hoped to elucidate the reaction mechanism further and calculate absolute rate constants for comparison with those obtained radiolytically.

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BIOCHEMISTRY

Molecular Weight of Urokinase

Urokinase is a proteolytic enzyme in human urine[1]. Its ability to activate plasminogen has stimulated interest in its potential clinical use as a thrombolytic agent.

Investigations were initiated in this laboratory to further characterize urokinase. Purified preparations of urokinase of specific activity approximately 7,000 Ploug units/mg dry weight (ref. 2) were obtained from Leo Laboratories (London) and other samples used were prepared from human urine. As a crystalline urokinase of about 100,000 C.T.A. units/mg protein (1 C.T.A. unit (ref. 3) = 0·7 Ploug units) has been described[4], the material we have employed cannot be regarded as pure.

We have estimated the molecular weight of urokinase by a gel-filtration method[5]. Columns of 'Sephadex *G*-75' and '*G*-100' were prepared and the elution volumes determined for a number of pure proteins by monitoring the ultra-violet absorption of the column effluent. The buffer routinely used was 0·05 M *tris*-HCl (*p*H 7·5) containing 0·1 M potassium chloride. Proteins used for calibration of the columns were cytochrome *C*, ribonuclease, myoglobin, soya-bean trypsin inhibitor, α-chymotrypsinogen, β-lactoglobulin, ovalbumin, serum albumin and γ-globulin. The relation between elution volume and $\log_{10}$ mol. wt. was linear over the molecular weight-range investigated (12,000–70,000) for both columns (Fig. 1).

The elution volume of various urokinase samples was determined on both '*G*-75' and '*G*-100 Sephadex' columns. As it was known that the enzyme preparation was impure, detection of protein in the column effluent by ultra-violet absorption was not used. Instead, the urokinase

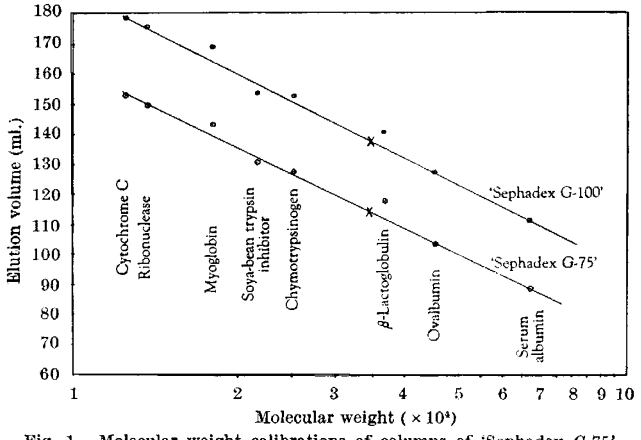

Fig. 1. Molecular weight calibrations of columns of 'Sephadex *G*-75' and '*G*-100' at *p*H 7·5. *X* indicates the position on the lines corresponding to the elution volume of urokinase

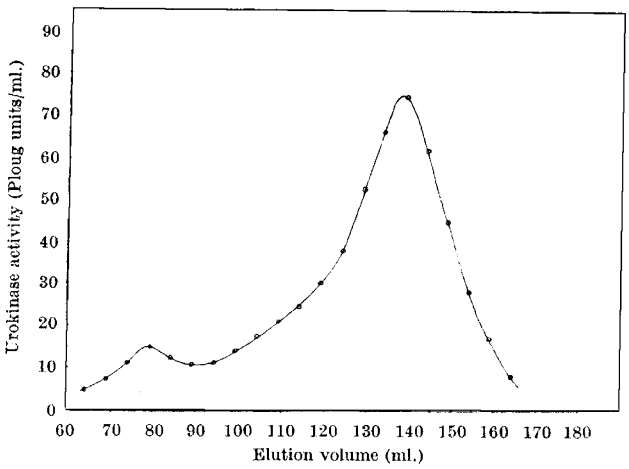

Fig. 2. The elution profile of a sample of urokinase on 'Sephadex *G*-100' at *p*H 7·5

present was assayed by its ability to activate plasminogen using the fibrin plate method[2]. The elution volume obtained on '*G*-75 Sephadex' was 114 ± 2 ml. and on '*G*-100 Sephadex' was 138 ± 2 ml. (Fig. 2). In each case this corresponds to a molecular weight of 34,500 ± 2,000 for urokinase.

Evidence that some urokinase samples were contaminated with a small quantity of high-molecular-weight material capable of activating plasminogen was observed; but the possibility that this represents urokinase bound to a high molecular weight material could not be excluded. This material was completely excluded from both gels and therefore had a molecular weight of at least 100,000. No contamination of the urokinase by other proteolytic enzymes of less than 100,000 molecular weight was observed.

In order to minimize the possibility of the observed urokinase elution volume being modified by absorption effects, a similar experiment was performed using a '*G*-75 Sephadex' column equilibrated with borate buffer at *p*H 10. An experiment was also performed on '*G*-100 Sephadex' at *p*H 7·5 in the presence of 0·7 M potassium chloride instead of 0·1 M potassium chloride. In neither case was any change observed in the estimated molecular weight of urokinase.

These experiments indicate that the molecular weight of urokinase is approximately 35,000. This is much lower than the value of 53,000 recently reported for a crystalline sample of urokinase by Lesuk *et al.*[4], based on ultracentrifugation measurements. The reason for the discrepancy between these two estimates is not clear.

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Localized Strand Separations within Deoxyribonucleic Acid during Selective Transcription

Molecules of deoxyribonucleic acid isolated under gentle conditions are found to possess a double-stranded helical structure, in which the bases of each DNA strand are exactly paired within the interior of the helix with