# Thiamin Diphosphate and Its Catalytic Functions

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# THIAMIN DIPHOSPHATE AND ITS CATALYTIC FUNCTIONS

E. R. SQUIBB LECTURES ON
CHEMISTRY OF MICROBIAL PRODUCTS


*Presented at the Institute of Microbiology*
*Rutgers, the State University of New Jersey*

*In recognition of the importance of cooperation between chemist and microbiologist the E. R. Squibb Lectures on Chemistry of Microbial Products were established with the support of The Squibb Institute for Medical Research in 1955. The lectures are presented annually in the fall at the Institute of Microbiology, Rutgers, the State University of New Jersey, New Brunswick, New Jersey.*

# PREFACE

The invitation to present the E. R. Squibb Lectures on the Chemistry of Microbial Products on November 6, 7, and 8, 1968 at the Institute of Microbiology at Rutgers State University gave an opportunity to consolidate the research information obtained over the past decade on the catalytic functions of thiamin diphosphate. Thiamin, vitamin $B_1$, was the first water-soluble vitamin discovered; nevertheless, knowledge of its exact mode of action was apparently more difficult to obtain than similar information about some of the other water-soluble vitamins. Even prior to the discovery of thiamin and to the knowledge that its pyrophosphate ester was a coenzyme in enzymatic reactions involving the cleavage of pyruvate, Carl Neuberg had postulated the existence of nascent acetaldehyde as an intermediate in many of these reactions. After the discovery of thiamin diphosphate as a coenzyme, but prior to the elucidation of its mode of action, nonspecific terms such as "active acetaldehyde" and "active

glycolaldehyde" were used to describe the unknown nature of the coenzyme adducts resulting from the cleavage of pyruvate and D-xylulose-5-phosphate.

It was not until Ronald Breslow found that carbanion formation occurred at carbon atom two of the thiazolium ring and proposed that this zwitterion catalyzed the formation of benzoin from benzaldehyde by a mechanism similar to that which occurs with the cyanide ion, that substantial progress was made with enzymatic conversions dependent upon thiamin diphosphate as a coenzyme.

In this series of lectures, I have attempted to describe the developments by several investigators which led to the elucidation of the catalytic functions of thiamin diphosphate.

I am grateful to the Squibb Institute for Medical Research for providing the opportunity to spend three stimulating days at the Institute of Microbiology. I also want to thank Professor J. O. Lampen for hospitality extended to me.

L. O. KRAMPITZ

# THIAMIN DIPHOSPHATE AND ITS CATALYTIC FUNCTIONS

CONTENTS

*Preface* vii

**Introduction** 1

CHAPTER 1. **Nonenzymatic Models** 4

CHAPTER 2. **Pyruvic Decarboxylase** 18

CHAPTER 3. **Acetoin and Acetolactic Synthetase** 27

CHAPTER 4. **Pyruvic Oxidase** 32

CHAPTER 5. **Transketolase** 38

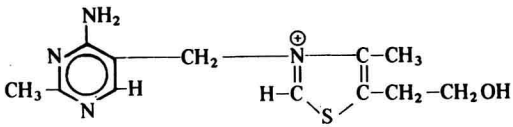
CHAPTER 6. **Phosphoketolase** 45

CHAPTER 7. **Phosphoroclastic Reaction** 51

CHAPTER 8. **Glyoxylic Acid Carboligase** 60

*Subject Index* 63

# INTRODUCTION



Thiamin

The history of the discovery and the chemical synthesis of vitamin $B_1$ is indeed a fascinating chapter in the story of the development of modern nutrition and biochemistry. Not long after thiamin was recognized as a nutritional requirement for many forms of life, its pyrophosphate ester, thiamin diphosphate, was found to be the coenzyme for enzymatic processes that cleaved α-keto acids. The mode of action of the coenzyme on a molecular level, however, was not elucidated as readily. Several theories were advanced which proposed that various functional groups of the molecule might be involved. Langenbeck proposed that a Schiffs base formation involving pyruvate and the amino group at the 4-position of the pyrimidine ring of thiamin diphosphate oc-

curred during the catalysis of the decarboxylation of pyruvate. The methylene group situated between the thiazolium and pyrimidine rings, the quatenary nitrogen, and the sulfhydryl group of the pseudobase form of the thiazolium ring as well as an oxido-reduction mechanism have also been proposed. None of these theories has been experimentally verified.

Two properties of the enzymatic systems which involve thiamin diphosphate (TDP) contributed immensely to the difficulty of formulation of experimental designs which would facilitate the elucidation of its mode of action. Very little is known concerning the nature of the binding of TDP to its enzymes. It is not bound covalently; nevertheless, the nature of the binding is such that the rate of dissociation of TDP from the protein is nil in some cases and extremely sluggish in others. As a consequence, the accumulation of intermediates of TDP with catalytic amounts of enzyme in quantities sufficiently great for analysis has been difficult. In addition the very nature of the enzymatic reactions for which TDP is a coenzyme has contributed to these difficulties. All of these reactions involve a cleavage of a keto substrate to an adduct of TDP and a residual moiety of the substrate. Following the cleavage reaction an acceptor for the adduct of TDP must be present in order for the total reaction to proceed and to regenerate the TDP.

For illustration purposes the following equations depict the action of transketolase on D-xylulose-5-phosphate.

