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.

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(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.

BIOCHEMISTRY DEPARTMENT
UNIVERSITY OF PITTSBURGH
SCHOOL OF MEDICINE
PITTSBURGH, PENNSYLVANIA

KLAUS HOFMANN
ALBERT JÖHL

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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.*

(1) This study has been supported by a grant from the United States Public Health Service (C-2291).

(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.

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TABLE I

COMPARISON OF O^{18} IN 4,5-DIMETHYLCATECHOL FORMED ENZYMICALLY FROM 3,4-DIMETHYLPHENOL[a] IN $O^{18}{}_2$[b] AND H_2O AND IN O_2 AND H_2O^{18}[c]

Experiment	Found	Atom % excess O^{18}[d] theoretical for uptake of one atom	No uptake
$O^{18}{}_2 + H_2O$	0.52	0.59	0.00
	.51		
	.56		
$O_2 + H_2O^{18}$	.00	0.59	0.00
	.00		

[a] Twenty-five ml. reaction volumes contained 0.3 mmole ascorbic acid, 1.3 mmole KH_2PO_4, 2.15 mmole K_2HPO_4, 0.45 mmole 3,4-dimethylphenol and 4.0 mg. purified[5] mushroom phenolase having 20–80 cresolase[6] and *ca.* 1000 catecholase[7] units/mg. dry wt. 4,5-Dimethylcatechol (30–50% yield) was isolated through its lead salt, from an ether extract of the reaction mixture, m.p. 84–86°. No hydroxylation occurred in the system when heat-denatured enzyme was substituted for active protein. [b] Prepared electrolytically. [c] Obtained from the Stuart Oxygen Company, containing 1.4 atom % O^{18}. [d] Mass spectrometry was performed by the Consolidated Engineering Corporation on carbon dioxide samples obtained by Unterzaucher pyrolysis[8] of 4,5-dimethylcatechol samples. Oxygen recovery was quantitative.

Since the phenolase complex is a cuprous protein[9,10,11,12] which is in the cupric form after each hydroxylation[13] and which combines with inhibitor CO in the ratio 2 Cu^+/CO,[9] hydroxylation by this enzyme system is describable as

(1) $\text{Protein}\langle^{Cu^+}_{Cu^+} + O_2 \longrightarrow \text{Protein}\langle^{Cu\text{---}}_{Cu\text{---}}{}^{O\text{---}O}$

(2) $\text{Protein}\langle^{Cu\text{---}}_{Cu\text{---}}{}^{O\text{---}O}$ + Monophenol $\longrightarrow$

$\text{Protein}\langle^{Cu^{++}}_{Cu^{++}}$ + Diphenol + H_2O

(3) $\text{Protein}\langle^{Cu^{++}}_{Cu^{++}} + 2e^- \longrightarrow \text{Protein}\langle^{Cu^+}_{Cu^+}$

The hydroxylative function of phenolase (eq. 1 and 2) is thus coupled to an electron source (eq. 3), *i.e.*, oxidation of *o*-diphenol to *o*-quinone, which may be linked in turn to the common pathways of metabolism through $TPNH^{+}$[14] or $DPNH^{+}$[15], possibly by quinone reductase.[16] The function of the phenolase complex as a terminal oxidase will be in demand during the biosynthesis of *o*-diphenols from monophenols. We propose that these *o*-diphenols are subsequently utilized to form flavonoids, lignins, tannins, cuticulation diphenols of arthropods, melanoproteins of chordates, and possibly adrenaline and noradrenaline.[12] Some instances of light-irreversible inhibition of terminal respiration by carbon monoxide[17,18] may be accounted for in these terms.

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DEPARTMENT OF BIOCHEMISTRY H. S. MASON
UNIVERSITY OF OREGON MEDICAL SCHOOL W. L. FOWLKS
PORTLAND, OREGON E. PETERSON

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BOOK REVIEWS

Annual Review of Physical Chemistry. Volume 5. G. K. ROLLEFSON, Editor, University of California, and R. E. POWELL, Associate Editor, University of California. Annual Reviews, Inc., Stanford, California. 1954. ix + 540 pp. 16 × 23 cm. Price, $7.00.

The fifth volume of these reviews maintains the excellence of former years. This series is now becoming very well known. Every chemistry and physics library must have these volumes on their shelves. Not only physical chemists and chemical physicists, but spectroscopists, nuclear, radiation and solid state physicists as well as biologists will do well to add this set to their book collection. These surveys offer the best possible means for keeping abreast in the many fields covered. The present volume includes the following topics: Thermochemistry and the Thermodynamic Properties of Substances, Heterogeneous Equilibria and Phase Diagrams, Solutions of Electrolytes, Solutions of Nonelectrolytes, Isotopes, Radioactivity and Nuclear Structure, Radiation Chemistry, Theory of Molecular Structure and Spectra, Spectroscopy, The Solid State, Kinetics of Reactions in Solution, Kinetics of Reactions in Gases, Properties of Macromolecules in Solution, Colloid Chemistry, Cryogenics, Nuclear Magnetic Resonance, Crystallography, Surface Chemistry and Catalysis, The Microwave Spectra of Gases, Experimental Molecular Structure, Ion Exchange, Statistical Mechanics of Transport and Non-equilibrium Processes, Modern Aspects of Electrode Kinetics.

The literature survey covers the year 1953. Some reviewers have chosen a few important papers, which are discussed at greater length. A complete coverage of all papers for the year would take too much space. The total number of literature citations is 3422! Naturally the style and mode of presentation differs for the various reviews. However, all of them are quite readable.

The authors and editors are to be congratulated upon this excellent compilation.

OFFICE OF ORDNANCE RESEARCH
BOX CM, DUKE STATION GEORGE GLOCKLER
DURHAM, N. C.