COMPREHENSIVE BIOCHEMISTRY

EDITED BY MARCEL FLORKIN AND ELMER H. STOTZ

VOLUME 18S

PYRUVATE AND FATTY ACID METABOLISM

ELSEVIER

COMPREHENSIVE BIOCHEMISTRY

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PYRUVATE AND FATTY ACID METABOLISM

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GENERAL PREFACE

The Editors are keenly aware that the literature of Biochemistry is already very large, in fact so widespread that it is increasingly difficult to assemble the most pertinent material in a given area. Beyond the ordinary textbook the subject matter of the rapidly expanding knowledge of biochemistry is spread among innumerable journals, monographs, and series of reviews. The Editors believe that there is a real place for an advanced treatise in biochemistry which assembles the principal areas of the subject in a single set of books.

It would be ideal if an individual or small group of biochemists could produce such an advanced treatise, and within the time to keep reasonably abreast of rapid advances, but this is at least difficult if not impossible. Instead, the Editors with the advice of the Advisory Board, have assembled what they consider the best possible sequence of chapters written by competent authors; they must take the responsibility for inevitable gaps of subject matter and duplication which may result from this procedure.

Most evident to the modern biochemists, apart from the body of knowledge of the chemistry and metabolism of biological substances, is the extent to which he must draw from recent concepts of physical and organic chemistry, and in turn project into the vast field of biology. Thus in the organization of Comprehensive Biochemistry, the middle three sections, Chemistry of Biological Compounds, Biochemical Reaction Mechanisms, and Metabolism may be considered classical biochemistry, while the first and last sections provide selected material on the origins and projections of the subject.

It is hoped that sub-division of the sections into bound volumes will not only be convenient, but will find favour among students concerned with specialized areas, and will permit easier future revisions of the individual volumes. Toward the latter end particularly, the Editors will welcome all comments in their effort to produce a useful and efficient source of biochemical knowledge.

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PREFACE TO SECTION IV

(VOLUMES 17–21)

Metabolism in its broadest context may be regarded as the most dynamic aspect of biochemistry, yet depends entirely for its advances on progress in the knowledge of the structure of natural compounds, structure–function relationships in enzymes, bioenergetics, and cytochemistry. Approaches to the study of metabolism range from whole organism studies, with limited possibility to reveal mechanisms, to cytochemical or even purified enzyme systems, sometimes with little attention to physiological conditions. Yet all approaches broaden our understanding of metabolism, and all of them may be recognized in the volumes assembled in Section IV on *Metabolism*. It is not unexpected then that previous sections of *Comprehensive Biochemistry* actually deal with some aspects under the broad heading of *Metabolism*, and certainly that the succeeding Section V on *Chemical Biology* should draw heavily on basic understanding of metabolism. Nevertheless Section IV attempts to bring together the broad outlines of the metabolism of amino acids, proteins, carbohydrates, lipids, and their derived products. The currently rapid advances in feed-back, hormonal, and genetic control of metabolism make it particularly difficult that these volumes be current, but the authors, editors, and publishers have made all possible efforts to include the most recent advances.

This supplementary volume to Section IV on *Metabolism* contains two essential chapters which were not available to the Editors earlier. The first, entitled "The Pyruvate Dehydrogenase Complex and the Citric Acid Cycle" by J. M. Lowenstein was originally planned for Volume 17 on *Carbohydrate Metabolism*, and the second, entitled "Fatty Acid Metabolism" by Salih J. Wakil and E. M. Barnes Jr., was planned for Volume 18 on *Lipid Metabolism*.

M. FLORKIN

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Chapter II. Fatty Acid Metabolism

by SALIH J. WAKIL AND EUGENE M. BARNES JR.

Chapter I

The Pyruvate Dehydrogenase Complex and the Citric Acid Cycle

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1. Pyruvate dehydrogenase

(*a*) *Mechanism*

The oxidative decarboxylation of pyruvate to acetyl-coenzyme A occurs according to the following reaction sequence (where TPP is thiamine pyrophosphate):

$$\text{Pyruvate} + \text{TPP} \rightarrow \text{1-hydroxyethyl-TPP} + CO_2 \quad (1)$$

$$\text{1-Hydroxyethyl-TPP} + \text{lipoic acid}_{\text{oxid.}} \rightarrow \text{acetyllipoic acid}_{\text{red.}} + \text{TPP} \quad (2)$$

$$\text{Acetyllipoic acid}_{\text{red.}} + \text{HS} \cdot \text{CoA} \rightarrow \text{acetyl} \cdot \text{S} \cdot \text{CoA} + \text{lipoic acid}_{\text{red.}} \quad (3)$$

$$\text{Lipoic acid}_{\text{red.}} + NAD^+ \rightarrow \text{lipoic acid}_{\text{oxid.}} + NADH + H^+ \quad (4)$$

