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Essays in Biochemistry

Volume 1 1965

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Edited for The Biochemical Society by

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Volume 1

1965

Published for The Biochemical Society by Academic Press
London and New York

Preface

The idea that a series of essays on biochemical subjects suitable for advanced students would be a useful innovation arose in the Committee of The Biochemical Society. There is a wealth of literature available to the biochemist, but many of the reviews now published, while excellent for the specialist, are too detailed for the student and are included in volumes too expensive for him to buy. The proposal, therefore, was for a series of small volumes of essays which might be read with pleasure and profit by the senior student and would be of a size and price suitable to his pocket. The project was approved at the 1964 Annual General Meeting of the Society and the Committee entrusted us with the Editorship.

Our first thought has been for the student trying to obtain a clear view of the various aspects of the subject. We hope, however, that the essays may prove helpful to teachers when dealing with parts of biochemistry on which they are not expert and to research workers who wish to obtain an up-to-date picture of topics outside their speciality—green mansions for the mitochondriac, for example. It seemed to us that an essay should preferably have a central theme, but that undue speculation might be confusing to the student. Informed surmises as to the future, however, should stimulate the minds of a generation subjected to the challenge of the squiggle, the screw and the cistron. Our suggestion to the authors has been therefore that they should present an overall view of their chosen subject, indicating its origin, present status and likely future development. Each contributor to Volume 1 has fulfilled this request. The essays are by no means stereotyped and for this we are most grateful. It may be, however, that one approach will prove more popular than another, and we should be very glad to receive all kinds of criticism and suggestions, so that we may try to provide what is required in future volumes. We are grateful to the authors for their extremely helpful co-operation. Not only were their essays provided on time but each has great merit, so that the volume fulfils all that we hoped for when the series was first planned.

We should like to add a word about the publishing arrangements. Academic Press Limited were invited to publish the series and have been most helpful in launching Volume 1. They take all the financial responsibility and pay to The Biochemical Society a royalty based on sales. We believe that this arrangement will ensure that the Members of the Society will have full control over all important matters while the expertise of Academic Press will enable the product to be made available

at a low price. The initial arrangements are for three years and the future will depend on the response of the Members of the Society. We hope that volumes will appear annually, not later than halfway through the academic year.

The cover bears a new monogram of The Biochemical Society. The emblem B ~ S was originally proposed, to symbolize the highly energetic nature of the Society. Modesty, however, prevailed.

We trust that the new venture may prove to be a worthy addition to the publications of the Society and that it may contribute to the pleasures of the study of biochemistry. Schiller[1] remarked that from the fiery mirror of truth joy smiles on the research worker. How much ultimate truth there is in these essays we cannot know, but we hope that in this instance adding to the literature, unlike multiplying the nation,[2] will increase the joy.

G. D. GREVILLE
P. N. CAMPBELL

December 1964

[1] *An die Freude*, ll. 49–50 (our translation).
[2] Isaiah, ch. 10, v. 3.

Conventions

The abbreviations, conventions and symbols used in these essays are those specified by the Editorial Board of *The Biochemical Journal* in *Suggestions to Authors, Chemical Nomenclature and Abbreviations, Symbols, Usages and Conventions, Revised 1957* (*Biochem. J.* **66**, 1) and *Suggestions and Instructions to Authors* [*Biochem. J.* **90**, 1 (1964)]. The following abbreviations of compounds, etc., are allowed without definition in *The Biochemical Journal* and are employed similarly in these essays.

ADP, CDP, GDP, IDP, UDP: 5′-pyrophosphates of adenosine, cytidine, guanosine, inosine and uridine
AMP, etc.: adenosine 5′-phosphate, etc.
ATP, etc.: adenosine 5′-triphosphate, etc.
CM-cellulose: carboxymethylcellulose
CoA and acyl-CoA: coenzyme A and its acyl derivatives
DEAE-cellulose: diethylaminoethylcellulose
DNA: deoxyribonucleic acid
DNP-: 2,4-dinitrophenyl-
EDTA: ethylenediaminetetra-acetate
FAD: flavin-adenine dinucleotide
FMN: flavin mononucleotide
GSH, GSSG: glutathione, reduced and oxidized
NAD: nicotinamide-adenine dinucleotide
NADP: nicotinamide-adenine dinucleotide phosphate
NMN: nicotinamide mononucleotide
RNA: ribonucleic acid
P_i, PP_i: orthophosphate, pyrophosphate
tris: 2-amino-2-hydroxymethylpropane-1,3-diol

The combination NAD^+, NADH is preferred.

