A CONVENIENT METHOD FOR THE PREPARATION OF S-PEPTIDE FROM BOVINE PANCREATIC RIBONUCLEASE

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Experiments with the 20 unit S-peptide from bovine pancreatic ribonuclease (Richards and Vithayathil, 1959) have permitted important insights into the active center of the enzyme (cf. W. H. Stein, 1965). In view of the constantly increasing number of requests for the peptide and in the absence of pure commercial preparations we wish to report a rapid and convenient method for the isolation and purification of ribonuclease S-peptide, which so far has been separated from S-protein by precipitation of the latter with trichloroacetic acid. We have now observed that ribonuclease-S is easily resolved into the components, S-protein and S-peptide, by gel filtration over Sephadex G-25.

Commercially available preparations of ribonuclease were purified by chromatography on Amberlite CG-50 (Hirs et al., 1953). Purified ribonuclease (658 mg) was dissolved in 5 ml of 0.1 N KCl solution and the pH adjusted to 8 by the addition of 0.1 N sodium hydroxide. To this solution was added 5 mg of Nagarse (Crystalline Bacterial Proteinase, obtained from Teikoku Chemical Industry Co. Ltd., 7, Itachibore Minamidori, 1-Chome, Nishi-Ku, Osaka, Japan, through Biddle Sawyer Corp., 20 Vesey Street, New York, N. Y.) A constant pH of 8 was maintained by adding alkali in a pH stat (Radiometer TTT1). As soon as the alkali consumption began to slow down, enzymatic degradation was stopped by the addition of 6 ml of 0.1 N

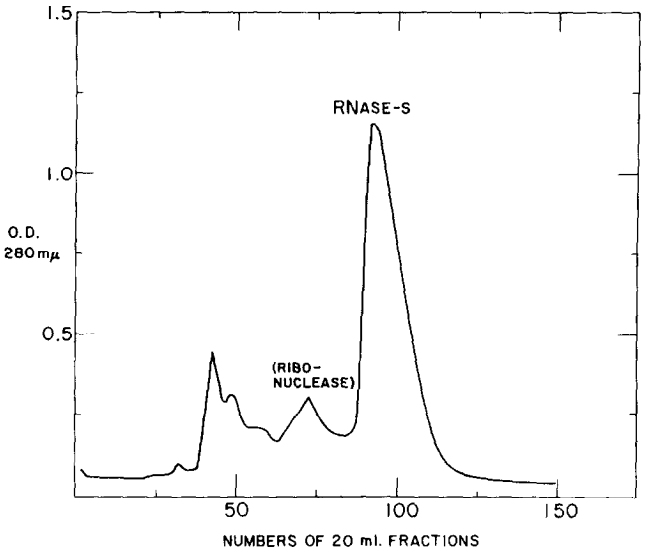


Fig. 1 Separation of RNase-S from ribonuclease and byproducts.

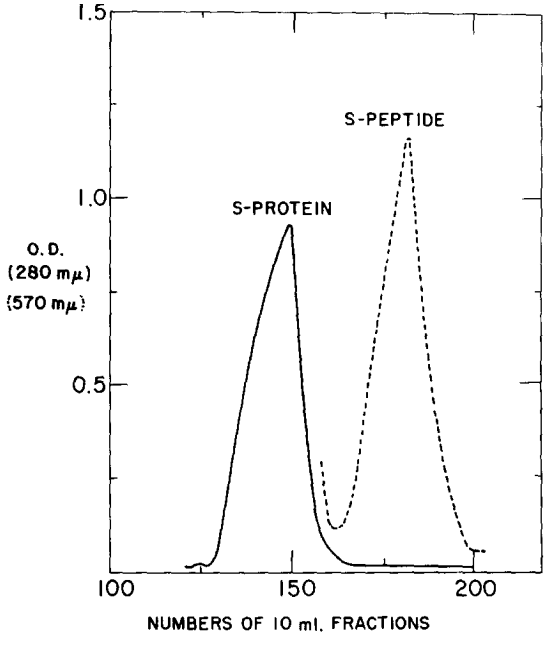
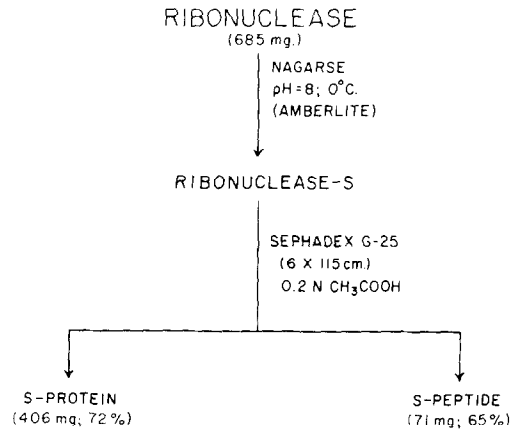


Fig. 2 The separation of RNase S-protein and RNase S-peptide.

hydrochloric acid. The reaction mixture was frozen and lyophilized and the residue applied to an Amberlite CG-50 column in order to separate ribonuclease S from unreacted ribonuclease and byproducts (Fig. 1). Material represented by the large peak (fractions 90-110) of Fig. 1 was collected and lyophilized. The dry product was then dissolved in 0.2 N acetic acid and filtered over a Sephadex G-25 column (6 x 120 cm) which was equilibrated with the same solvent. Figure 2 shows the resulting chromatographic pattern: S-peptide and S-protein are well separated from each other. The pooled fractions of the first peak gave 406 mg (72%) of S-protein and those of the second peak 71 mg (65%) of S-peptide. The scheme of fractionation is summarized in Figure 3.



When the amino acid composition of the S-peptide was determined, high values were obtained for serine. The isolated S-peptide is a mixture of two homologous peptides differing by one residue of serine, namely the one occupying position 21 in native ribonuclease. These investigations will be described in a forthcoming publication.[1]

(1) Similar observations have been made by M. Doscher and C. H. W. Hirs to whom we are obligated for informing us about their findings prior to publication.

REFERENCES

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