# Chemical Synthesis of Bovine Pancreatic Ribonuclease A

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*Summary* Bovine pancreatic ribonuclease, which consists of 124 amino acids, was synthesized in a conventional manner by assembling relatively small 30-peptide fragments of established purity.

IN 1969, two groups of investigators reported the synthesis of materials with partial enzyme activity of ribonuclease (RNase) A. Gutte and Merrifield,[1] using automated solid phase synthesis, reported that they obtained a supernatant solution with a specific activity of 78%, after ammonium sulphate fractionation of the trypsin-resistant material. The Merck group[2] undertook the synthesis of S-protein and obtained a solution containing *ca.* $2\gamma$ of RNase S′ activity, upon combination with the S-peptide[3] derived from the natural source. The final product in both syntheses was not chemically characterized and an unambiguous synthesis of RNase thus remained to be accomplished.

Our synthesis of RNase was performed in a conventional manner, using methanesulphonic acid (MSA) as a deprotecting reagent[4] in the final step. Thus, amino acid derivatives bearing protecting groups removable by MSA were employed; *i.e.*, Arg(MBS),[5] Lys(Z), Cys(MBzl), Glu(OBzl), and Asp(OBzl) (MBS = *p*-methoxybenzenesulphonyl, Bzl = benzyl, Z = benzyloxycarbonyl, MBzl = *p*-methoxybenzyl). Relatively small 30-peptide fragments of established purity were used to construct the entire amino acid

sequence of RNase using Rudinger's azide procedure[6] as the main tool. Such small acyl components used in an excess in each condensation were easily removed by precipitation from appropriate solvents or occasionally by gel-filtration on Sephacryl S-200. Because of the poor solubility of the protected intermediates with increasing chain length, reactions were performed in a mixture of three solvents, *NN*-dimethylformamide–dimethyl sulphoxide–hexamethylphosphoramide. After condensation, the purity of each product was assessed by amino acid analysis, in which Phe was selected as a diagnostic amino acid, since this amino acid occurs only three times (at positions 8, 46, and 120) (Figure).

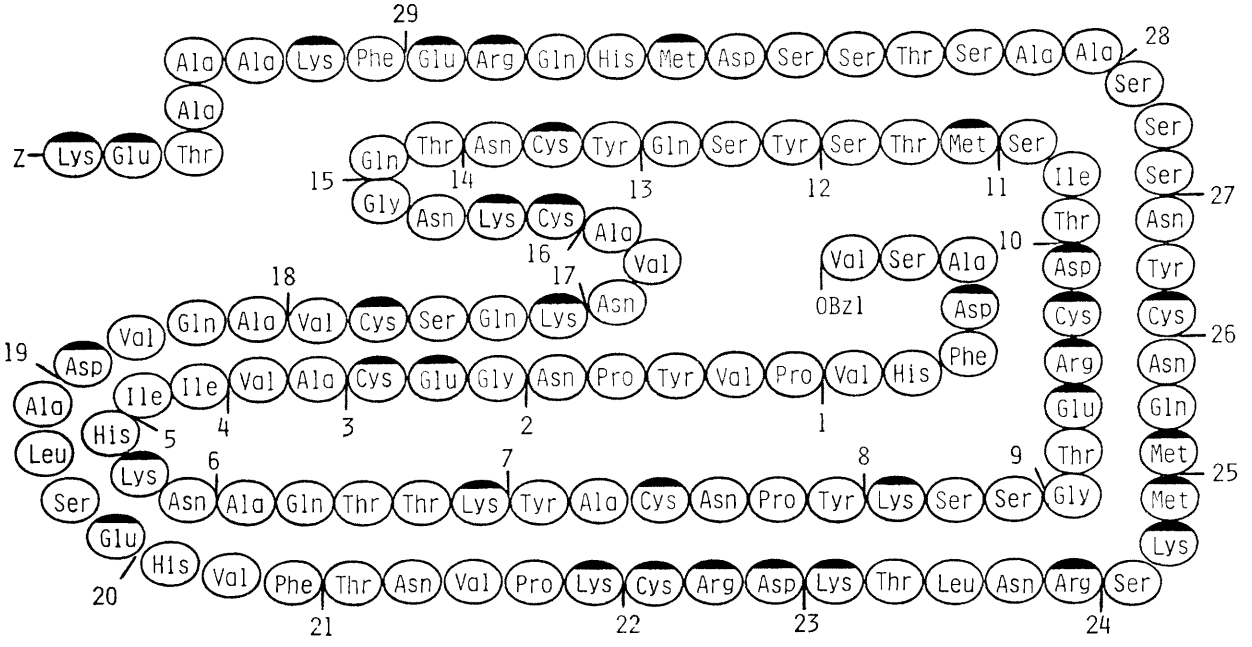


FIGURE. Partial shading indicates protected amino acids: Asp(OBzl), Glu(OBzl), Lys(Z), Cys(MBzl), Arg(MBS), Met(O), Glu(OBu) (position 2). | indicates positions of fragment condensation.

In the final step, the Cys(MBzl) sulphoxide which had partially formed during the synthesis was reduced by benzenethiol.[7] The protected RNase thus obtained was treated with MSA in the presence of *m*-cresol to remove all protecting groups. The deprotected peptide was reduced with 2-mercaptoethanol at pH 8·6 and subsequently submitted to air oxidation to form 4 disulphide bridges according to the method of Anfinsen and Haber.[8] The crude product, isolated after gel-filtration on Sephadex G-75, exhibited an activity of 12% against yeast RNA.[9] Affinity chromatography on Sepharose-(4B)-5′-(4-aminophenylphosphoryl)-uridine-2′(3′)-phosphate[10] then brought the activity of the air oxidized product up to 82%. After ion-exchange chromatography on CM-cellulose[11] using 0·1 M sodium phosphate buffer (pH 7·5) as eluant, we succeeded in obtaining a synthetic protein with essentially the same enzymic activity (104—107%) as that of natural bovine pancreatic RNase A (Sigma, Lot. 47C-0422). The synthetic protein exhibited a single band on disc electrophoresis at pH 4·3 and its amino acid ratios in 6N HCl hydrolysate were in excellent agreement with those of natural enzyme. The specific activity of our synthetic protein was further confirmed by assaying against 2′,3′-cyclic cytidine phosphate[12] and its physicochemical properties {u.v. spectra ($\epsilon_{max}$ 9700 at 277·5 nm), rotation ($[\alpha]_D$ −70·8° in 0·1 M KCl at 23 °C), and the Michaelis constant ($K_M$ 1·22 mg per ml)} were identical with those of natural RNase.[11,13]

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