References are given in the form used in *The Biochemical Journal*, the titles of journals being abbreviated in accordance with the system employed in the *World List of Scientific Periodicals*, 3rd ed., 1952, and 4th ed., vol. 1, A–E, 1963 (London: Butterworths).

Enzyme Nomenclature

At the first mention of each enzyme in each essay there is given, whenever possible, the number assigned to it in *Report of the Commission on Enzymes of the International Union of Biochemistry, 1961* (Oxford:

Pergamon Press), or in *Enzyme Nomenclature: Recommendations of the International Union of Biochemistry on the Nomenclature and Classification of Enzymes, together with their Units and the Symbols of Enzyme Kinetics, 1964* (to be published). The names used by the authors of the essays are not necessarily those recommended by the International Union of Biochemistry.

Contents

The Role of CO_2 Fixation in Metabolism

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I. Introduction

The process of CO_2 utilization or fixation is of prime importance in photosynthetic and autotrophic organisms since all of the carbon atoms of these organisms are derived from this single source. The role of CO_2 fixation in heterotrophic organisms is less dramatic and more difficult to demonstrate since these organisms normally produce large amounts of CO_2 from other substrates, thereby masking the smaller amounts of CO_2 uptake. Before tracers were available detection of CO_2 fixation was dependent on rare examples where a net CO_2 uptake could be detected or where the presence of CO_2 was clearly essential for the growth of an organism. The availability of carbon isotopes made the detection much simpler but elucidation of the actual mechanisms has come only through detailed studies of metabolic pathways at the enzymatic level. The phenomenon of CO_2 fixation in heterotrophic organisms was recognized

about 30 years ago by Wood and Werkman when they noted that propionic acid bacteria growing on glycerol actually used sizable amounts of CO_2 as a substrate. The enzymatic basis of CO_2 fixation was slow to emerge, but since 1945 about a dozen distinct reactions and enzymes have been found to contribute to this process. It is an interesting sidelight that the most recent of these enzymes to be recognized is probably the agent responsible for the initial observation of CO_2 fixation in the propionic acid bacteria. As will emerge from this essay, it is the authors' opinion that CO_2 fixation reactions play important, although somewhat specialized, roles in a variety of metabolic areas and that, considering the variety and essential nature of these processes, it is not too much to say that most, if not all, heterotrophic cells are dependent on CO_2 fixation reactions.

The present essay will be limited to a discussion of the probable nature and metabolic significance of well established enzymes from heterotrophic cells capable of forming a carbon–carbon bond from CO_2 and an acceptor molecule. This limitation excludes other groups of enzymes where CO_2 is affixed to a non-carbon atom, e.g. the formation of carbamyl phosphate from CO_2. The limited scope of this essay precludes an extensive bibliography and citations will usually be made to a review article where the original references are to be found.

II. Enzymes, Reactions and Mechanisms of CO_2 Fixation

The first enzyme to be implicated in CO_2 fixation was an oxaloacetate decarboxylase from *Micrococcus lysodeikticus* described by Krampitz, Wood and Werkman in 1940. The actual experimental observation was limited to an exchange reaction between oxaloacetate and CO_2 and no *net* fixation of CO_2 could be demonstrated, but these findings provided much impetus for further work on CO_2 fixation. It is curious to record that none of the various enzymes capable of oxaloacetate formation which have since been discovered appears to bear any relationship to the reaction observed in *M. lysodeikticus*, and the most likely explanation is that the exchange reaction simply reflects a reversible portion of a decarboxylation reaction as suggested below.

$$\text{Enz} + \text{oxaloacetate} \overset{Mn^{2+}}{\rightleftharpoons} \text{Enz-pyruvate} + CO_2$$
$$\downarrow$$
$$\text{Enz} + \text{pyruvate}$$

A number of enzymes capable of *net* fixation of CO_2 have been described and it is convenient to consider these enzymes in three general groupings: (a) fixation of CO_2 by reduction, (b) fixation of CO_2 by

phosphoenolpyruvate, and (c) fixation of CO_2 by biotin-containing enzymes. The enzymes making up these groups, the reactions they catalyse, and possible reaction mechanisms are discussed in the succeeding sub-sections.

