A NEW COENZYME FOR PHOSPHORYLATION*

by

PADMASINI AYENGAR, DAVID M. GIBSON AND D. R. SANADI

Institute for Enzyme Research, University of Wisconsin, Madison, Wisconsin (U.S.A.)

The system which couples the breakdown of succinyl coenzyme A to the phosphorylation of adenosine diphosphate (ADP) consists of at least two distinct enzyme components (designated A and B). The formation of an intermediate in this reaction, involving a trace contaminant in commercial ADP, was suggested by the liberation of CoA from succinyl CoA in amounts proportional to but not stoichiometric with ADP[1]. This conclusion has been confirmed by the isolation of a nucleotide which is essential for the phosphorylation reaction (Equation 1).

$$\text{Succinyl CoA} + \text{ADP} + P_i \rightleftarrows \text{ATP} + \text{Succinate} + \text{CoA} \qquad (1)$$

The reaction is followed spectrophotometrically at 340 mμ by coupling with the α-ketoglutaric dehydrogenase system (Equation 2). It is seen from curve 1, Fig. 1 that when highly purified CoA

$$\alpha\text{-Ketoglutaric acid} + \text{CoA} + \text{DPN}^+ \rightleftarrows \text{Succinyl CoA} + \text{DPNH} + H^+ + CO_2 \qquad (2)$$

and ADP are used, the system has an absolute requirement for the phosphorylation coenzyme (CoP). As shown in Table I the same requirement obtains for the synthesis of succinyl CoA from ATP, CoA and succinate (reverse reaction) as measured by the disappearance of –SH of CoA. The presence of CoP in the CoA preparation[3] as well as in commercial ATP is indicated by the data.

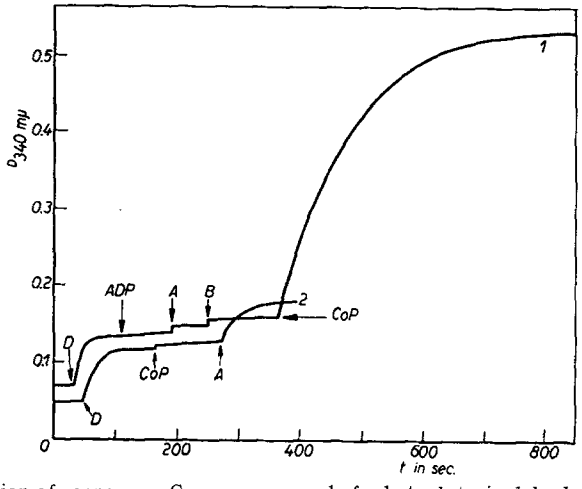

Fig. 1. Demonstration of coenzyme. Curve 1: 0.01 ml of α-ketoglutaric dehydrogenase (D) was added to a mixture of 5 μmoles of KG, 0.1 mg CoA, 5 μmoles of cysteine, 1 μmole of DPN, 10 μmoles of $MgCl_2$ and 100 μmoles of phosphate in 3.0 ml. The other additions were 0.15 μmole of pure ADP, 0.4 mg enzyme A, 0.25 mg enzyme B and 0.015 μmole CoP as indicated. The optical density was followed continuously in the Beckman spectrophotometer with automatic recording arrangement. Curve 2: Similar except 0.03 μmole of CoP.

