

method¹¹ using *d*-ribose as standard, give the ratios: base/sugar/phosphorus = 1:0.99:0.96.

TABLE II

Bases and nucleotides	RF in HCl-Isopropanol ^a
Guanine	0.35
Hypoxanthine	0.40
Adenine	0.55
Compound III	0.55
CMP	0.61
UMP	0.68
TMP	0.80

It is concluded that the material isolated is an adenine ribonucleoside-monophosphate peptide. The peptide, as far as we know, has not previously been described in this form.

It is possible that these nucleotide peptides are involved in protein synthesis, as has been postulated by HOAGLAND¹² for active amino acids. If this is the case, these peptides must be in an active form when bound to a nucleotide, and should be considered as "activated" peptides.

The sequence of the amino acids and the type of union between the nucleotide and the peptide is under investigation in this laboratory.

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Received August 17th, 1960

Biochim. Biophys. Acta, 44 (1960) 381-383

The amino acid sequence of peptide B of co-fibrin

Evidence obtained from peptide fragments identified in pepsin digests of desulfated Peptide B is presented here in support of a proposed complete amino acid sequence for this peptide.

Peptide B is one of the two large acidic peptides released from the N-terminal portion of bovine fibrinogen through the hydrolytic action of bovine thrombin¹. The complete amino acid sequence of the other acidic peptide, Peptide A, has been

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Proposed sequence:	$\text{N-acetyl-Thr-Glu-Phe-Pro-Asp-Tyr-Asp-Glu-Gly-Glu-Asp-Arg-Pro-Lys-Val-Gly-Leu-Gly-Ala-Arg}$ $\text{SO}_4 \text{---}$	
Peptide fragments identified in peptic digests	<div>N-acetyl-Thr-Glu (Thr-Glu, Phe, Pro, Asp)Tyr Phe</div> <div>Pro-Asp-Tyr Pro-Asp-Tyr(Asp, Glu) Tyr-Asp-Glu Tyr-Asp-Glu-Gly(Glu, Asp) Tyr-Asp-Glu-Gly(Glu, Asp, Asp, Arg, Pro, Lys, Val, Gly)Leu Asp-Glu Gly-Glu-Asp Gly-Glu-Asp-Asp(Arg, Pro, Lys, Val, Gly)Leu Asp-Arg-Pro-Lys-Val-Gly-Leu Gly-Ala-Arg</div>	
Fragments in chymotryptic digests ³	(Thr, Phe, Tyr, Glu ₃ , Asp ₄ , Arg, Pro ₂ , Lys, Val, Gly ₂)Leu Gly-Ala-Arg	
Fragments in tryptic digests ³	(Thr, Phe, Tyr, Glu ₃ , Asp ₄ , Arg, Pro ₂ , Gly, Lys) Val-Gly-Leu-Gly-Ala-Arg	

Fig. 1. Summary of data leading to the proposed amino acid sequence of Peptide B.

reported². A partial sequence of Peptide B was formulated from fragments obtained following digestion by trypsin and chymotrypsin³. Recently BLOMBÄCK AND SJÖQUIST⁴ have reported the location of several more amino acid residues in this peptide.

The proposed amino acid sequence of Peptide B is presented in Fig. 1 along with a listing of peptide fragments which have been identified in peptic digests of the desulfated peptide.

The sulfate was quantitatively removed from the tyrosine sulfate residue of peptide B⁵ without cleavage of peptide bonds by treatment with 1 N HCl at 25° for 20 h⁶. Peptic digestion was carried out at 37° in 0.01 N HCl with a 1:150 molar ratio of enzyme to substrate. Peptide fragments were separated and purified by chromatography on Dowex 50,X2 followed by paper chromatography or paper electrophoresis.

The peptide fragment, which appears to be N-acetyl-Thr-Glu, was isolated and identified by methods similar to those employed by NARITA for studies of the N-acetyl dipeptide from digests of tobacco-mosaic-virus protein⁷. A 72-h peptic digest of Peptide B was passed into a column of Dowex 50 (X2, H⁺ form). The ninhydrin-negative fragment was detected by chromatographic analysis of acid-hydrolyzed portions of water-effluent fractions; equimolar quantities of glutamic acid and threonine were found in the hydrolyzates. Treatment of the fragment with high concentrations of carboxypeptidase A for long periods resulted in the release of small amounts of glutamic acid. Chromatography of a hydrazinolysate of this ninhydrin-negative peptide fragment, employing the pyridine-aniline-water and collidine-water systems of NARITA⁷, led to the identification of free glutamic acid (R_F , 0.30 and 0.0 in the two systems, respectively) and threonine hydrazide (R_F , 0.55 and 0.15, respectively). A compound having the R_F of acetyl hydrazide was observed in each chromatographic system in approximately equimolar concentration to threonine hydrazide.

N-acetylseryltyrosine has been identified in the α -melanocyte-stimulating hormone of pig pituitary⁸ as well as in tobacco-mosaic-virus protein⁷ as the N-terminal sequence. The present work strongly supports the existence of an N-acetyl blocking group at the N-terminus of Peptide B, and hence at one of the N-termini of bovine fibrinogen. Furthermore, the presence of this grouping explains the previous failure to find an N-terminal residue in Peptide B by usual methods of N-terminal analysis^{1,3,4}.

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Received September 5th, 1960