and tyrosine or the imidazole ring of histidine are present in the purified tetratetracontapeptide ester is supported by the results of the hydrazinolysis step.

Acknowledgment. We thank Mr. Carl Homnick for the expert execution of the amino acid analyses reported here. We are also greatly indebted to Messrs. S. M. Miller and R. Pospolita for the large-scale preparation of NCA's and NTA's.

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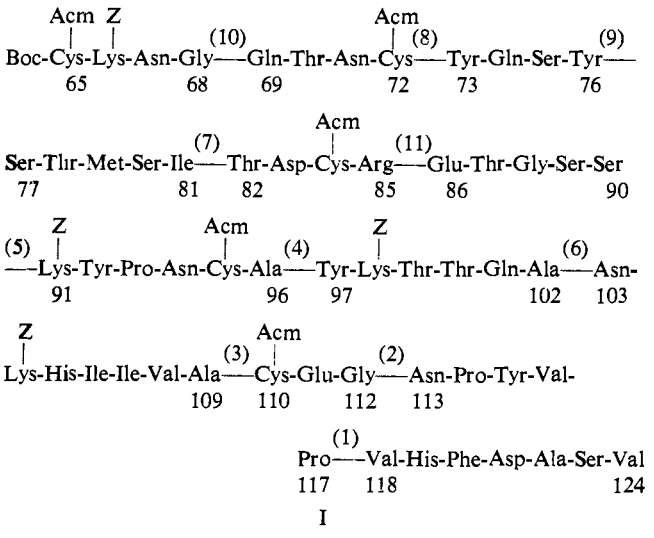
Studies on the Total Synthesis of an Enzyme. III. Synthesis of a Protected Hexacontapeptide Corresponding to the 65–124 Sequence of Ribonuclease A

Sir:

We report herewith the preparation of the protected hexacontapeptide fragment 65–124 (I) of RNase A. This polypeptide was prepared by the addition of the



I

N-protected azide of the heneicosapeptide 65–85 to the nonatriacontapeptide 86–124. Coupling reactions involving peptidal azides in which the carboxy-terminal amino acid is unprotected arginine[1] had not, to our knowledge, been previously employed, but we had successfully carried out such reactions in a synthesis of porcine calcitonin[2] and in other instances.[2] The fragments were prepared and the azide coupling reactions were carried out by the procedures described in the two preceding communications.[3,4] The azide couplings of the fragments were performed in the order indicated by the numerals in parentheses above the appropriate bonds in I.

The heneicosapeptide 65–85 was synthesized *via* two routes. In one of these the protected octapeptide azide of fragment 69–76 was allowed to react with 1 equiv of fragment 77–85. The resulting heptadecapeptide, obtained in about 60% yield, was treated with trifluoroacetic acid to remove the butyloxycarbonyl blocking group, and the resulting salt was dissolved in hexamethylphosphoramide and treated with 2 equiv of a solution of the azide of the blocked tetrapeptide[5] 65–68 in dimethylformamide at 5° for 2 days. The product (fragment 65–85) was obtained in about 70% yield.

An alternate route to the heneicosapeptide 65–85 involved the addition of the above blocked tetrapeptide azide in 20% excess to the octapeptide 69–76 to give the desired dodecapeptide in >80% yield. The coupling of the protected azide of fragment 65–76 with the nonapeptide fragment 77–85 proceeded in 15–30% yield, and isolation of pure product generally required purification on Sephadex G-50.

In the preparation of the nonapeptide it is noteworthy that the methyl ester of fragment 77–81, though carboxy terminal in isoleucine, could nevertheless be converted quantitatively to the hydrazide on treatment at 25° with a 25% solution of hydrazine in dimethylformamide overnight and that the azide derived therefrom smoothly reacted with fragment 82–85 at −20° in 60% yield.

