group A streptococci. It is largely responsible for a zone of beta-haemolysis surrounding colonies on blood agar media (Loridan and Alouf, 1986). Streptolysin S is so-called because it is produced in serum and is oxygen-stable in contrast to streptolysin S, the oxygen labile, sterol-dependent xytolysin also produced by streptococci (Wannamaker, 1983).

Proteins are usually fragile molecules that often require great care during purification to ensure that they remain intact and fully active. Removal of proteins from the cellular environment subjects them to a variety of conditions and processes that can lead to loss of activity of alteration of their structure (Deutscher, 1990). These include d[i]lution, change in solution conditions, exposure to degradative enzymes, oxygen, heavy metals, and surfaces and changes in physical conditions (e.g. freezing and thawing), SLS is normally assayed by its haemolytic activity and this property is remarkably labile (Bernheimer, 1967).

We report here a stability study of SLS, during storage under a variety of condilions.

Materials and Methods

The method used for toxin production was that of London and Alouf (1986). After very step of the purification procedure, the stability and storage properties of SLS were determined. For this purpose small aliquots of purified SLS were stored at room temperature, 4°C, -20°C, and -70°C or in a freeze-dryed state and with or without different stabilizing agents. These agents included glycerol (10%, 20% and 40%) or the addition of an extraneous protein such as bovine serum albumin (BSA, Sigma) in different concentrations (0.1% 0.5% and 1%). A mixture consisting of 10% glycerol plus either 0.1%, 0.5% or 1% BSA was used to stabilize the SLS. In the same manner 20% and 40% of glycerol were tested with BSA.

Results

SLS is usually labile (Bernheimer, 1983) and its instability through manipulative steps, probably due to its hydrophobicity, has hampered its purification and characterization (Ginsburg, 1970); Lai et al., 1978, Lothian and Alouf, 1986). Aliquots of SLS were stored at a range of different temperatures with or without a supplement of glycerol or bovine serum albumin (Table). The results suggested that SLS is completely stable for more than six months when stored with a supplement of 0.1% bovine serum albumin plus 20% glycerol at -20°C. When bovine serum albumin alone (0.1% or 0.5%) was used for the storage of SLS without glycerol, there was 99% loss of SLS activity within 12h. SLS in buffer (400 mM potassium phosphate - 100mM ammonium acetate, (pH 7.0) alone at 37°C, 4°C or -20°C lost 99% of its activity in 12h.

Discussion

The results presented above show that SUS lost almost all haemolytic activity at 37°C, 4°C, or -20°C, with or without glycerol in 12 h. However, SLS retained 100% haemolytic activity for more than six months if stored in the presence of 0.1% extraneous protein (bovine serum albumin) plus 20% glycerol at - 20°C. If one is primarily interested in studying enzyme activity, the presence of serum albumin may not matter. In contest, the presence of extraneous protein is undesirable in structural studies. The presence of 20% glycerol may also be undesirable. Although glycerol can be removed by dialysis, it is likely that biological activity would be lost during this process. The role of serum albumin in stabilising the activity of SLS is not known.

Table
Loss of SLS activity under different storage conditions

Temperature	Time in Hours	SLS activity HU/ml*
Immediately after passing through column	0	16384
Room temperature	12	128
37°C	do	128
+4°C	do	128
-20°C	do	128
With Glycerol		
5% - +4°	do	16
5% - 020°C	do	16
10% - +4°C	do	16
10% - -20°C	do	16
50% - +4°C	do	16
50% - -20°C	do	16
In Ice Box	do	256
With Bovine Serum Albumin (BSA)		
0.1% BSA +20 glycerol (-20°C)	6 month	100% stable (16384 HU/ml)
0.1% BSA (-20°C)	12 h	128
0.5% BSA (-20°C)	12 h	128

*One HU (Haemolytic unit) was defined as the amount of test material which caused 50% lysis of the Sheep red blood cells (SRBC) (Loridan and Alouf, 1986).

Poor stability of SLS has been the subject of interest by previous investigators. According to Ginsburg (1970), haemolysin completed with serum albumin, detergent or RNA-core is very unstable in aqueous solutions and it loses of its activity on standing at 25°C for a few hours. According to Koyama and Egami (1963), in the purified state, the haemolytic activity rapidly decreased even at ice-cold temperature and at neutral pH only 10 percent of original activity remained after an overnight. However, in partially purified preparations, the haemolytic activity could be conserved by lyophilzation without any appreciable inactivation. Lai et al. (1978), Bernheimer (1983), and Alouf and Loridan (1986) reported that SLS was unusually labile and its instability through manipulative steps was probably due to its hydrophobicity.

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