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Physical Behavior of Streptolysin S

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Streptolysin S, a cytolytic streptococcal growth product of current interest, has been the subject of two comprehensive reviews (H. Okamoto, Ann. Rept. Res. Inst. Tuberc. Kanazawa Univ. **19**:165, 1962; I. Ginsburg and T. N. Harris, Ergeb. Mikrobiol. Immunol. Exptl. Therap. **38**:198, 1964). As commonly prepared, it exists as a polypeptide attached to oligoribonucleotide along with various amounts of contaminating oligoribonucleotide [J. Koyama and F. Egami, J. Biochem. (Tokyo) **53**:147, 1963; J. Koyama, J. Biochem. (Tokyo) **54**:146, 1963]. The active component of the complex appears to be the polypeptide, and the oligoribonucleotide functions as a carrier. The polypeptide moiety may not be capable of existing alone in an active state for appreciable periods of time, but it can form active complexes with other kinds of carrier molecules as serum albumin and substituted oxyethylene polymers (I. Ginsburg and T. N. Harris, Ergeb. Mikrobiol. Immunol. Exptl. Therap. **38**:198, 1964).

We have attempted to estimate the molecular weight of "nucleic acid streptolysin S" by virtue of its ease of detection and by taking advantage of the methods and principles of gel filtration described by P. Andrews (Biochem. J. **91**:222, 1964). Streptolysin S was prepared as described previously (A. W. Bernheimer, J. Exptl. Med. **90**:373, 1949) and then passed through a water-jacketed column of Sephadex G-75, Superfine (Pharmacia, Uppsala, Sweden), equilibrated with 0.025 M phosphate (*p*H 6), 0.1 M KCl, and 5% glycerol. Streptolysin S content of effluent fractions was estimated by measuring hemolytic activity (A. W. Bernheimer, J. Exptl. Med. **90**:373, 1949), substituting rabbit for human erythrocytes. Crystalline reference proteins in effluent fractions were estimated by absorption of light at 280 mμ. Streptolysin S had an effluent volume of 17.6 ml (Fig. 1), which corresponds on an Andrews plot (Fig. 2) to a molecular weight of 12,000.

The sedimentation coefficient of streptolysin S was estimated by the method of R. G. Martin and B. N. Ames (J. Biol. Chem. **236**:1372, 1961), which has been shown to be applicable to relatively small macromolecules (J. L. H. O'Riordan et al., Science **154**:885, 1966). Samples (0.1 ml) were layered onto 2-ml linear gradients of 5 to 20% sucrose in 0.025 M phosphate (*p*H 7), and were centrifuged at 39,000 rev/min for 23 hr in an SW39 rotor (Spinco Div., Beckman Instruments, Inc., Palo Alto, Calif.). The streptolysin S (0.2 mg) and reference proteins (4 mg) were run in separate tubes, and the contents of each of the tubes were fractionated (Fig. 3). The distance moved by streptolysin S was 19 mm, which is an average of 18.5, 20, and 18.5 mm in three trials,

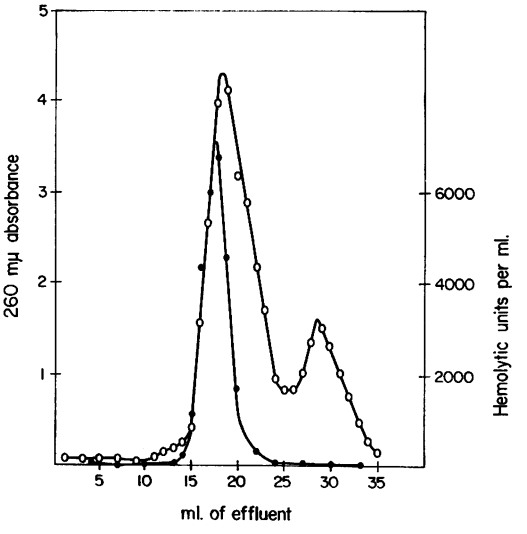

FIG. 1. *Distribution of streptolysin S (hemolytic) activity (●) and absorption of light at 260 mμ (○) in Sephadex G-75 effluent. About 60% of the hemolytic activity of the sample applied to the column was recovered in the effluent fractions.*

and corresponds to an $S_{20,w}$ of 2.4. Comparison of this value with those of proteins and transfer ribonucleic acids having sedimentation coefficients of the same order of magnitude suggests a molecular weight of the order of 20,000. In view of the uncertainties inherent in such a comparison, as well as the lack of precision of the methods, this value is in reasonable agreement with that found by gel filtration.

According to Koyama [J. Biochem. (Tokyo) **54**:146, 1963], the molar ratio of polypeptide to oligonucleotide of highly purified streptolysin S

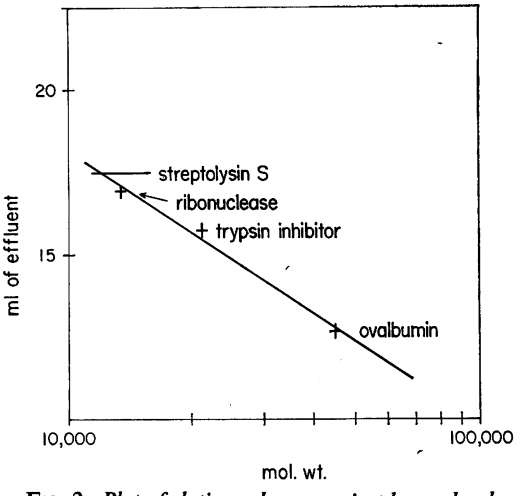

FIG. 2. *Plot of elution volumes against log molecular weight. Void volume was 10.3 ml.*

is 0.3. With the gel filtration figure, the molecular weight of the polypeptide moiety is 0.3/1.3 × 12,000 or 2,800, and it should therefore consist of about 28, perhaps fewer, amino acid residues of which glutamic acid (or glutamine) and serine are the most abundant. The small size of the active moiety may account for some of the properties of streptolysin S, most notably its inability to induce formation of neutralizing antibody.

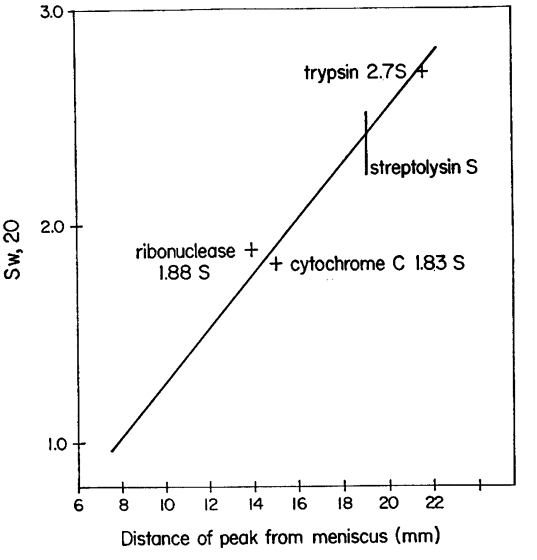

FIG. 3. *Plot of sedimentation coefficients against distance of peaks from meniscus.*

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