A. FIXATION OF CO_2 BY REDUCTION

The first demonstration of a net CO_2 fixing reaction was that of Ochoa[1] in 1945, who showed that the reaction catalysed by isocitrate dehydrogenase (EC 1.1.1.42) was reversible.

$$\text{L}_S\text{-Isocitrate} + \text{NADP}^+ \xrightleftharpoons[\text{dehydrogenase}]{\text{Isocitrate}} \alpha\text{-oxoglutarate} + CO_2 + \text{NADPH}$$

Two years later Ochoa, Mehler and Kornberg[1] discovered that the malate enzyme (EC 1.1.1.40) catalysed a similar oxidative decarboxylation of malate.

$$\text{L-Malate} + \text{NADP}^+ \xrightleftharpoons[\text{enzyme}]{\text{Malate}} \text{pyruvate} + CO_2 + \text{NADPH}$$

This enzyme has been shown to be distributed widely in animal tissues, plants and micro-organisms, and the freely reversible reaction offers a mechanism for the formation of dicarboxylic acids from CO_2 and pyruvate. The two reactions just shown bear a close resemblance to another dehydrogenation reaction, that of 6-phospho-D-gluconate.

$$\text{6-Phospho-D-gluconate} + \text{NADP}^+ \xrightleftharpoons[(\text{EC } 1.1.1.44)]{\text{6-Phosphogluconate dehydrogenase}} \text{D-ribulose-5-phosphate} + \text{NADPH} + CO_2$$

All three of the above reactions are shown with NADP, but analogous reactions involving NAD have also been reported.

Although the malate enzyme and isocitrate dehydrogenase have been brought to reasonably high states of purity and have been studied intensively, the intimate mechanism of the reactions remains obscure. Since these reactions all involve a dehydrogenation and a decarboxylation step (in the oxidative direction), the β-keto acid would be expected to be an intermediate: oxaloacetate with malate enzyme, oxalosuccinate with isocitrate dehydrogenase, and 3-oxo-6-phospho-D-gluconate with 6-phosphogluconate dehydrogenase. Evidence from isotope experiments indicates that this is not the case; when the oxidation of the ^{14}C-substrate is carried out in the presence of a pool of the keto acid no ^{14}C is trapped in the keto acid. However, oxaloacetate and oxalosuccinate are decarboxylated by malate enzyme and isocitrate dehydrogenase, respectively, but a malate enzyme (EC 1.1.1.39) isolated by Saz and

Hubbard[1] from *Ascaris* did not decarboxylate oxaloacetate, suggesting that this reaction is not a necessary attribute of the enzyme. Isocitrate dehydrogenase catalyses the reduction of oxalosuccinate by NADPH yielding isocitrate but the malate enzyme does not catalyse the reduction of oxaloacetate. It seems likely that the appropriate keto acid is enzyme-bound as is illustrated below for isocitrate dehydrogenase.

$$\text{Enz} + \text{L}_s\text{-isocitrate} \underset{\text{NADPH}}{\overset{\text{NADP}^+}{\rightleftharpoons}} \text{Enz} - \text{oxalosuccinate} \rightleftharpoons \text{Enz} + \alpha\text{-oxoglutarate} + CO_2$$

$$\text{Enz} + \text{oxalosuccinate}$$

The only other partial reaction demonstrated thus far is that shown by Rose[2] in which a tritium atom bound to C-3 of α-oxoglutarate is labilized,

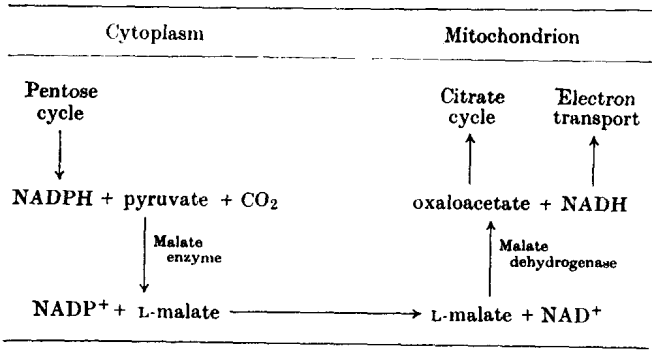


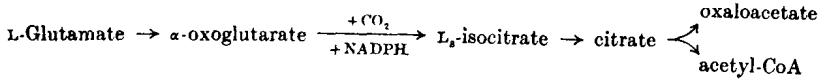
FIG. 1. Hypothetical role of the malate enzyme in hydrogen transport.

