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SYNTHESIS OF AN ENZYMATICALLY ACTIVE ORN^{10}-S-PEPTIDE OF RIBONUCLEASE-S

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In previous papers[1-5] we described the synthesis of some peptides related to the N-terminal eicosapeptide of RNase A *.

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*) The following abbreviations will be used: RNase-A, the principal chromatographic component of beef pancreatic Ribonuclease; RNase-S, subtilisin-modified RNase-A; S-peptide, the eicosapeptide obtained from RNase-S; S-protein, the protein component obtained from RNase-S; RNase-S', the reconstituted enzyme obtained by mixing equimolar amounts of S-peptide and S-protein; Orn^{10}-S-peptide, the synthetic eicosapeptide where the Arg^{10} is substituted by Orn; Orn^{10}-RNase-S', the reconstituted enzyme obtained by mixing equimolar amounts of Orn^{10}-S-peptide and S-protein; RNA, ribonucleic acid.

The results obtained by Richards et al.[6,7] and by Crestfield, Moore and Stein[8,9] on the active portion of RNase A, indicated that the S-peptide is of essential importance for the knowledge of Ribonuclease chemistry. In fact the S-peptide contains one histidine residue in position 12, which is directly involved in the active site of the enzyme[7,9]. Moreover this fragment provides a relatively simple model for studying the binding sites responsible for the formation of the active complex with S-protein.

Recently Hofmann et al.[10-15] published the synthesis of several shorter sequences of the S-peptide. Among them the 1-13 sequence, after recombination with S-protein, in the ratios 1:3, 1:10 and 1:100 generated respectively 50%, 68-72% and 80% of the enzymic activity of RNase S', against yeast RNA.

In this preliminary note we wish to report the first synthesis of an enzymatically active modified S-peptide in which an ornityl residue substitutes arginine in position 10.

The scheme of the synthesis, shown in the figure †, has been planned in order to facilitate modifications of particular residues connected either with the active site or with the binding sites of the molecule. The stepwise elongations as well as the condensations of the peptide subunits have been carried out using those procedures which give the best guaranties to preserve the optical purity.

The key peptide, in the achievement of this synthesis, was the esapeptide N^{α},benzyloxycarbonyl,N^{ε},t-butyloxycarbonyllysyl-phenylalanyl-γ,t-butylglutamyl-N^{δ},t-butyloxycarbonylornityl-glutamminyl--histidine methyl ester (I) corresponding to the sequence 7-12.

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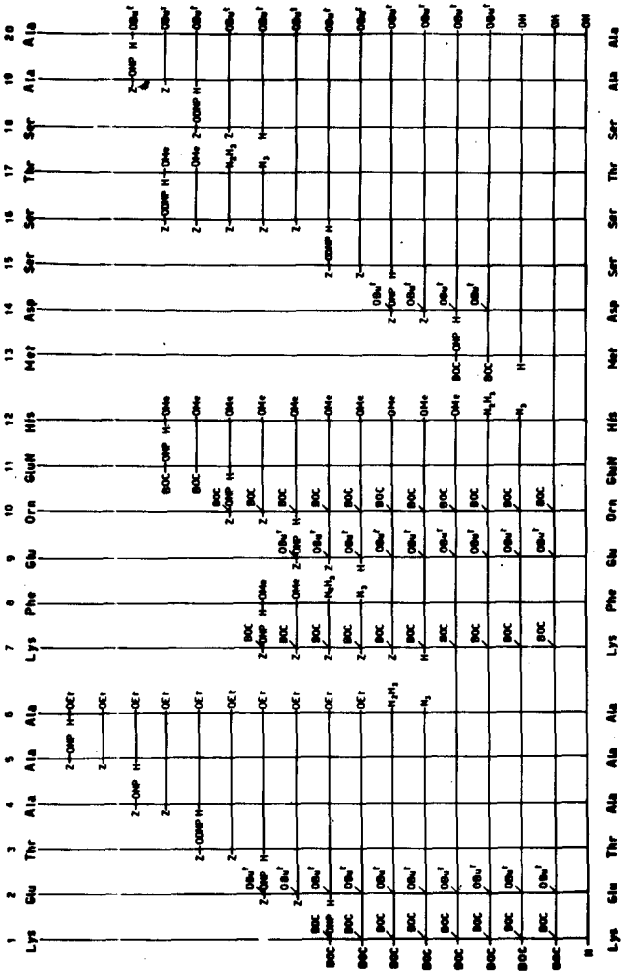
†) The peptides and the peptide derivatives mentioned are of the L-configuration. For a simpler description the customary L-designation for individual amino acid residues is omitted. Z,benzyloxycarbonyl; BOC,t-butyloxycarbonyl; OMe,methyl ester; OEt,ethyl ester; OBu^{t},t-butyl ester; ONP,p-nitrophenyl ester; ODNP,2,4 dinitro-phenyl ester.



[Anal. Calcd. for $C_{59}H_{87}N_{11}O_{16}$: C 58.73 H 7.27 N 12.77; Found: C 57.93 H 7.35 N 12.50; $[\alpha]_D^{20}$ - 19.1 ± 0.2, c 1.6% in dimethylformamide; single chlorine[16] and Pauly positive spot Rf_1 0.64, Rf_2 0.75; amino acids ratios in acid hydrolysate‡ $(Lys + Orn)_{2.10}$ $Phe_{0.95}Glu_{2.07}His_{1.10}$].