Sum:

$$\text{Pyruvate} + \text{HS} \cdot \text{CoA} + NAD^+ \rightarrow \text{acetyl} \cdot \text{S} \cdot \text{CoA} + CO_2 + NADH + H^+ \quad (5)$$

These reactions represent the mechanism of pyruvate oxidation in animal tissues and in many microorganisms. The sequence, which was discovered during the early nineteen fifties[1-8], consists of at least four steps and involves five different cofactors, namely thiamine pyrophosphate (TPP), lipoid acid, coenzyme A, FAD, and NAD. The four steps are catalyzed by an enzyme complex which has been highly purified from a variety of sources.

(*i*) *Pyruvate decarboxylase*

This member of the pyruvate dehydrogenase complex catalyzes the reaction

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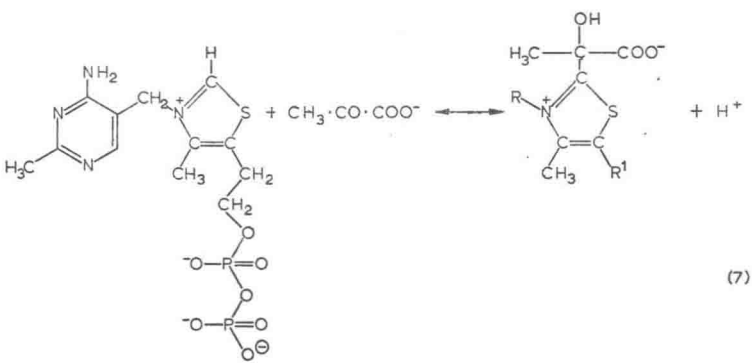
between pyruvate and TPP, which results in the decarboxylation of pyruvate and the formation of the intermediate 1-hydroxyethyl-TPP (reaction 6).

$$\begin{array}{l} \mathrm{COOH} \\ | \\ \mathrm{CO} \\ | \\ \mathrm{CH_3} \end{array} + \mathrm{TPP} \leftrightarrow \begin{array}{l} \mathrm{CHO[TPP]} \\ | \\ \mathrm{CH_3} \end{array} + \mathrm{CO_2} \qquad (6)$$

Evidence in support of this formulation includes the observation that TPP is required for the exchange of $^{14}CO_2$ into pyruvate which is catalyzed by preparations of pyruvate dehydrogenase from pig heart. The exchange reaction does not require coenzyme A and NAD[9-11]. It is not inhibited by arsenite, which indicates that dihydrolipoic acid is not involved[12]. Moreover, pyruvate decarboxylase obtained by resolution of the pyruvate dehydrogenase complex from *Escherichia coli*, which catalyzes reaction 2, does not contain lipoic acid[13]. The mammalian and the bacterial pyruvate dehydrogenase complexes, as well as pyruvate decarboxylase derived from the complex, catalyze pyruvate oxidation in the presence of ferricyanide as electron acceptor. This reaction requires TPP but occurs in the absence of CoA and NAD[2,4,14,15]. The acyl acceptor is water, and the product is acetate. Similar results have been obtained in studies of α-ketoglutarate oxidation linked to ferricyanide reduction[3,15].

The initial step of reaction 1 involves the addition of pyruvate to the



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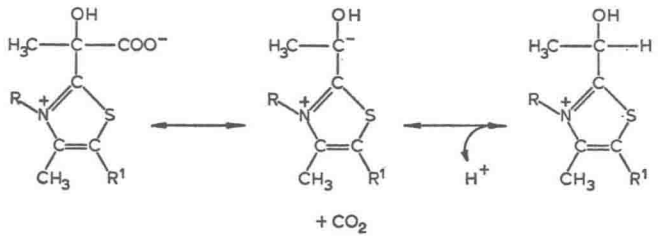
2′-position of TPP. This is followed by the decarboxylation reaction, the product being 2′-(1-hydroxyethyl)-TPP[17-20]. Both the pyruvate adduct of

