The formation of N-terminal alanine is in agreement with the latest results of Smyth, Stein, and Moore.[3] However, no traces of N-terminal tyrosine could be detected in the cleavage products. The fact that in oxytocin the cysteinyl–tyrosine bond is cleaved in a 40% yield[2] demonstrates that such sequences can be detected by our procedure. The present studies suggest the presence in ribonuclease of the sequence (Cys–Gly), possibly derived from positions 72 and 73 in the polypeptide chain.

Supporting evidence for the suggested sequence was obtained by tryptic digestion[14] of acetylated S-(β-aminoethyl)RNase (V). End-group determination by the cyanate method gave all the amino acid residues following cysteine and arginine in the published formula, except for the appearance of glycine instead of tyrosine.

In view of the above findings, we are now engaged in the determination of the origin of the apparent sequence Cys–Gly in order to elucidate whether it corresponds to positions 72–73 in the ribonuclease polypeptide chain or whether it is the product of a side reaction.

Acknowledgments.—We thank Professor E. Katchalski and Professor A. Berger for their interest in this work.

(14) M. A. Rasterg and D. Cole, *Biochem. Biophys. Res. Commun.*, **10**, 467 (1963).

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Chemical Interactions between Lysine and Dehydroalanine in Modified Bovine Pancreatic Ribonuclease[1]

Sir:

When S-dinitrophenylated reduced RNase (I)[2] was treated with alkali, and the derivative thus obtained (II) subjected to acid hydrolysis, a new amino acid could be separated from the amino acids normally present in RNase, on the short column of the Spinco automatic amino acid analyzer[3] using citrate buffer (pH 5.28). This amino acid emerged before lysine as shown in Fig. 1.

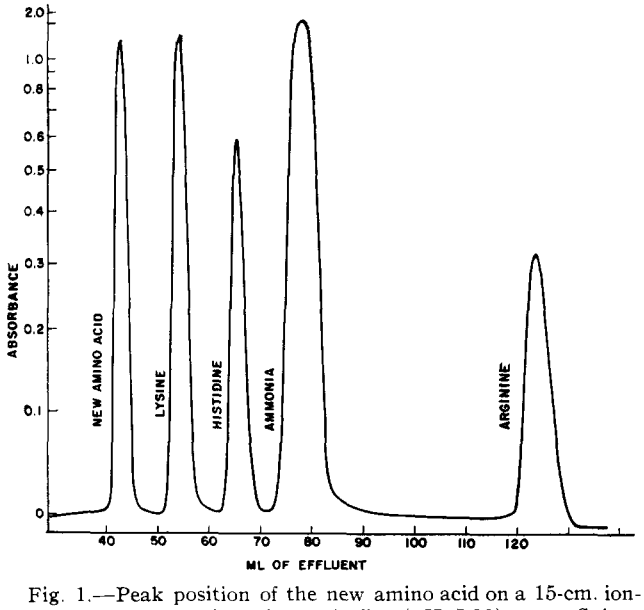
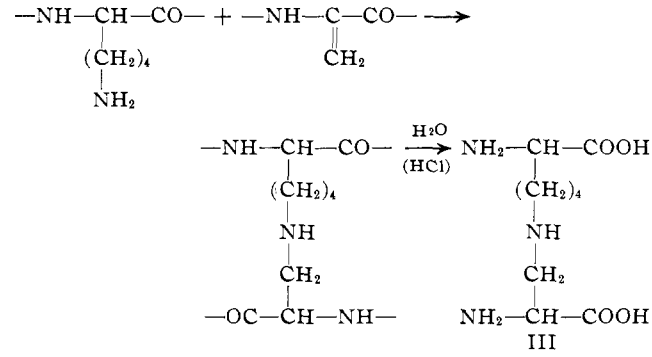


Fig. 1.—Peak position of the new amino acid on a 15-cm. ion-exchange column using citrate buffer (pH 5.28) on a Spinco automatic amino acid analyzer.

We propose that this amino acid[4] (III) is formed as a result of the reaction of ε-amino groups of lysine residues with dehydroalanine residues leading to DL-α-amino-β(-ε-N-L-lysine)propionic acid[5] according to the following scheme.



