COMMUNICATIONS TO THE EDITOR

STUDIES ON POLYPEPTIDES. VI. SYNTHETIC CONFIRMATION OF *N*-TERMINAL, AMINO ACID SEQUENCE OF CORTICOTROPIN-A[1]

Sir:

White and Landmann[2] isolated from a 24-hour peptic digest of hog corticotropin-A a pentapeptide to which they assigned the structure of seryltyrosylserylmethionylglutamic acid (I). The fact that seryltyrosine had been previously identified as the *N*-terminus of corticotropin-A,[3,4] characterized the pentapeptide (I) as the *N*-terminal sequence of this pituitary hormone. The same *N*-terminal amino acid sequence is also present in sheep α-corticotropin[5] and porcine β-corticotropin.[6] Since the ultimate proof of the structure of the pentapeptide (I) depended on a comparison of the natural material with a synthetic specimen of established chemical structure, we have undertaken the synthesis of (I) by methods known not to cause racemization.

Carbobenzoxymethionylglutamic acid, diethyl ester, was prepared from carbobenzoxymethionine and diethyl glutamate, by the mixed anhydride procedure, and this material was converted into methionylglutamic acid (II), $[\alpha]^{25}D$ $+16.6°$ (in *N* HCl). *Anal* Calcd. for $C_{10}H_{18}O_5N_2S$: C, 43.2; H, 6.5; N, 10.1. Found: C, 43.5; H, 6.7; N, 10.0, by saponification and decarbobenzoxylation. The dipeptide (II) was then treated with carbobenzoxyserine azide[7] to give carbobenzoxyserylmethionylglutamic acid. Serylmethionylglutamic acid (III), from ethanol, $[\alpha]^{25}D$ $-26.1°$ (in H_2O). *Anal.* Calcd. for $C_{13}H_{23}O_7N_3S$, C_2H_5OH: C, 43.8; H, 7.1; N, 10.2. Found: C, 44.5; H, 6.7; N, 10.8, was prepared from the acylated tripeptide by reduction with sodium in liquid ammonia. The coupling of (III) with the azide of carbobenzoxyseryltyrosine afforded carbobenzoxyseryltyrosylserylmethionylglutamic acid which was again decarbobenzoxylated with sodium in liquid ammonia, to form (I). The crude pentapeptide was repeatedly precipitated from water by ethanol, and was finally obtained as its crystalline monohydrate by slowly cooling a concentrated aqueous solution: $[\alpha]^{25}D$ $-20.6°$ (in 2 *N* HCl). *Anal.* Calcd. for $C_{25}H_{37}O_{11}N_5S$, H_2O: C, 47.4: H, 6.2; N, 11.1. Found: C, 47.0; H, 6.4; N, 11.6. The peptide gave a positive ninhydrin reaction and produced a dark purple color with diazotized sulfanilic acid in sodium carbonate solution.

The paper chromatographic comparison of the synthetic product with the natural material in the Partridge[8] and 2-butanol–ammonia systems,[9] revealed identical behavior of both compounds. Their R_f value in the Partridge system was 0.51, and both peptides appeared between glutamic acid and lysine in the latter system. Paper chromatography of a 1:1 mixture of the peptides produced one spot only.

The behavior of the synthetic specimen on treatment with carboxypeptidase and aminopeptidase[10] duplicated that observed with the natural material.[2] The four constituent amino acids were liberated in the expected molar ratios by both enzymes. The recovery of serine from an acid hydrolyzate (18 hours at 105° with 6 *N* HCl) was only 90% of the theoretical, reflecting the well-known lability of this compound to acid. Excellent recoveries of tyrosine, methionine, and glutamic acid were realized.[11]

These results establish the structure and optical purity of the synthetic peptide, and substantiate structure (I) for the pentapeptide resulting from the peptic digestion of corticotropin-A. A detailed description of our experiments will be presented at a later date.

(1) The authors wish to express their appreciation to Armour and Company for their generous support of this investigation.

(2) W. F. White and W. A. Landmann, THIS JOURNAL, **77**, 771 (1955).

(3) W. A. Landmann, M. P. Drake and W. F. White, *ibid.*, **75**, 4370 (1953).

(4) W. F. White and W. A. Landmann, *ibid.*, **76**, 4193 (1954).

(5) J. Harris and C. H. Li, *ibid.*, **76**, 3607 (1954).

(6) P. H. Bell, *ibid.*, **76**, 5565 (1954).

(7) J. S. Fruton, *J. Biol. Chem.*, **146**, 463 (1942).

(8) S. M. Partridge, *Biochem. J.*, **42**, 238 (1948).

(9) J. Roland and A. Gross, *Anal. Chem.*, **26**, 502 (1954).

(10) D. H. Spackman, E. L. Smith and D. M. Brown, *J. Biol. Chem.*, **212**, 255 (1955).

(11) We wish to express our thanks to Dr. W. F. White of the Armour Laboratories for comparing these properties of the natural and synthetic peptides.

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OXYGEN TRANSFER AND ELECTRON TRANSPORT BY THE PHENOLASE COMPLEX[1]

Sir:

The metabolic role of the phenolase complex[2] is controversial, particularly in respect to an hypothesis suggesting that this enzyme system catalyses terminal transfer of electrons to oxygen.[3] In order to throw some light on the problem and to understand the molecular events occurring at the catalytic configuration of the enzyme complex, we have studied its hydroxylative phase using O^{18}_2 and H_2O^{18} as tracers.

We find that all oxygen enzymically introduced as hydroxyl into the benzene ring of the substrate comes from molecular oxygen (Table I). None comes from solvent water. Hydroxylation mechanisms inconsistent with this observation[4] must accordingly be incorrect. The phenolase complex is an *oxygen transferase.*

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(2) We mean by "phenolase complex" that pair of enzymic activities occurring together, associated with copper-protein, and responsible for phenol *o*-hydroxylation and for dehydrogenation of *o*-diphenols to *o*-quinones.

(3) W. O. James, "Plant Respiration," Oxford Press, New York, N.Y., 1953.

(4) D. Kertesz, *Biochim. et Biophys. Acta*, **9**, 170 (1952).