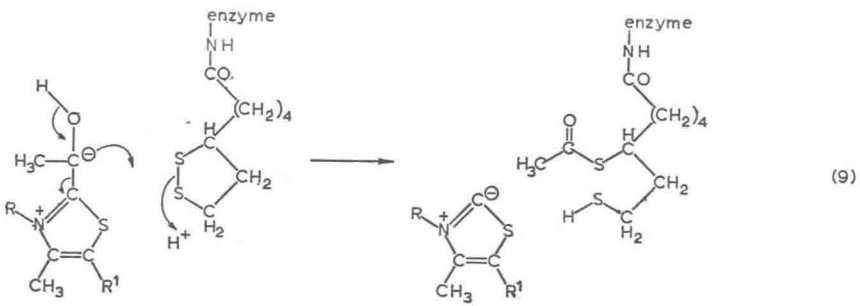


(8)

TPP and hydroxyethyl-TPP have been isolated from incubation mixtures containing pyruvate and pyruvate dehydrogenase[21,22]. Synthetic hydroxyethyl-TPP gives rise to acetyl-coenzyme A when it is incubated with pyruvate dehydrogenase, NAD and coenzyme A[23]. This reaction is much slower than the overall reaction with pyruvate[24], but this does not necessarily argue against the participation of hydroxyethyl-TPP. The hydrogen atom on the 2′-carbon of TPP is particularly labile, and exchanges non-enzymatically with labeled hydrogen from water[17,25]. The rate of non-enzymatic hydrogen exchange at the 2′-position is increased by magnesium ions[26], which are also required for the enzymatic reaction.

(ii) Lipoyl reductase

This enzyme catalyzes the transfer of the hydroxyethyl group from hydroxyethyl-TPP to lipoic acid. In the course of the transfer reaction the hydroxyethyl group is oxidized to an acetyl group with the concomitant reduction of the lipoate to dihydrolipoate. The products are enzyme-bound 6-*S*-acetyldihydrolipoate and TPP. The process can be imagined to be initiated by the removal of a proton from hydroxyethyl-TPP, which then reacts with lipoate as follows:



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To complete the reaction the TPP–carbanion is protonated to yield TPP.

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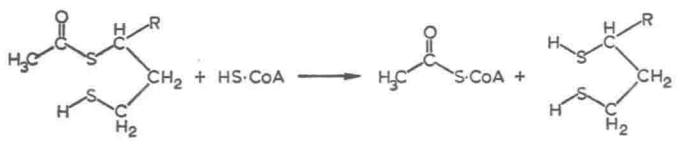
This stage does not involve coenzyme A, since it has been found that the analogous reaction with α-ketoglutarate yields a succinyl derivative which readily forms succinomonohydroxamate in the presence of hydroxylamine and in the complete absence of coenzyme A[27]. The inhibition of pyruvate and α-ketoglutarate oxidation by arsenite is additional evidence for the involvement of acyl dihydrolipoate in this process[8,27]. The arsenite inhibition requires α-ketoacid to generate acyl dihydrolipoate, and coenzyme A to convert the acyl-dihydrolipoate to dihydrolipoate. The latter is then rendered inactive by reaction with arsenite to form a stable cyclic thioarsenite[28].

The lipoic acid is covalently bound to the enzyme[5,8,14,29–32]. In the case of the bacterial enzyme, the carboxyl group of lipoic acid is in an amide linkage with an ε-amino group of a lysine residue[32]. Work with the pyruvate dehydrogenase complex has failed to demonstrate the occurrence of free acetyl-dihydrolipoate. No exchange between added lipoate and enzyme-bound lipoate could be observed[31,32]. For further details on the chemistry and function of lipoic acid the reader is referred to the special chapter on this topic[8].

(iii) Acetyl transferase

The enzyme catalyzes the transfer of the acetyl group from the thiol group of dihydrolipoate to the thiol group of coenzyme A. The products are dihydrolipoate and acetyl-coenzyme A[33]:



(10)

Reversibility of the reaction can be demonstrated with added dihydrolipoic acid using an acetyl-coenzyme A generating system consisting of acetyl phosphate and coenzyme A[4]. The natural isomer, (−)-lipoate, is acetylated much faster than (+)-lipoate[8]. The product of the reverse reaction is 6-*S*-acetyllipoate[33].

(iv) Dihydrolipoate dehydrogenase[8]

The enzyme has been highly purified[13,34] and separated from other members of α-ketoacid dehydrogenase complexes. It contains FAD, and catalyzes

the reversible oxidation of dihydrolipoate to lipoate using NAD as hydrogen acceptor (reaction 4). Free dihydrolipoate and lipoate serve as substrates for the pig-heart enzyme[35]. However, as has already been stated, in the pyruvate dehydrogenase complex the carboxyl group of lipoate exists as an amide with ε-amino groups of lysine residues of lipoyl reductase.

