[1] D. T. GISH, L. K. RAMACHANDRAN AND W. M. STANLEY, *Arch. Biochem. Biophys.*, 78 (1958) 433.
[2] L. K. RAMACHANDRAN AND D. T. GISH, *J. Amer. Chem. Soc.*, 81 (1959) 884.
[3] J. E. FOLK AND J. A. GLADNER, *J. Biol. Chem.*, 231 (1958) 398.
[4] A. PATCHORNIK, W. B. LAWSON AND B. WITKOP, *J. Amer. Chem. Soc.*, 80 (1958) 4747.
[5] A. C. CHIBNALL AND R. G. WESTALL, *Biochem. J.*, 26 (1932) 122.
[6] H. WILSON AND R. CANNAN, *J. Biol. Chem.*, 119 (1937) 309.
[7] P. B. HAMILTON, *J. Biol. Chem.* 158 (1945) 375.
[8] K. NARITA *Biochim. Biophys. Acta*, 30 (1958) 352.
[9] H. WILSON AND R. CANNAN, *J. Biol. Chem.*, 119 (1937) 309.
[10] P. B. HAMILTON, *J. Biol. Chem.*, 158 (1945) 375.
[11] D. H. SPACKMAN, W. H. STEIN AND S. MOORE, *Anal. Chem.*, 30 (1958) 1190.
[12] W. E. STONE, *Proc. Soc. Exptl. Biol. Med.*, 93 (1956) 589.
[13] T. W. GOODWIN AND R. A. MORTON, *Biochem. J.*, 40 (1946) 628.
[14] J. R. SPIES AND D. C. CHAMBERS, *Anal. Chem.*, 21 (1949) 1249.
[15] H. FRAENKEL-CONRAT AND B. SINGER, *J. Amer. Chem. Soc.*, 76 (1954) 180.
[16] L. K. RAMACHANDRAN, *Biochim. Biophys. Acta*, 32 (1959) 557.
[17] R. G. SHEPHERD, K. S. HOWARD, D. S. DAVIES, E. A. EIGNER AND N. E. SHAKESPEARE, *J. Am. Chem. Soc.*, 78 (1956) 5067.
[18] C. H. W. HIRS, S. MOORE AND W. H. STEIN, *J. Biol. Chem.*, 219 (1956) 623.

Received July 20th, 1959

Enzymic formation of adenylserine and an unknown carboxyl-activated compound

The first step in protein synthesis is thought to be a carboxyl activation of amino acids. This reaction[1], which is analogous to that for activation of fatty acids, is as follows:

$$\text{Enzyme} + \text{ATP} + \text{amino acid} \overset{Mg^{++}}{\rightleftharpoons} \text{enzyme (adenyl amino acid)} + \text{pyrophosphate}$$

This communication deals with the identification of adenylserine and the observation of an unknown carboxyl-activated compound as products of a reaction mixture containing large amounts of a purified serine-activating enzyme[2].

20–50 mg enzyme, 0.2 μmole of DL-[3-^{14}C]serine containing 120,000 counts/min, 1–2 μmoles ATP, $MgCl_2$, Tris(hydroxymethyl)aminomethane buffer (pH 7.4), and crystalline pyrophosphatase were incubated in 2.7 ml at 37° for 5 min. After the enzyme was precipitated by cold trichloroacetic acid, the supernatant was adjusted to pH 3.0 and chromatographed at 4° on Dowex-1.

Two radioactive compounds were separated from serine (Fig. 1). Compound I (Peak I) chromatographed identically with synthetic adenylserine and contained 990–1200 counts/min. When incubated in neutral NH_2OH, it formed another compound which chromatographed on Dowex-50 identically with synthetic serine hydroxamate. Furthermore, Compound I incorporated label simultaneously from [8-^{14}C]ATP and [3-^{14}C]serine and the stoichiometry of this incorporation was 1:1. Radioactivity from either DL-[1-^{14}C]serine or [α-^{32}P]ATP was also incorporated into Compound I. When Compound I, derived from [α-^{32}P]ATP, was subjected to paper electrophoresis in 0.05 *M* citrate buffer, pH 3.9, the radioactivity migrated toward the cathode and coincided with the ultraviolet absorption of synthetic adenylserine. The foregoing evidence strongly suggests that Compound I was adenylserine. This represents the second instance in which an amino acid acyl adenylate has been isolated from an enzymic reaction mixture and identified[3,4].