When enzyme A and CoP are added to the α-ketoglutaric dehydrogenase system at equilibrium, there is further reduction of DPN (Fig. 1, curve 2) indicating liberation of CoA from succinyl CoA. The DPNH formed is equivalent to the CoP added within the limits of the measurement. On addition of pure ADP at this stage (without enzyme B) no further change is produced. Therefore, as previously suggested, the change in DPNH with enzyme A and commercial ADP must be due to the presence of CoP in the latter[1]. The small increment in DPNH on adding enzyme A alone to the α-ketoglutaric dehydrogenase system[1] is again due to the presence of CoP in some batches of CoA. Using reprecipi-

* Supported by a grant from the American Heart Association.

tated CoA, it has been found that the arsenolysis of succinyl CoA[1] is independent of CoP which might indicate that the reaction catalyzed by enzyme preparation A is complex. The sequence of reactions in phosphorylation as known at present may be indicated as follows:

$$\text{Succinyl CoA} \xrightarrow[\text{CoP, } PO_4^{\equiv}, Mg^{++}]{\text{Enzyme A}} \text{CoP-Intermediate} \xrightarrow[\text{ADP}]{\text{Enzyme B}} \text{ATP}$$

TABLE I

COENZYME REQUIREMENT FOR SUCCINYL CoA SYNTHESIS

μmoles

ATP commercial	*ATP rechromatographed*	*CoA*	*CoA reprecipitated*	*CoP*	*-SH*
—	—	—	0.32	—	0.00
—	0.50	—	0.32	—	0.02
—	0.50	—	0.32	0.0015	0.11
—	0.50	—	0.32	0.006	0.18
0.50	—	—	0.32	—	0.14
—	—	0.47	—	—	0.00
—	0.50	0.47	—	—	0.18

The reaction mixture consisting of KBH_4 (1 μmole), $MgCl_2$ (2 μmoles), THAM buffer (6 μmoles), succinate (20 μmoles), enzyme A (0.1 mg), enzyme B (0.025 mg) and above components in 0.5 ml at pH 7.5 was incubated for 10 minutes at 30°. The -SH was determined by the nitroprusside reaction[2].

REFERENCES

1 D. R. SANADI, D. M. GIBSON, P. AYENGAR AND L. OUELLET, *Biochim. Biophys. Acta*, 13 (1954) 146.
2 R. R. GRUNERT AND P. H. PHILLIPS, *Arch. Biochem.*, 30 (1951) 217.
3 H. BEINERT, R. W. VON KORFF, D. E. GREEN, D. A. BUYSKE, R. E. HANDSCHUMACHER, H. HIGGINS AND F. M. STRONG, *J. Biol. Chem.*, 200 (1953) 385.

Received January 10th, 1954

BOOK REVIEWS

The Harvey Lectures, delivered under the Auspices of the Harvey Society of New York, 1951–1953, Series XLVII, Academic Press Inc. Publishers, New York, 1953. Price $ 7.50.

This series of Harvey Lectures contains four lectures of great biochemical interest. A. R. TODD (The nucleotides: some recent chemical research and its biological implications) gives a survey of the work carried out in the Department of Organic Chemistry in Cambridge on the structure of nucleotides, including co-enzymes of nucleotide-like structure, and of the macromolecular nucleic acids. The contribution of I. L. CHAIKOFF (Metabolic blocks in carbohydrate metabolism in diabetes) not only gives a lucid account of the numerous investigations on the metabolic disturbances of carbohydrate and fat metabolism in diabetes by the author and his co-workers, but also clearly shows the importance of lipogenesis as a pathway in glucose metabolism. The complexity of the phenomenon of blood coagulation is once more revealed by the lecture, entitled "Coagulation of the blood" by WALTER H. SEEGERS, who reviews 15 years' work of strenuous experimental work with its pitfalls and successes. This appears to be the first comprehensive survey of SEEGERS' work, published in about 70 original papers. L. ZECHMEISTER (Biochemical studies and chromatography) treats his investigations by means of chromatographic procedures of the carotenoids and of colourless fluorescent polycyclic hydrocarbons, including the strongly carcinogenic 3,4-benzpyrene, occurring in marine animals, barnacles.

Most of the other lectures of this series (F. R. WINTON, Hydrostatic pressures affecting the flow of urine and blood in the kidney; H. W. MAGOUN, An ascending reticular activating system in the brain stem; W. BARRY WOOD, Jr., Studies on the cellular immunology of acute bacterial