a reaction which requires the presence of NADPH and Mn^{2+} but not of CO_2. It is interesting to note that in the analogous reaction with malate enzyme,[2] the labilization of a tritium atom from pyruvate does not occur unless NADPH, Mn^{2+} and CO_2 are all present, an observation which suggests that the reaction mechanisms of isocitrate dehydrogenase and malate enzyme are not identical. The possibility must be considered that these reactions involve concerted mechanisms without the formation of detectable intermediates and that the decarboxylations do not reflect the actual mechanisms.

Although it is obvious that these enzymes have the potential for fixing CO_2, it is not certain that they function metabolically in the direction of carboxylation. The reaction catalysed by malate enzyme is the only clearly demonstrated pathway by which dicarboxylic acids can be

formed in certain tissues, such as skeletal and cardiac muscle, and it is possible to postulate that the enzyme is responsible for the maintenance of the oxaloacetate needed for the citrate cycle. Likewise in tissues such as liver and kidney, it has been suggested that the malate enzyme plays a part via fixation in the gluconeogenic process but more recent evidence which will be discussed later does not support this mechanism. Alternative views are that malate enzyme functions primarily as a NADPH-generating system or takes part in an intracellular hydrogen transport system as illustrated in Fig. 1.

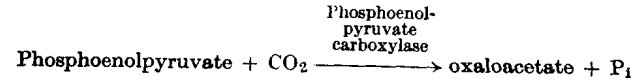
Evaluation of the physiological significance of carboxylation by isocitrate dehydrogenase and 6-phosphogluconate dehydrogenase is even more difficult. With the former enzyme, a metabolic route involving the carboxylation of α-oxoglutarate demands a source of the latter compound and a metabolic outlet for the product, isocitrate. A possible series of reactions which meets these requirements involves the citrate-splitting enzyme (EC 4.1.3.8) of Srere and is shown below:



All of the enzymes of the above pathway are found in the soluble part of the cell and it is postulated that glutamate could be supplied via the citrate cycle of the mitochondria. The mechanism would provide an extra-mitochondrial pathway for oxaloacetate and acetyl-CoA formation. The latter could be used for fatty acid synthesis while the oxaloacetate could either be converted to malate and re-enter the mitochondria or in some tissues be converted to phosphopyruvate.

B. FIXATION OF CO_2 BY PHOSPHOENOLPYRUVATE

This group of enzymes catalyses the formation of oxaloacetate with phosphoenolpyruvate as the CO_2 acceptor. The first two enzymes of this type, phosphoenolpyruvate carboxylase (EC 4.1.1.31) and phosphoenolpyruvate carboxykinase (EC 4.1.1.32), were discovered almost simultaneously in 1953 by Bandurski and Greiner and by Utter and Kurahashi, respectively.[1] The third member of the group, phosphoenolpyruvate carboxytransphosphorylase (EC 4.1.1.38) was found only recently by Siu and Wood.[3]



$$\text{Phosphoenolpyruvate} + CO_2 + GDP \xrightleftharpoons[\text{Phosphoenolpyruvate carboxykinase}]{} \text{oxaloacetate} + GTP$$

$$\text{Phosphoenolpyruvate} + CO_2 + P_i \xrightleftharpoons[\text{Phosphoenolpyruvate carboxytransphosphorylase}]{} \text{oxaloacetate} + PP_i$$

These reactions appear to be variants of a carboxylation mechanism in which the phosphate acceptors differ as illustrated below.

$$CO_2 + \text{phosphoenolpyruvate} + (H_2O, \text{GDP or } P_i) \rightleftharpoons \text{oxaloacetate} + (P_i, \text{GTP or } PP_i)$$