The tetrapeptide fragment 65–68 which was required for this synthesis contains an asparaginylglycine sequence whose rearrangement to β-aspartylglycine, presumably *via* a succinimide intermediate, has been described.[6] Our protected tetrapeptide ester and hydrazide were shown to be single components by tlc in two systems, and the absence of a carboxylate anion in the hydrazide was shown by electrophoresis at pH 9. Infrared spectroscopy showed no evidence for the presence of a succinimido moiety[6] in the tetrapeptide ester. The integrity of the asparaginylglycine sequence is also supported by enzymic degradation studies on a sample of the tetrapeptide hydrazide which had been deblocked with anhydrous HF.[3] After incubation with aminopeptidase M at 36° the resulting hydrolysate was indistinguishable by tlc from a synthetic mixture of the component amino acids, and only 2% of β-aspartylglycine was detected in the hydrolysate by Spinco amino acid analysis. Finally, the protected tetrapeptide ester was stable in 50% acetic acid for 2 days. It may be concluded, therefore, that the Asp-Gly bond is reasonably stable to the reaction conditions to which it was to be exposed in our scheme[3] for the synthesis of S-protein.

The synthesis of the nonatriacontapeptide 86–124

(1) Treatment of the carbobenzyloxylated, acetamidomethylated reduced S-protein with trypsin should afford fragment 86–124 in which the sulfhydryl groups and the ε-amino functions of lysine are protected. This material could serve as a relay. Work along these lines is in progress. For this reason an azide coupling with carboxy-terminal arginine-85 became attractive.

(2) Unpublished results from these laboratories.

(3) R. G. Denkewalter, D. F. Veber, F. W. Holly, and R. Hirschmann, *J. Am. Chem. Soc.*, **91**, 502 (1969).

(4) R. G. Strachan, W. J. Paleveda, Jr., R. F. Nutt, R. A. Vitali, D. F. Veber, M. J. Dickinson, V. Garsky, J. E. Deak, E. Walton, S. R. Jenkins, F. W. Holly, and R. Hirschmann, *ibid.*, **91**, 503 (1969).

(5) The synthesis of this fragment, but with different protecting groups, has been described by L. A. Stchukina, V. G. Degtyar, and E. I. Boltyanskaya, *Khim. Prir. Soedin.*, 37 (1967). The synthesis of a cyclic disulfide linked octapeptide corresponding to residues 65–72 has also been described by M. A. Ruttenberg, *J. Am. Chem. Soc.*, **90**, 5598 (1968). See also L. A. Stchukina and V. G. Degtyar, *Khim. Prir. Soedin.*, 39 (1968).

(6) E. E. Haley and B. J. Corcoran, *Biochemistry*, **6**, 2668 (1967). See also K. Weber and W. Konigsberg, *J. Biol. Chem.*, **242**, 3563 (1967), and M. A. Ondetti, A. Deer, J. T. Shechan, J. Pluščec, and O. Kocy, *Biochemistry*, **7**, 4069 (1968), and references cited therein.

