enzyme. We find that addition to S-protein[4] of synthetic lysylglutamylthreonylalanylalanylalanyllysylphenylalanylglutamylarginylglutaminylhistidylmethionine (I) ($[\alpha]^{27}D$ $-64.9°$ in 10% acetic acid; R^2_f 0.4 x his[5]; single ninhydrin, Pauly, Sakaguchi and methionine positive spot on paper electrophoresis at pH 1.9, 3.5 and 6.5; amino acid ratios in acid hydrolysate $lys_{2.09}glu_{3.09}thr_{0.95}ala_{2.95}phe_{1.04}arg_{1.00}his_{1.04}met_{0.95}$) produces a ribonuclease analog which exhibits 68–72% the biological activity of RNase-S′ with yeast RNA as substrate.[6] The reconstructed enzyme attains maximal catalytic activity when approximately 10 moles of peptide are added per mole of S-protein, but an enzyme exhibiting approximately 50% the activity of RNase-S′ results at a molar ratio of 3:1. For maximum activity (62–65% that of RNase-S′) toward uridine-2′:3′-cyclic phosphate[7] approximately 30 moles of I per mole of S-protein are required.

The following peptides, which correspond to sections of the N-terminal sequence proposed for beef RNase-A, fail to generate enzymic activity[6] upon addition to S-protein in molar ratios as high as 100 to 1: histidylmethionine (II) (*Anal.* Found: C, 46.1; H, 6.4; N, 19.4; $[\alpha]^{28}D$ $-15.4°$ in water; single ninhydrin, methionine and Pauly positive spot; R_f^1 0.31; R_f^2 2.1 x his); phenylalanylglutamylarginylglutaminylhistidylmethionine (III) ($[\alpha]^{26}D$ $-35.6°$ in 10% acetic acid; single ninhydrin, methionine, Pauly and Sakaguchi positive spot on paper electrophoresis at *p*H 1.9, 3.5 and 6.5; R_f^2 1.5 x his; amino acid ratios in acid hydrolysate $phe_{1.00}glu_{2.07}arg_{0.98}his_{0.97}met_{1.00}$); aspartylserylserylthreonylserylalanylalanine (IV) ($[\alpha]^{26}D$ $-63.5°$ in water; single ninhydrin positive spot on paper electrophoresis at *p*H 1.9, 3.5 and 6.5; amino acid ratios in acid hydrolysate $asp_{0.94}ser_{3.10}thr_{0.94}ala_{2.03}$); lysylglutamylthreonylalanylalanylalanyllysine (V) ($[\alpha]^{28}D$ $-60.8°$ in 10% acetic acid; single ninhydrin positive spot on paper electrophoresis at *p*H 1.9, 3.5 and 6.5; amino acid ratios in acid hydrolysate $lys_{2.03}glu_{0.97}thr_{0.93}ala_{3.07}$; amino acid ratios in LAP digest $lys_{2.07}glu_{0.97}thr_{0.93}ala_{3.00}$); and lysylglutamylthreonylalanylalanylalanyllysylphenylalanylglutamylarginylglutamine (VI) ($[\alpha]^{25}D$ $-79.2°$ in 10% acetic acid; single ninhydrin and Sakaguchi positive spot; R_f^2 0.4 x his; single spot on paper electrophoresis at *p*H 1.9, 3.5 and 6.5; amino acid ratios in acid hydrolysate $lys_{2.06}arg_{1.02}thr_{0.95}glu_{2.96}ala_{3.05}phe_{0.95}$). Combinations of peptides (V + III), (VI + II) and (II + IV) in the molar ratios 100:100:1 are inactive.

These results support the revised amino acid sequence for positions 1 to 13 in RNase-A[8] and demonstrate that a partially synthetic RNase from which a sizable fragment of the covalent peptide chain (amino acid residues 14 to 20) is eliminated exhibits significant enzymic activity.

Special importance has been attributed to the sequence aspartylserine for catalytic activity of a number of proteolytic and esteratic enzymes.[9] Our experiments unequivocally eliminate aspartylserine (positions 14 to 15) as essential for RNase activity. The ability of peptide I to activate S-protein and the lack of this property in peptide VI emphasizes the significance for catalytic activity of the histidylmethionine portion in the ribonuclease molecule.[10]

We have confirmed the results of Vithayathil and Richards[11] that performic acid-oxidized S-peptide when added to S-protein at a molar ratio of 3:1 regenerates 90% of the activity of RNase-S′ toward RNA. However, oxidation of I with hydrogen peroxide[12] or performic acid[13] results in marked diminution of its ability to activate S-protein against this substrate. Exposure to thioglycolic acid (45–50° for 24 hours) regenerates essentially the full activity of the hydrogen peroxide-treated peptide. These observations implicate the methionine sulfur as the site for the reversible oxidation–reduction behavior and suggest that the thioether sulfur of peptide I is important for maximal activation of S-protein. The ability of the 13α-aminobutyric acid analog of I to regenerate enzymic activity with S-protein is being investigated.

Correlation between ability of S-peptide derivatives to activate S-protein and their capacity to bind to this RNase fragment may contribute significantly toward understanding of protein–peptide interaction on the one hand and topography of the active site on the other. Studies along these lines are in progress.

Current theories pertaining to the active site of RNase may need revision and future ones will have to take into consideration the experimental findings which are presented in this communication.

Acknowledgment.—The skillful technical assistance of Mrs. Chizuko Yanaihara, Mrs. Maria Gunther, Miss Ursula Egli and Mr. John L. Humes is gratefully acknowledged.

(4) F. M. Richards, *Proc. Natl. Acad. Sci. U. S.*, **44**, 162 (1958); the abbreviations used are: RNase-S, subtilisin-modified beef ribonuclease 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 proportions of S-peptide plus S-protein; RNA, ribonucleic acid; LAP, leucine aminopeptidase.

(5) R_f^1 values refer to the Partridge system (S. M. Partridge, *Biochem. J.*, **42**, 238 (1948)); R_f^2 values refer to the system 1-butanol–pyridine–acetic acid–water, 30:20:6:24 (S. G. Waley and J. Watson, *ibid.*, **55**, 328 (1953)).

(6) RNase determinations were carried out with a Cary 14 recording spectrophotometer using a scale expander (range 0–0.1 optical density) essentially as described by M. Kunitz, *J. Biol. Chem.*, **164**, 563 (1946).

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(10) Participation of the histidine residue in position 12 in the catalytic function of RNase has been suggested by Richards.[4]

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AROMATIC SILICON SYSTEMS—A REINVESTIGATION

Sir:

In attempting to extend the investigation we recently described under the heading of Aromatic Silicon Systems,[1] we have discovered major discrepancies in the original work. While it is still too early to be able to state precisely what the difficulties are, it is clear that a significant portion to the published work cannot be duplicated. We deem it advisable to call attention to this fact immediately.

The entire work is under reinvestigation in our laboratory and we hope to report our new findings as soon as possible.

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RECEIVED FEBRUARY 8, 1963

(1) R. A. Benkeser, *et al.*, *J. Am. Chem. Soc.*, **84**, 4723, 4727 (1962). A preliminary announcement of this work appeared in *J. Am. Chem. Soc.*, **83**, 3716, 5029 (1961).