Dihydrolipoate dehydrogenase possesses a reactive disulfide which participates in the oxidation reaction. The overall dehydrogenase reaction (4) involves a transfer of two electrons from dihydrolipoate to NAD. Spectral evidence indicates that a biradical, comprising a flavin semiquinone and possibly a sulfide radical (R–S ·) are involved in the electron-transfer reaction[36,37]. The enzyme is inhibited by arsenite and by cadmium ions provided NADH is present. The inhibition is reversed readily by BAL (British Antilewisite) and also by monothiols[14,27,38,39]. Addition to the enzyme of NADH results in the appearance of two additional sulfhydryl groups per mole of flavin, suggesting that a disulfide is reduced by this treatment which is then susceptible to arsenite inhibition[39,40].

Straub's diaphorase[41], an enzyme preparation capable of oxidizing NADH in the presence of certain artificial electron acceptors, and dihydrolipoate dehydrogenase have been demonstrated to be the same enzyme[16,42,43]. For further details the reader is referred to the special article by Reed[8] elsewhere in this series, and to the review by Massey[7].

(*v*) *Molecular architecture*[44,45]

The pyruvate dehydrogenase complex from *E. coli* has been isolated in pure form and possesses a molecular weight of about 4.8 million (refs. 44,46). It has been resolved into three separate enzymes, namely pyruvate decarboxylase, lipoyl reductase–acetyl transferase, and dihydrolipoate dehydrogenase. The complex can be reconstituted by combining the separate enzymes[13]. Lipoyl reductase–acetyl transferase can be separated further into two enzymes by calcium phosphate gel chromatography at pH 9.5.

The component enzymes occur in the complex in the following proportions: pyruvate decarboxylase, 12 molecules of molecular weight 183 000; lipoyl reductase–acetyl transferase, 24 subunits of molecular weight 70 000; and dihydrolipoate dehydrogenase, 6 molecules of molecular weight 112 000 (ref. 44). The total molecular weight calculated from these figures is 4.6 million. Dihydrolipoate dehydrogenase contains two moles of FAD per mole of enzyme[13], or twelve moles of FAD per mole of native dehydrogenase complex. Lipoyl reductase–acetyl transferase contains approximately one

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lipoic acid residue per 35 000 g of protein, or 48 residues per mole of the enzyme or of the native dehydrogenase complex. Treatment with acetic acid at pH 2.6 dissociates lipoyl reductase–acetyl transferase into inactive subunits possessing a molecular weight of 70 000. Rapid dilution of these subunits into buffer at pH 7.0 leads to restoration of enzymatic activity[44,47]. Attempts at partial reconstitution show that pyruvate decarboxylase and dihydrolipoate dehydrogenase do not combine with each other. However, either of these enzymes combines with lipoyl reductase–acetyl transferase[13,44]. When all three are mixed together, a pyruvate dehydrogenase complex is produced which is similar to the native complex.

Electron micrographs of the pyruvate dehydrogenase complex, of lipoyl reductase–acetyl transferase, of the pyruvate dehydrogenase component, and of dihydrolipoate dehydrogenase, prepared from *E. coli*, show that the enzymes of the complex are assembled in an orderly array with a diameter of 300–350 Å (Fig. 1). Phosphotungstate-stained preparations of the whole complex show a polyhedral structure in the center of which can be seen a tetrameric arrangement of subunits. The latter appears to be square when viewed end-on. The tetrameric center is symmetrically surrounded by twelve further subunits which are 60–90 Å in diameter. The outer subunits appear to be organized in the form of staggered rings, although other, more complex, arrangements cannot be ruled out at present.

Preparations of lipoyl reductase–acetyl transferase negatively stained with phosphotungstate reveal tetramers which closely resemble those seen in the center of the pyruvate dehydrogenase complex (Fig. 2). The sides of the tetramers measure 120–140 Å approximately. In addition to the square tetramers, some molecules appear as two parallel strands which are more than two subunits in length. A few appear more complex, possibly in the shape of hexagons. Reed and co-workers[44,45,47] interpret these pictures as indicating that the subunits are arranged into cubes consisting of two tetramers which appear as squares when viewed end-on, as parallel strands when viewed with one edge towards the viewer, and as hexagons when viewed with one corner towards the viewer.

Preparations of the pyruvate dehydrogenase component of the complex show a variety of images under the electron microscope which suggest this component consists of a tetrahedral arrangement of four subunits. Recent studies indicate that these subunits are composed of two pairs of different polypeptide chains, each with a molecular weight of about 45 000 (ref. 45).

A tentative model of the whole complex which is based on the electron