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# Coenzyme A Structure Delineated, Biochemical Behavior Partially Explained

CHICAGO.—Progress in the study of coenzyme A (CoA), pursued in the laboratory of its discoverer, Fritz Lipmann, and in other locations, has proceeded far enough since the actual discovery in 1945 that a final structure has been established and evidence in support of the structural formula is rapidly accumulating. CoA has now been purified to the extent that a product well over 90% in activity has been isolated. The coenzyme is isolated from a *Streptomyces fradiae* fermentation, and purified through steps involving adsorption and elution from charcoal, reduction with zinc and hydrochloric acid, and chromatography in a resin column.

Research reported by G. David Novelli of Massachusetts General Hospital at the recent meeting of the American Society of Biological Chemists here has established that coenzyme A is a fairly typical dinucleotide having at one end of the molecule some features in common with TPN. It consists of the vitamin, pantothenic acid, joined through a peptidic link to $\beta$-mercaptoethylamine. Assuming one molecule of pantothenate per mole of CoA analysis indicates that the product contains one mole of adenine, three moles of phosphate of which one mole is a monoester, and one mole of sulfur. The structure was established through a combination of enzymatic degradation and resynthesis experiments.

It has been determined that CoA can be produced from pantetheine through the action of several catalysts. An orderly stepwise biosynthesis can be carried out in the supernatant of avian or mammalian liver. The first reaction is a phosphorylation of pantetheine by ATP catalyzed by pantetheine kinase, to yield phosphopantetheine. The latter condenses with ATP, splitting out inorganic pyrophosphate and forming dephospho-CoA through a new pyrophosphate bridge between adenylic acid and phosphopentetheine. Finally, dephospho-CoA is again phosphorylated by ATP on the ribose moiety to form CoA.

Large-scale preparation of the enzyme has become a possibility since the discovery that both rat and hog supernatants are excellent sources of enzyme. The easy availability of hog liver allows production in large quantities by a procedure involving precipitation with protamine sulfate, ammonium sulfate fractionation, and adsorption of inactive protein with calcium phosphate gel.

The reactive site of CoA, that part of the molecule which enters into reciprocal action with the substrate, has been shown to be the sulfhydryl group. This discovery evolved from work on active acetate; the latter material, according to F. Lynen of the University of Munich, Germany, is now believed to be an acetylated derivative of CoA, although proof is still lacking.

During attempts to isolate active acetate from respiring yeast, a test for the detection of active acetate was based on enzymatic reaction with sulfanilamide. In the presence of a fraction of pigeon liver, it was found, the acetylation of sulfanilamide increases with increasing additions of active acetate. Since this reaction is quantitative, purification of active acetate can be accomplished through this procedure. Chemical investigation of crude preparations as well as other preparations about 40% pure showed that the active substance is identical with S-acetyl-coenzyme A. Proof of structure was secured by several means, among which was a consideration of the kinetics of color development in the nitroprusside reaction and another the change in short ultraviolet light absorption after exposure to alkali. Acetyl-coenzyme A was thus shown to be an acyl mercaptan, and its extraordinary susceptibility to alkali was attributed to the splitting of the thioester bond.

The peculiarity of the acyl mercaptan bond is the fact that in spite of the high energy of hydrolysis it is completely stable in aqueous solution at neutral pH and room temperature, a property which permits the functioning of these compounds in cellular metabolism. Under physiological conditions the energy of the bond is realized only in the presence of specific proteins.

**Participants in the symposium on coenzyme A were: H. Beinert of the University of Wisconsin; Mary E. Jones of Massachusetts Hospital; and symposium chairman, Fritz Lipmann of Harvard Medical and the Massachusetts General Hospital**