Short Communications and Preliminary Notes

PRELIMINARY STUDIES ON RIBONUCLEASE STRUCTURE

by

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Previous studies[1] have indicated an unequal labeling of alanine, aspartic acid, or glutamic acid residues isolated from different positions in the structure of radioactive ovalbumin prepared *in vitro* and *in vivo*. These results support an hypothesis of stepwise protein synthesis involving peptide intermediates and make unlikely, at least in the case of ovalbumin, a process involving the simultaneous "template" combination of free amino acids. To test the generality of these conclusions we have begun similar studies on ribonuclease, a protein particularly suited to such work because of its low molecular weight, partially determined amino acid composition[2], and relative ease of preparation in pure radioactive form[3]. Assuming a molecular weight of 13,500 g/*M*, it follows from the analyses of BRAND *et al.*[2] that ribonuclease contains three residues each of glycine and phenylalanine. The present studies are concerned with the separation of these residues for the purpose of specific activity comparisons through isolation of specific peptide fragments of the protein chain containing them.

Silica gel chromatography[4] of a number of 6 *N* HCl and 12 *N* HCl hydrolysates of dinitrophenyl-ribonuclease have resulted in the finding of bis-dinitrophenyl-lysine as the only N-terminal amino acid in this protein and quantitative spectrophotometric data indicate the presence of only one N-terminal amino acid per mole (0.3–0.96 moles bis-dinitrophenyl-lysine/mole ribonuclease). These results suggest that ribonuclease is composed of a single chemical peptide chain, as distinct from the 5 crystallographic chain reported by CARLISLE AND SCOULOUDI[5]. This conclusion must, of course, be tested by the use of other terminal group methods in addition of the dinitro-fluorobenzene method. Preliminary experiments with seven times recrystallized carboxypeptidase under a variety of conditions have shown that valine is rapidly released from the C-terminal portion of ribonuclease. This finding supports the single chain structure of ribonuclease although traces of other amino acids (alanine, glutamic acid, aspartic acid, serine, threonine) in addition to the strong valine spot were also observed.

Pepsin digestion of ribonuclease results in the reproducible formation of 13–15 peptide fragments with an *average* peptide chain length of 8–9 amino acids[6]. Paper chromatography of the digest using a variety of solvents leads to only a partial separation of these peptides.

Peptic digests have been subjected to filter paper electrophoresis[7] to facilitate the separation of relatively large quantities of material for study. Completely reproducible patterns are obtained (0.04 *M* phosphate buffer; pH 7.20, 250 mv, 20 mamp., Munktell paper #20/150, 8.5 inches × 20 inches, 16 hours at 5° C), and 10–30 mg quantities may be run at once. Five components separate well under these conditions leaving a partially resolved complex of neutral material (Table I). The amino acid periodicities of peptides #3 and #5 are under particular investigation since they are most cleanly separated, and are probably single components, as evidenced by their paper chromatographic homogeneity. In addition, they each contain phenylalanine and glycine, amino acids we have employed as isotopically labelled precursors in the preparation of radioactive ribonuclease for the specific radioactivity distribution studies mentioned above.

TABLE I

RESULTS OF FILTER PAPER ELECTROPHORESIS OF RIBONUCLEASE DIGESTS UNDER THE CONDITIONS DESCRIBED IN THE TEXT

Component	*Charge*	*Distance of Migration**	*Qualitative Composition***
1	—	7.5	ala, asp, glu, gly, val
2	—	5.5	
3	—	3.5	ala, arg, asp, glu, gly, iso, lys, phe, tyr, val
4	—	1.3	
Neutral Complex	±	—0.5 to +2.5	
5	+	3.5	ala, arg, asp, gly, lys, met, phe, thr

* Measured in inches from the point of zero mobility.

** The abbreviations used for the amino acids are those of E. BRAND AND J. T. EDSALL, *Ann. Rev. Biochem.*, 16 (1947) 224.

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Received August 20th, 1952

ADENINE AS A PRECURSOR OF ACID SOLUBLE NUCLEOTIDES IN THE RAT

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Adenine-8-^{14}C [1] (0.2 mMole/kg) has been given intraperitoneally to adult male rats. Two hours after injection, the livers and other internal organs (kidney, spleen, testis, and washed small intestine) were separately pooled, extracted with cold 7 % aqueous trichloracetic acid, and the ice-cooled extract freed from acid by continuous ether extraction. Following mercuric precipitation and treatment with hydrogen sulphide, the nucleotides were separated on Dowex 2 (formate) by an adaptation of the method of COHN AND CARTER[2], using formic acid/formate mixtures whose volatility simplified purification and counting of the adenosine phosphate fractions. Details of the specific activities of the various fractions are tabulated (Table I).