NOTES
Streptolysin O: Sedimentation Coefficient and Molecular Weight Determinations
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The sedimentation coefficient of streptolysin O as determined by sucrose density gradient ultracentrifugation is 3.7S. An approximate molecular weight of 60,500 was calculated from the sedimentation velocity, and a similar value was obtained by Sephadex gel filtration. There was no measurable difference in the sedimentation coefficient of streptolysin O in either the active or reversibly inactive forms, indicating that there were at most only minor conformational differences between the two forms.

Sedimentation properties and molecular weight estimations of the group A streptococcal lysisin, streptolysin O, by ultracentrifugation and Sephadex gel filtration have varied from a sedimentation coefficient of less than 1S with a corresponding molecular weight of less than 10,000 (3), to molecular weights of 80,000 (2) and 107,000 (1). In this communication, sucrose density gradient centrifugation was used to determine the sedimentation coefficient and approximate molecular weight (4, 7).

Succrose density gradient centrifugation was carried out by using a 4.8-ml 5 to 20% sucrose gradient with a 0.2-ml sample volume. Centrifugation was executed in a Beckman Spinco model L ultracentrifuge with an SW39 rotor at 35,000 rev/min (100,000 × g). Human gamma-globulin was used as a marker, assuming a sedimentation coefficient of 6.8S (4) and a molecular weight of 150,000 (6). Three different streptolysin O preparations were used: two from commercial sources (Certified Blood Donor Service Inc., Woodbury, N.Y. and Hyland Laboratories, Los Angeles, Calif.) and a third which was prepared in this laboratory by 60% ammonium sulfate precipitation of a culture supernatant fluid from a Bernheimer C203U group A Streptococcus pyogenes variant producing no streptolysin S. Lysis by all three preparations was blocked by anti-streptolysin O.

Streptolysin O activity was assayed by the microtiter technique, making twofold dilutions and measuring hemolysis of rabbit red blood cells (RBC). Each dilution was activated by reduction with equal volumes of 0.005 M cysteine HCl, allowing a reaction time of 10 min at room temperature. After the addition of 0.025 ml of a 2% rabbit RBC solution, the microplates were incubated at 37° C for 45 min and hemolysis was measured as a function of dilution by appearance of free hemoglobin.

Since the sedimentation coefficient as a function of time is linear (7), an accurate evaluation of this velocity can be determined from the data in Fig. 1 by using the equation of Martin and Ames (7) and substituting the number of fractions from the meniscus for the distance from the meniscus, 

\[ S_i/F_i = S_S/F_S \]

The average sedimentation coefficient for the 12 determinations was 3.7S with a standard deviation of 0.03S. By using the formula (7) \( S_i/S_f = \text{(molecular weight}_i/\text{molecular weight}_f)^{3/4} \), the molecular weight was estimated to be 60,500.

Another estimate of molecular weight was provided by Sephadex G-100 chromatography of streptolysin O, bovine serum albumin (BSA), and ovalbumin eluted with phosphate-buffered saline (pH 7). The peak elution fraction for BSA (molecular weight 69,000) was number 78, whereas that for ovalbumin (molecular weight 40,000) was fraction 88. Streptolysin O activity was greatest in fraction 80. If we assume that the fraction number is inversely proportional to the logarithm of the molecular weight (5), the molecular weight of streptolysin O would be approximately 61,000. This lends additional support to the value obtained by sucrose density gradient centrifugation.
To determine whether there was a change in size, shape, or polymerization created by streptolysin O activation, another sucrose density gradient experiment was carried out with a mixture of active and reversibly inactive streptolysin O. The mixture showed hemolysis when diluted to 1:4 without the addition of cysteine-hydrochloride, representing the active form of streptolysin O. With the addition of cysteine-hydrochloride, hemolysis occurred up to a dilution of 1:32, representing the active plus the previously inactive streptolysin O. Fractions from a 29-hr sucrose density gradient centrifugation were titered before and after activation, and the difference between the two titers was used to determine the sedimentation coefficient of the inactive form of streptolysin O. There was no significant difference between the sedimentation coefficient of the active and the inactive forms (Fig. 2), indicating little or no change in size or shape associated with enzyme activation. These observations excluded the possibility that activation resulted in aggregation or separation of enzyme subunits, since they would be separated by sucrose density gradient centrifugation. Therefore, the sedimentation coefficient as calculated is representative of both active and inactive streptolysin O.

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