The following evidence is offered to support this proposal. When I was treated with alkali (0.1 *N* sodium hydroxide, 30 min. at room temperature), 3 moles of the new amino acid was formed on acid hydrolysis (assuming a ninhydrin color yield of twice that of leucine), and only 6.5 moles of lysine (out of the 10 moles initially present) and 5.5 moles of dehydroalanine residues (out of the 8 moles expected) were found.[6] However, fully acetylated S-DNP RNase[7] (IV), in which all the ε-amino groups of lysine residues are blocked, did not yield the new amino acid under the above conditions. Moreover, practically all the expected lysine residues (9.4 out of 10) and dehydroalanine residues (7.6 out of 8) were determined after acid hydrolysis.

It thus seems that 3 moles of the unprotected lysine residues reacted with 3 moles of dehydroalanine residues to yield 3 moles of the new amino acid.

The above reaction was also carried out on model compounds. N-Acetyl-S-DNP-L-cysteinyl-L-lysine methyl ester hydrobromide,[8] as well as a copolymer of S-DNP-cysteine and lysine (1:4),[8] yielded the new amino acid on treatment with alkali. The product obtained behaved in the automatic amino acid analyzer identically with that obtained from I, and its electrophoretic behavior was found to be as expected on the basis of the proposed structure.

The new amino acid (LAL) was also formed when S-DNP lysozyme and S-DNP cytochrome C were exposed to 0.1 *N* NaOH and totally hydrolyzed. Native RNase under these conditions also yielded LAL (see

(1) This investigation was supported by Research Grant AM-5098 from the National Institutes of Health, U. S. Public Health Service.

(2) M. Sokolovsky and A. Patchornik, *J. Am. Chem. Soc.*, **86**, 1859 (1964).

(3) Quantitative amino acid analyses were performed on a Spinco automatic amino acid analyzer.

(4) The same amino acid seems to have been isolated from alkali treated RNase by Z. Bohak, who also established its structure [private communication, Zvi Bohak at the Rockefeller Institute, on leave from the Hebrew University, Jerusalem.]

(5) We wish to propose the name Lysalanine (abbreviated LAL) for the new amino acid.

(6) A. Patchornik and M. Sokolovsky, *J. Am. Chem. Soc.*, **86**, 1206 (1964).

(7) The abbreviation used is DNP, 2,4-dinitrophenyl.

(8) M. Sokolovsky, Ph.D. Thesis submitted to the Hebrew University, Jerusalem, Israel, 1963.

TABLE I

DEHYDROALANINE, LYSINE, AND NEW AMINO ACID CONTENT OF MODIFIED RNase

Compound	Moles of dehydroalanine per mole of protein[a] Calcd.	Found	Moles of lysine Calcd.	Found	Moles of compound III
S-DNP RNase	8	5.5	10	6.5	3
Acetylated S-DNP RNase	8	7.6	10	9.4	0
Native RNase	...	2.2	10	8.5	2.1
Oxidized RNase[9]	...	0.2	10	9.6	0

[a] The amount of dehydroalanine present was determined by estimation of the pyruvic acid formed upon acid hydrolysis.

Table I). This protein, as do cystine derivatives,[10] at high pH yields dehydroalanine which can undergo the reaction described above. Oxidized RNase, which does not undergo β-elimination of its cysteic acid residues, yields almost no dehydroalanine and no LAL. The finding[11] that alkali-treated wool is no longer soluble in cuprammonium–sulfite–urea mixture may be due to the fact that the above reaction between the ϵ-group of lysine and dehydroalanine residues results in extensive cross linking.

Application of the specific scission of the dehydroalanine residues in peptide chains makes it possible to determine at which points of the peptide chain the above reaction occurs. Evidently only the residues next to the carboxyl group of unreacted dehydroalanine residues will appear as N-terminal end groups after the specific fragmentation. A comparison of N-terminal end groups appearing after cleavage of acetylated protein will, therefore, indicate the dehydroalanine residues which reacted with ϵ-amino groups of lysine. The N-terminal groups[12] present after cleavage of RNase derivatives are listed in Table II.