Phosphoenolpyruvate carboxylase, where water serves as the phosphate acceptor, has been found only in plants [4] and a few species of autotrophic bacteria, and is essentially irreversible. Phosphoenolpyruvate carboxykinase was first detected in liver and kidney and subsequently has been reported to be present in a variety of plants and micro-organisms [4] although it has not been found in other animal tissues. The animal enzyme appears to use GDP as phosphate acceptor and IDP is also active whereas the plant and microbial enzymes utilize ADP in most instances. This reaction is freely reversible; in fact, the rate of phosphoenolpyruvate formation is somewhat more rapid than the rate of oxaloacetate synthesis [5] and the question must be raised here, as it was with malate enzyme, whether this enzyme's main function is in the direction of carboxylation. Phosphoenolpyruvate carboxytransphosphorylase, which utilizes P_i as the phosphate-acceptor, has been reported thus far only in the propionic acid bacteria. This reaction is also reversible and conceivably could serve as a mechanism for the formation of phosphoenolpyruvate from PP_i as will be discussed later. All enzymes of this group are biotin-independent and the basic mechanism differs from that of enzymes discussed in the following sub-section. No partial reactions have yet been demonstrated with certainty for any enzyme of this group, suggesting either that the reactions are concerted in mechanism or, less likely, that the reaction sequences are ordered in such a way that partial reactions are masked by the need for other reaction components. The studies by Maruyama and Lane [6] offer support of the concerted mechanism for phosphoenolpyruvate carboxylase. These workers studied the incorporation of $NaHC^{18}O_3$ into oxaloacetate and P_i and found that ^{18}O appeared in both products in a ratio of approximately 2:1. This result is consistent with the concerted mechanism shown in Fig. 2. The results also show that HCO_3^- rather than CO_2 is the active species since the equivalent of three oxygen atoms appear in the product. Similar results with $NaHC^{18}O_3$ have been obtained by

Kaziro and co-workers with propionyl carboxylase, as will be noted later.

It appears probable that phosphoenolpyruvate carboxylase may play a significant role in the non-photosynthetic carboxylation reactions in

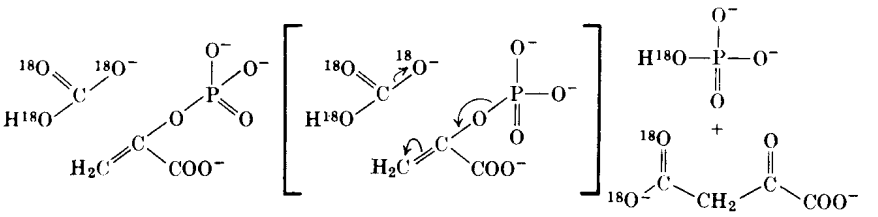


FIG. 2. Evidence with $HC^{18}O_3$ of a concerted mechanism in the phosphoenolpyruvate carboxylase reaction.

plants (cf. review by Walker[4]). The physiological significance of the other two members of this group will be discussed in some detail in a later section.

C. FIXATION OF CO_2 BY BIOTIN-CONTAINING ENZYMES

1. *Introduction and Historical Developments*

The most recent and exciting event in relation to fixation of CO_2 has been the elucidation of the mechanism of action of biotin. Biotin was isolated in 1936 by Kögl and Tönnis[7] as one of the components of the "bios" factor required by yeast and it was then found by György and du Vigneaud[7] and their co-workers to be identical with "vitamin H" which was known to protect rats and other animals against the toxicity of raw egg white. The toxic material in the egg white is avidin, a protein which forms a stoicheiometric complex with biotin. Since the complex is not split by the enzymes of the digestive tract, biotin deficiency develops when raw egg white is included in the diet. Avidin has been found to inhibit all biotin enzymes and is now used as a diagnostic tool for detection of such reactions. Wessman and Werkman[3,7] were probably the first to use avidin for this purpose.

The discovery of the role of biotin in CO_2 fixation is all the more interesting because its function had been suspected since 1940. Several studies showed that the fixation of CO_2 into aspartate is dependent on biotin and that the requirement of biotin for growth of yeast can be replaced in part by aspartate. Since vitamins such as thiamine, niacin and riboflavin were known to function as coenzymes, it was quite natural to propose that biotin would have a coenzyme function in CO_2 fixation,

but the solution of the problem defied investigators for many years. A more complete discussion of these early developments may be found in previous reviews.[3,7,8] In the meantime, numerous reactions by which CO_2 is fixed were discovered, and at one time hopes were raised that "activated CO_2" would be identified as carbonyl phosphate[1] and again as adenyl carbonate[1] but these hopes proved to be false. It is quite certain now that biotin covalently bound to lysine of the enzyme molecule is the coenzyme form of this vitamin and that carboxybiotin-enzyme is the long sought "activated CO_2". Some confusion still remains, however, since two different structures of carboxybiotin-enzyme have been presented as "activated CO_2".