The tetrapeptide (9-12) N^{α},benzyloxycarbonyl,γ,t-butylglutamyl-N^{δ},t-butyloxycarbonylornityl-glutamminyl-histidine methyl ester was built up by stepwise elongation, starting from the C-terminal residue, using the p-nitrophenyl ester method. After removal of the N^{α} protecting group the tetrapeptide was condensed, by azide procedure, with the dipeptide N^{α},benzyloxycarbonyl,N^{ε},t-butyloxycarbonyllysyl-phenylalanine hydrazide (7-8).

The N-terminal esapeptide (1-6) N^{α},N^{ε},di-t-butyloxycarbonyllysyl-γ,t-butylglutamyl-threonyl-alanyl-alanyl-alanine ethyl ester (II), was synthetized by stepwise elongation starting from the C-terminal residue using the p-nitrophenyl esters (2,4 dinitrophenyl ester for the threonine residue) as the acylating component. [Anal. Calcd. for $C_{40}H_{71}N_7O_{14}$: C 54.96 H 8.19 N 11.22; Found: C 54.30 H 8.06 N 11.10; $[\alpha]_D^{20}$ - 21.8 ± 0.2, c 1.85% in dimethylformamide; single chlorine[16] positive spot Rf_1 0.80, Rf_2 0.95; amino acid ratios in acid hydrolisate $Lys_{1.10}Glu_{1.00}Thr_{0.98}Ala_{2.90}$].

The esapeptide (II) was transformed into the corresponding hydrazide and condensed, by using the azide procedure, with the N^{ε},t-butyloxycarbonyllysyl-phenylalanyl-γ,t-butylglutamyl-N^{δ},t-bu-

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‡) Ascending thin layer chromatography was performed on Silica Gel G using the following solvent systems: Rf_1 n-butanol: glacial acetic acid: water = 4:1:1 v/v; Rf_2 ethyl acetate: pyridine: glacial acetic acid: water = 60:20:6:14 v/v. The acid hydrolysis were carried out in 6N HCl for 22 hours at 110°C. The amino acid composition of acid hydrolisates was determined according to the method of D.H. Spackman, S. Moore and W.H. Stein[17].

tyloxycarbonylornityl-glutamminyl-histidine methyl ester obtained from (I) by catalytic hydrogenation. The protected dodecapeptide (1-12) (III) [Single chlorine[16] positive spot Rf_1 0.65, Rf_2 0.80; amino acid ratios in acid hydrolisate $(Lys + Orn)_{3.10}Glu_{3.05}Thr_{0.95}Ala_{3.00}Phe_{1.05}His_{1.05}$] has been transformed into the corresponding hydrazide and condensed, by azide procedure, with the completely unprotected C-terminal octapeptide (13-20) (IV).

The 13-20 sequence N^{α},t-butyloxycarbonylmethionyl-β,t-butyl-aspartyl-seryl-seryl-threonyl-seryl-alanyl-alanine t-butyl ester (V) [Anal. Calcd. for $C_{41}H_{72}N_8O_{17}S$: C 50.18 H 7.40 N 11.42 S 3.27; Found: C 49.04 H 7.50 N 10.94 S 3.26; $[\alpha]_D^{20}$ - 11.77 ± 0.2, c 1.1% in dimethylformamide; single chlorine[16] positive spot Rf_1 0.75, Rf_2 0.83; amino acid ratios in acid hydrolisate $Met_{1.10}Asp_{1.03}Ser_{2.90}Thr_{0.95}Ala_{2.10}$] was built up starting with the condensation, through the azide procedure, of the dipeptide N^{α},benzyloxycarbonylseryl-threonine hydrazide (16-17) with the tripeptide seryl-alanyl-alanine t-butyl ester (18-20). From the protected pentapeptide (16-20) so obtained, the N^{α} protecting group was removed by catalytic hydrogenation and the elongation by stepwise procedure with the p-nitrophenyl ester method (2,4 dinitro-phenylester for the serine residue) yielded the octapeptide.

The fully protected octapeptide (V), after treatment with trifluoro acetic acid gave a chromatographically homogeneous material (single ninhydrin and chlorine[16] positive spot Rf_1 0.2) which, after condensation with the N-terminal dodecapeptide (III) yielded the 1-20 sequence. The eicosapeptide so obtained, was first treated with trifluoro acetic acid, and then purified by chromatography, using an Amberlite IRC-50 column, and by gel filtration on Sephadex G-25. $[\alpha]_D^{20}$ - 57 ± 0.2, c 0.162% in water; amino acid ratios in acid hydrolisate $(Lys + Orn)_{3.15}Glu_{3.10}Thr_{1.95}Ala_{4.95}Phe_{1.15}His_{0.97}Met_{1.05}Asp_{1.10}Ser_{3.10}$.

The homogeneity of the synthetic Orn^{10}-S-peptide has been checked by paper electrophoresis at pH 2, 3.7 and 8. In all these conditions the peptide proved to be an homogeneous material as evidentiated by a single ninhydrin, and Pauly positive spot.

Its chromatographic and electrophoretic behaviour, was perfectly similar to that of the native S-peptide.

The enzymic activity of the Orn^{10}-S-peptide after recombination with S-protein in the molar ratios 1:1 and 1:10, was checked against yeast RNA according to the literature[18]. The recovery of biological activity for the reconstituted modified enzyme (Orn^{10}-RNase S') was respectively 70% and 74% of the activity of RNase S'.

As a conclusion the replacement of the Arginyl residue in position 10 with an Ornityl residue, yielded a synthetic eicosapeptide bearing a very high enzymic activity.

The details of the synthesis will be published elsewhere.

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