Table I. Amino Acid Analyses of Fragments

Fragment[a]	Spinco amino acid analysis
H(118–124)OH	$Val_{2.00}His_{0.98}Phe_{0.98}Asp_{1.00}Ala_{1.00}Ser_{0.99}$
H(118–124)OH[b]	$Val_{2.00}His_{0.99}Phe_{0.98}Asp_{0.98}Ala_{0.99}Ser_{0.97}$
Boc(113–117)$NHNH_2$	$Asp_{1.05}Pro_{2.04}Tyr_{0.98}Val_{0.97}$
Boc(113–124)OH	$Asp_{2.11}Pro_{1.99}Tyr_{0.99}Val_{2.97}His_{0.94}Phe_{1.02}Ala_{1.02}Ser_{0.97}$
Boc(110–112)OEt	$Glu_{1.01}Gly_{1.00}$
Boc(110–124)OH	$^1/_2$-$Cys_{0.83}Glu_{1.13}Gly_{1.06}Asp_{2.02}Pro_{2.08}Tyr_{0.99}Val_{2.82}His_{0.90}Phe_{0.94}Ala_{0.94}Ser_{1.04}$
Boc(103–109)OMe[c]	$Asp_{0.99}Lys_{0.99}His_{0.99}Ile_{1.95}$[d]$Val_{1.01}Ala_{1.00}$
Boc(103–124)OH	$Asp_{3.16}Lys_{0.97}His_{1.86}Ile_{1.09}Val_{4.11}Ala_{2.06}$ $^1/_2$-$Cys_{0.75}Glu_{0.97}Gly_{0.94}Pro_{2.07}Tyr_{0.99}Phe_{0.96}Ser_{0.98}$
Boc(103–124)OH[k]	$Asp_{3.04}Lys_{0.98}His_{2.00}Ile_{1.94}Val_{4.04}Ala_{2.05}$ $^1/_2$-$Cys_{0.19}Glu_{0.97}Gly_{0.66}Pro_{2.26}Tyr_{0.98}Phe_{1.01}Ser_{0.66}$
Boc(97–102)OMe	$Tyr_{0.98}Lys_{1.00}Thr_{1.99}Glu_{1.03}Ala_{1.01}$
Boc(91–96)OMe	$Lys_{0.98}Tyr_{0.98}Pro_{1.85}$[e]$Asn_{1.05}Ala_{1.01}$
	Acm
H(91–96)OMe[f,g]	$Lys_{1.08}Tyr_{0.98}Pro_{1.04}Asn_{0.84}$[i]$Cys_{1.08}$[h,i]$Ala_{0.99}$
Boc(91–96)$NHNH_2$	$Lys_{1.00}Tyr_{0.99}Pro_{1.22}$[e]$Asp_{1.00}$ $^1/_2$-$Cys_{0.72}Ala_{1.01}$
Boc(91–102)OMe	$Lys_{2.02}Tyr_{2.02}Pro_{1.02}Asp_{1.04}$ $^1/_2$-$Cys_{0.95}Ala_{1.96}Thr_{1.96}Glu_{1.02}$
Boc(86–90)$NHNH_2$	$Glu_{1.02}Thr_{1.03}Gly_{1.00}Ser_{1.95}$
Boc(86–102)$NHNH_2$	$Glu_{1.97}Thr_{3.03}Gly_{0.97}Ser_{2.02}Lys_{1.97}Tyr_{0.93}Pro_{0.88}Asp_{1.01}Ala_{2.02}$
Boc(86–124)OH	$Glu_{3.10}Thr_{3.15}Gly_{2.00}Ser_{3.04}Lys_{2.94}Tyr_{2.89}Pro_{3.29}Asp_{3.96}Ala_{4.10}His_{1.86}Ile_{1.13}Val_{3.87}Phe_{0.98}$
Boc(86–124)OH[k]	$Glu_{3.11}Thr_{2.87}Gly_{2.00}Ser_{2.19}Lys_{3.07}Tyr_{2.96}Pro_{3.24}$[e]$Asp_{4.00}Ala_{4.13}His_{1.85}Ile_{1.91}$[d]$Val_{3.88}Phe_{0.98}$
Boc(82–85)OEt	$Thr_{1.02}Asp_{1.00}Arg_{0.99}$
Boc(77–81)$NHNH_2$	$Ser_{2.03}Thr_{1.05}Met_{0.97}Ile_{0.95}$
Boc(77–85)OEt	$Ser_{2.01}Thr_{2.03}Met_{0.91}Ile_{0.99}Asp_{1.01}Arg_{1.00}$
Boc(73–76)OEt	$Tyr_{2.05}Glu_{1.00}Ser_{0.98}$
Boc(69–72)OEt	$Glu_{1.00}Thr_{1.00}Asp_{1.00}Cys_{0.86}$
Boc(69–76)$NHNH_2$	$Glu_{2.09}Thr_{0.99}Asp_{1.00}Tyr_{1.90}Ser_{1.01}$
Boc(65–68)OH	$Cys_{0.84}$[j]$Lys_{1.00}Asp_{1.01}Gly_{1.01}$
Boc(65–76)$NHNH_2$	$Lys_{1.00}Asp_{2.07}Gly_{1.00}Glu_{1.99}Thr_{1.01}Tyr_{1.78}Ser_{0.94}$
Boc(65–85)OEt	$Lys_{0.99}Asp_{2.98}Gly_{1.00}Glu_{2.02}Thr_{3.07}Tyr_{1.78}Ser_{2.91}Met_{0.96}Ile_{1.00}Arg_{1.04}$
Boc(65–85)$NHNH_2$	$Cys_{1.74}Lys_{1.06}Asp_{3.10}Gly_{0.94}Glu_{1.85}Thr_{3.12}Ser_{3.00}Met_{0.90}Ile_{1.00}Arg_{0.91}$
Boc(69–85)OEt	$Glu_{1.91}Thr_{3.19}Asp_{2.04}Tyr_{1.96}Ser_{2.90}Met_{1.01}Ile_{1.01}Arg_{0.96}$
Boc(65–124)OH[l]	$Asp_{7.02}Gly_{2.99}Glu_{5.03}Thr_{6.24}Tyr_{4.82}Ser_{6.03}Met_{0.99}Ile_{2.09}Pro_{3.16}Ala_{3.78}Val_{3.45}Phe_{0.93}$
Boc(65–124)OH[k]	$Lys_{4.29}Asp_{7.24}Gly_{3.43}Glu_{5.15}Thr_{5.72}Tyr_{4.95}Ser_{5.24}Met_{1.14}Ile_{2.95}Arg_{0.86}Pro_{2.67}Ala_{4.09}His_{2.57}$-$Val_{3.62}Phe_{0.86}$