TABLE II

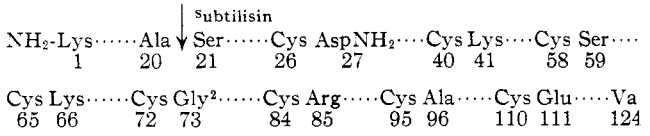
YIELDS OF N-TERMINAL RESIDUES AFTER CLEAVAGE OF RNase DERIVATIVES

Amino acid	Mole/mole of S-DNP RNase (I)	Mole/mole of acetylated S-DNP RNase (IV)	Mole/mole of S-DNP "S-protein"[13]
Lysine	0.07	0.95	0.02
Arginine[a]	0.16	0.15	..
Aspartic acid	0.31	0.34	0.34
Serine	0.47	0.42	1.34
Glutamic acid	0.20	0.52	0.40
Alanine[b]	0.26	0.24	0.23
Glycine[b]	0.42	0.36	0.36

[a] Arginine was found to give low yields by the cyanate method as found with arginine amide. [b] Values corrected for the nonspecific formation of additional quantities of these amino acids in the cyanate method.[8]

The N-terminal amino groups appearing after cleavage of IV have been described previously.[2] It can be seen that the only differences between the acetylated and nonacetylated derivative are in the lysine and glutamic acid values.

(9) C. H. W. Hirs, *J. Biol. Chem.*, **219**, 611 (1956).
(10) R. Cecil and J. R. McPhee, *Advan. Protein Chem.*, **14**, 306 (1959).
(11) J. M. Swan, *Australian J. Chem.*, **14**, 69 (1961).
(12) The N-terminal groups were identified by the cyanate method [G. H. Stark and D. G. Smyth, *J. Biol. Chem.*, **238**, 214 (1963)].
(13) F. M. Richards and P. J. Vithayathil, *ibid.*, **234**, 1459 (1959).



The nonappearance of Lys-41 and Lys-66 (see abbreviated formula of reduced RNase) and the decrease in the value for Glu-111 indicate that the dehydroalanine residues derived from Cys-40, Cys-65, and Cys-110 were involved in the reaction described. The dissappearance of Lys-1 also implicates this residue in the reaction. It seems probable that, because of spatial proximity, the pairs 40–41 and 65–66, respectively, reacted (*cf.* the reaction of N-acetyl-S-DNP-L-cysteinyl-L-lysine methyl ester hydrobromide quoted above). The third pair would then be Lys-1 and dehydroalanine-110. A specific interaction of two residues so remote from each other may be due to a specific spatial configuration. This view is supported by the finding that reduced and S-dinitrophenylated "S-protein," in which residues 1 to 20 are absent, gave a yield of N-terminal Glu-111 similar to that obtained from IV (see Table II; in this case an additional serine group due to residue 21 is found).

The approach described here might be of possible value as a tool in the determination of the chemical topography within a protein molecule.

Acknowledgments.—We thank Professor E. Katchalski and Professor A. Berger for their interest in this work.

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Formyl, a Novel NH_2-Terminal Blocking Group in a Naturally Occurring Peptide. The Identity of *seco*-Gramicidin with Desformylgramicidin[1,2]

Sir:

Gramicidin, a peptide antibiotic which contains no free amino or carboxyl group, was isolated in 1941 by Hotchkiss and Dubos,[3] who initially assumed a cyclic structure on the basis that hydrolysates of gramicidin contained only neutral amino acids, but no fatty acids or alcohols.[4] Later the discovery of ethanolamine in gramicidin hydrolysates prompted Synge[5] to propose an *ortho*-peptide bond[6] for the linkage of ethanolamine to explain the neutral character of gramicidin and the free hydroxyl, the only functional group present.

We have now found that gramicidin A[7] contains an N-formyl group. Two-hour hydrolysis of gramicidin at 110° in 50% sulfuric acid, followed by distillation at room temperature under high vacuum into a trap cooled to −80°, yielded a solution of formic acid in water as distillate. The formic acid was identified

(1) Gramidicin A. III. Preceding papers: S. Ishii and B. Witkop, *J. Am. Chem. Soc.*, **85**, 1832 (1963); **86**, 1848 (1964).
(2) Presented in part at the IUPAC Meeting, Kyoto, April, 1964.
(3) R. D. Hotchkiss and R. J. Dubos, *J. Biol. Chem.*, **141**, 155 (1941).
(4) R. D. Hotchkiss, *ibid.*, **141**, 171 (1941).
(5) R. L. M. Synge, *Biochem. J.*, **39**, 355 (1945).
(6) R. L. M. Synge, *Cold Spring Harbor Symp. Quant. Biol.*, **14**, 191 (1950).
(7) J. D. Gregory and L. C. Craig, *J. Biol. Chem.*, **172**, 839 (1948).