Numerous other metabolic pathways have been reported to involve biotin in some way but thus far the only direct demonstration of a role of biotin has been in the fixation of CO_2 or in transcarboxylation.

The discovery of the malate enzyme provided the first opportunity to study the relationship of biotin to a specific enzyme. When Ochoa and co-workers found that the amount of the malate enzyme was depressed in the livers of biotin-deficient turkeys while other dehydrogenases were present at normal levels, it seemed that direct evidence for the postulated role of biotin was forthcoming. Assay of the purified malate enzyme showed, however, that it did not contain biotin nor was the activity of the enzyme stimulated by biotin.[1]

Concurrent with the above studies, oleic acid and other unsaturated fatty acids were found to have a sparing effect on the biotin requirement of the lactic acid bacteria.[7] In fact, when oleate was added together with aspartate, there was no requirement for biotin in the growth medium. Biotin thus was linked to the metabolism of fatty acids, but 10 years were to elapse before the function of biotin in the synthesis of fatty acids was clarified.

2. *The Biotin Enzymes*

These enzymes catalyse reactions in which, with one exception, a coenzyme-A ester participates. The reactions are believed to take part in two steps as follows:

$$\text{Enz-biotin} + HCO_3^- + \text{ATP} \rightleftharpoons \text{Enz-biotin} \sim CO_2^- + \text{ADP} + P_i \quad (1)$$

$$\text{Enz-biotin} \sim CO_2^- + R\cdot CH_2\cdot COSCoA \rightleftharpoons \text{Enz-biotin} + R\cdot \overset{COO^-}{\overset{|}{C}}H\cdot COSCoA \quad (2)$$

$$\text{Net: } HCO_3^- + \text{ATP} + R\cdot CH_2\cdot COSCoA \rightleftharpoons R\cdot \overset{COO^-}{\overset{|}{C}}H\cdot COSCoA + \text{ADP} + P_i \quad (3)$$

The R can be a hydrogen atom as in acetyl-CoA or a $-CH_3$ group as in propionyl-CoA.

$$HCO_3^- + ATP + CH_3 \cdot COSCoA \xrightleftharpoons{\text{Acetyl-CoA carboxylase (EC 6.4.1.2)}} ADP + P_i + {}^-OOC \cdot CH_2 \cdot COSCoA$$

Malonyl-CoA

$$HCO_3^- + ATP + CH_3 \cdot CH_2 \cdot COSCoA \xrightleftharpoons{\text{Propionyl-CoA carboxylase (EC 6.4.1.3)}} ADP + P_i + CH_3 \cdot \overset{COO^-}{\overset{|}{C}}H \cdot COSCoA$$

Methylmalonyl-CoA

β-Methylcrotonyl-CoA and pyruvate likewise serve as acceptors.

$$HCO_3^- + ATP + CH_3 \cdot \overset{CH_3}{\overset{|}{C}} : CH\text{-}COSCoA \xrightleftharpoons{\beta\text{-Methylcrotonyl-CoA carboxylase (EC 6.4.1.4)}} ADP + P_i + CH_3 \cdot \overset{CH_2 \cdot COO^-}{\overset{|}{C}} : CH \cdot COSCoA$$

β-Methylglutaconyl-CoA

$$HCO_3^- + ATP + CH_3 \cdot CO \cdot COO^- \xrightleftharpoons{\text{Pyruvate carboxylase (EC 6.4.1.1)}} ADP + P_i + {}^-OOC \cdot CH_2 \cdot CO \cdot COO^-$$

In these substrates a hydrogen atom is activated by the carbonyl group (ester or ketone) which is adjacent or separated from it by a vinyl group.