[a] Fragments refer to formula I and carry the substituents shown therein, unless otherwise indicated. Except as noted in the table, analyses were carried out after 20-hr acid hydrolysis. [b] Submitted to enzymatic degradation with leucine aminopeptidase. [c] 100-hr acid hydrolysis. [d] Plus 0.07 alloisoleucine. [e] The proline value can be variably high, because of the presence of unoxidized cysteine. [f] Blocking groups removed with anhydrous HF. [g] Submitted to enzymatic degradation with aminopeptidase M. [h] Acetamidomethylcysteine emerges with aspartic acid on the amino acid analyzer. [i] No aspartic acid was seen by tlc after enzymic digestion. [j] As cysteic acid after performic acid oxidation. [k] 70-hr hydrolysis. [l] Satisfactory values for the basic amino acids were obtained on a separate specimen.

involved the addition of 2 equiv of the azide of the protected fragment 86–102 to the nucleophile 103–124 at 5°. The yield (based on the latter) was about 50% after purification on Sephadex G-50. The azide couplings (indicated in formula I) which were required for the preparation of fragment 86–102 proceeded without difficulty. The docosapeptide 103–124 was prepared *via* three azide couplings as shown in formula I. Because of the low solubility in dimethylformamide, the nucleophile 118–124[7] was dissolved in hexamethylphosphoramide for the azide couplings. Only the docosapeptide required gel filtration for purification.

In the final coupling reaction the nucleophilic nonatriacontapeptide 86–124 in hexamethylphosphoramide was added to a fourfold excess of the protected azide of fragment 65–85 in dimethylformamide. About 40% of the nonatriacontapeptide was converted to product at −20°. An aliquot of the product was treated with a large excess of hydrazine in dimethylformamide at room temperature for 3 min. After removal of solvents, the recovered polypeptide appeared to be unchanged and did not react with Tollens reagent. This result supports the view that the hexacontapeptide contains neither ester linkages nor amide bonds involving the imidazole ring of histidine and that the pyrimary amide groups in asparagine and glutamine are stable under these conditions.

Acknowledgment. We thank Mr. Carl Homnick for the expert execution of the amino acid analyses reported here. We are also greatly indebted to Messrs. S. M. Miller and R. Pospolita for the large-scale preparation of NCA's and NTA's.

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(7) J. E. Shields and H. Renner, *J. Am. Chem. Soc.*, **88**, 2304 (1966), have already described the synthesis of this heptapeptide.

Studies on the Total Synthesis of an Enzyme. IV. Some Factors Affecting the Conversion of Protected S-Protein to Ribonuclease S′

Sir:

In two of the three preceding communications we described the preparation of the protected tetratetracontapeptide fragment 21–64[1] of RNase A as well as the

(1) R. G. Strachan, W. J. Paleveda, Jr., R. F. Nutt, R. A. Vitali, D. F. Veber, M. J. Dickinson, V. Garsky, J. E. Deak, E. Walton, S. R. Jenkins, F. W. Holly, and R. Hirschmann, *J. Am. Chem. Soc.*, **91**, 503 (1969).