Abbreviations: ATP, adenosine triphosphate; RNA, ribonucleic acid.

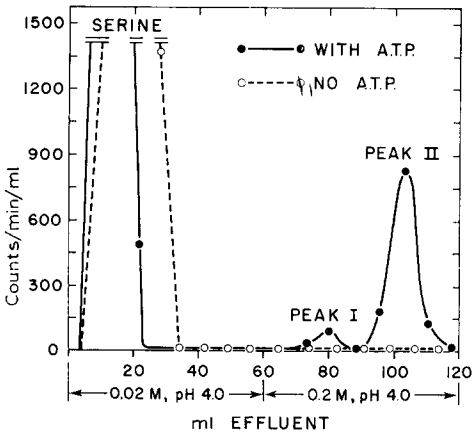

Fig. 1. Separation on Dowex-1 (acetate) of DL-[3-^{14}C]serine from two other compounds. (Radioactivity eluted with ammonium formate buffers and determined with a Tri-Carb qliuid scintillation counter.)

Compound II (Peak II, Fig. 1) contained 4,800 to 12,000 counts/min when either DL-[1-^{14}C]serine or DL-[3-^{14}C]serine were used as substrates. It was stable to ribonuclease and to incubation in 0.1 *N* alkali or 1.0 *M* neutral NH_2OH (38°, 15 min). It separated chromatographically from O-phosphoserine and was converted to serine by acid hydrolysis. When either [8-^{14}C]ATP, [α-^{32}P]ATP, or [β,γ-$^{32}P_2$]ATP were used as substrates, radioactivity was not incorporated into this compound. Paper electrophoresis at pH 3.9 indicated a strong negative charge. The compound derived from [3-^{14}C]serine did not form H^{14}CHO with periodate indicating that the amino or hydroxyl group of serine was blocked.

Compound II, which is not carboxyl activated, is apparently derived from a carboxyl-activated compound in the trichloroacetic acid supernatant. For example, Compound II disappeared when NH_2OH was incubated with the supernatant prior to chromatography. Under these conditions the supernatant yielded approximately the same amount of ^{14}C in serine hydroxamate as was isolated in Compounds I and II. The unknown carboxyl-activated compound probably is not RNA serine because preincubation of the enzyme with ribonuclease to digest contaminating RNA did not prevent appearance of Compound II. Neither is it a small nucleotide complex (*e.g.*, a derivative of adenylserine) because the appearance of Compound II was unaffected by charcoal treatment of the TCA supernatant. Attempts to isolate and characterize this carboxyl-activated compound and to establish its biological role are in progress.

This investigation was partially supported by The Division of Research Grants of the National Institutes of Health, Public Health Service, RG-5303-Cl.

Department of Biochemistry, School of Medicine, Western Reserve University, Cleveland, Ohio (U.S.A.)

LESLIE T. WEBSTER, JR.*
EARL W. DAVIE

[1] M. B. HOAGLAND, E. B. KELLER AND P. C. ZAMECNIK, *J. Biol. Chem.*, 218 (1956) 315.
[2] L. T. WEBSTER, JR. AND E. W. DAVIE, *Federation Proc.*, 18 (1959) 348.
[3] H. S. KINGDON, L. T. WEBSTER, JR. AND E. W. DAVIE, *Proc. Natl. Acad. Sci. U.S.*, 44 (1958) 757.
[4] M. A. KARASEK, P. CASTELFRANCO, P. R. KRINSHNASWAMY AND A. MEISTER, *J. Am. Chem. Soc.*, 80 (1958) 2335.

Received July 21st, 1959

* Senior Research Fellow, SF-309, Public Health Service.