Pyruvate carboxylase has an absolute requirement for a catalytic amount of acetyl-CoA or other CoA compound when catalysed by the liver enzyme but the apparently analogous enzyme obtained from a pseudomonad by Seubert and Remberger has no such requirement.[9]

It should be emphasized that the role of biotin as a transcarboxylating agent is the most distinctive part of the mechanism. This function of biotin is more clearly illustrated by the reaction catalysed by oxaloacetate transcarboxylase (EC 2.1.3.1) which occurs in the propionic acid bacteria.[1] Here Mg^{2+} and ATP are not required and the reactions are as follows:

$${}^-OOC \cdot CH_2 \cdot CO \cdot COO^- + \text{Enz-biotin} \xrightleftharpoons{\text{Oxaloacetate transcarboxylase}} CH_3 \cdot CO \cdot COO^- + \text{Enz-biotin} \sim CO_2^-$$

$$\text{Enz-biotin} \sim CO_2^- + CH_3 \cdot CH_2 \cdot COSCoA \rightleftharpoons \text{Enz-biotin} + CH_3 \cdot \overset{COO^-}{\overset{|}{C}}H \cdot COSCoA$$

$$\text{Net: } {}^-OOC \cdot CH_2 \cdot CO \cdot COO^- + CH_3 \cdot CH_2 \cdot COSCoA \rightleftharpoons CH_3 \cdot CO \cdot COO^- + CH_3 \cdot \overset{COO^-}{\overset{|}{C}}H \cdot COSCoA$$

Since all the biotin enzymes which have been purified have been found to be of high molecular weight, approximately 700,000, with about 4 moles of biotin per mole of enzyme, it seems likely that such enzymes may consist of aggregates of 4 sub-units with a molecular weight of about 170,000.[3] In every case that has been investigated the biotin is bound in a very stable peptide linkage to the ϵ-amino group of a lysyl group (Fig. 3).

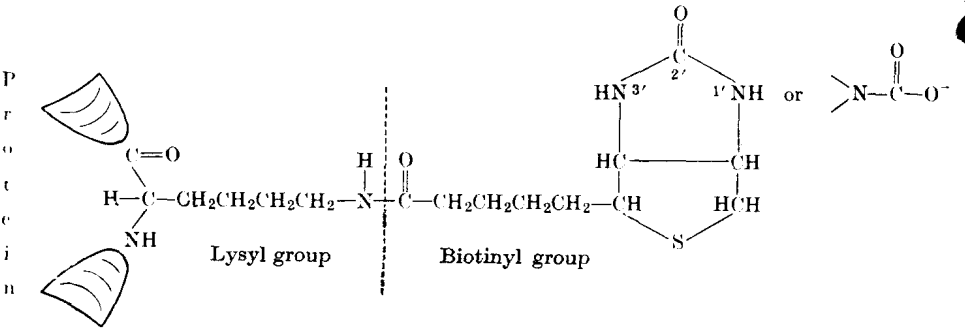


FIG. 3. The linkage of biotin in enzymes of CO_2 fixation and transcarboxylation. The carboxylated enzyme has a carboxyl group in place of the H at the 1′-nitrogen atom.

3. *The Mechanism of Action of Biotin*

The breakthrough on the role of biotin in CO_2 fixation came about through a fortunate property of β-methylcrotonyl-CoA carboxylase. Lynen and co-workers wished to study the properties of the carboxylated-biotin enzyme but, having insufficient enzyme for such studies, used free biotin as the substrate, in the hope that it would be carboxylated. It was found[1,3,7] that free biotin, when present in high concentration, was carboxylated as illustrated below.

$$\text{ATP} + {}^{14}CO_2 + \text{Enz-biotin} \rightleftharpoons \text{ADP} + P_i + \text{Enz-biotin} \sim {}^{14}CO_2$$

$$\text{Enz-biotin-}{}^{14}CO_2 + \text{biotin} \rightleftharpoons \text{Enz-biotin} + \text{biotin} \sim {}^{14}CO_2$$

$$\text{Net:} \quad \text{ATP} + {}^{14}CO_2 + \text{biotin} \rightarrow \text{ADP} + P_i + \text{biotin} \sim {}^{14}CO_2$$

This was indeed a fortunate coincidence because we now know that no other biotin enzyme carboxylates free biotin. The mechanism by which free biotin can substitute for β-methylcrotonyl-CoA is still mysterious, but the reaction is by no means insignificant and has been observed by Himes and co-workers[3] with extensively purified enzyme.

The carboxybiotin was found to be very unstable, decomposing almost instantly at pH 2 and with a half-life of about 20 min. at neutral or slightly