D-xylulose-5-phosphate + TDP—E ⇌ glycolaldehyde-TDP—E + glyceraldehyde-3-phosphate (1)

glycolaldehyde-TDP—E + ribose-5-phosphate ⇌ sedoheptulose-7-phosphate + TDP—E (2)

Sum: D-xylulose-5-phosphate + ribose-5-phosphate → sedoheptulose-7-phosphate + glyceraldehyde-3-phosphate (3)

Transketolase (E) with its bound TDP catalyzes a cleavage of D-xylulose-5-phosphate to a glycolaldehyde adduct of TDP and glyceraldehyde-3-phosphate. Unless the aldehydic acceptor, ribose-5-phosphate, is present the reaction will not proceed. With the latter present the glycoladelhyde-adduct with TDP will condense with ribose-5-phosphate to form sedoheptulose-7-phosphate and regeneration of enzyme-bound TDP. Because the rate of dissociation of TDP, or its adduct from the enzyme, is extremely slow, the addition of substrate quantities of TDP does not aid in the accumulation of the intermediates of the initial cleavage, i.e., reaction (1).

In most TDP-dependent reactions the identification of the acceptor has been more readily accomplished than the identification of the adduct with TDP. Descriptive terms such as "active glycolaldehyde" in the case of transketolase and "active acetaldehyde" for those reactions involving the cleavage of pyruvate, were employed to describe the unknown nature of the adducts.

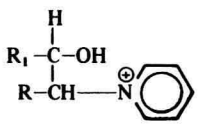
Nonenzymatic model systems gave the first insight into the nature of these cleavages and condensations catalyzed by thiamin. Not only did these model systems aid in the elucidation of the mode of action of TDP in enzymatic systems, they also suggested procedures by which the various adducts of TDP could be prepared and subsequently tested enzymatically. These model systems will be discussed first and a discussion of the mode of action of TDP in enzymatic systems will follow.

CHAPTER

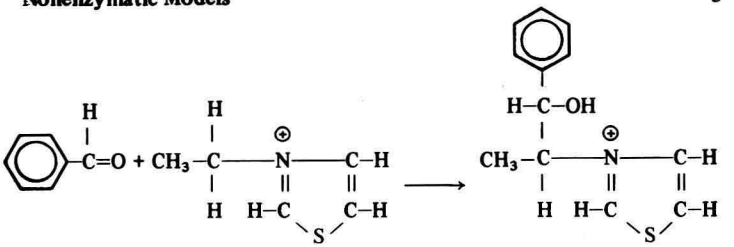
1

# NONENZYMATIC MODELS

Pyridinium compounds, with an alkyl or aryl substitution on the quatenary nitrogen, condense with aldehydes to form the following types of compound:



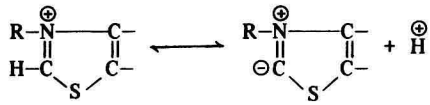
Ugai and his co-workers [1,2], recognizing the similarity between pyridinium and thiazolium compounds, attempted to demonstrate a condensation of benzaldehyde with N-ethyl thiazolium bromide, expecting to obtain the product depicted in the equation:



Instead of obtaining the benzaldehyde condensation product with the methylene group adjacent to the quatenary nitrogen of the thiazolium ring, they obtained benzoin, indicating that the thiazolium compound had catalyzed the condensation of 2 moles of benzaldehyde to benzoin. A number of thiazolium compounds, including thiamin, were reacted with benzaldehyde and many of them were found to form benzoin.

The catalysis of benzoin formation from benzaldehyde by the thiazolium ion was recognized by Mizuhara and co-workers [3,4] as a model system similar to the TDP-dependent enzymatic system which forms acetoin from pyruvate. They demonstrated that pyruvate was decarboxylated nonenzymatically by thiamin at pH 8.4 and room temperature, and small quantities of acetoin were formed. When pyruvate and acetaldehyde were added to thiamin under the same conditions considerably more acetoin was formed. Inasmuch as the optimum pH for acetoin formation is 8.4, which is near the pK of pseudobase formation by thiazolium compounds, they proposed that an adduct of pyruvate with the pseudobase occurred. This mechanism of action had been previously suggested by Karrer [5]. Ingraham and Westheimer [6] and Breslow [7] examined the possibility that the methylene group situated between the two resonating heterocyclic rings of thiamin might be involved in the catalysis. They showed that deuterium of $D_2O$ did not exchange with the hydrogens of the methylene group, thus eliminating this possibility. Breslow [8] however, continuing this type of investigation, found that 3,4-dimethyl thiazolium

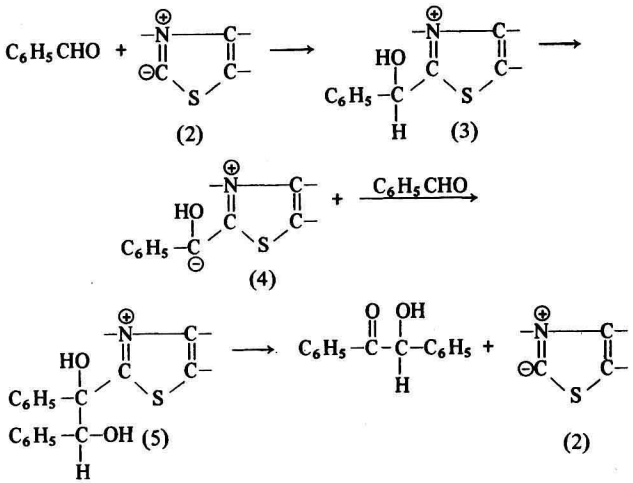
bromide and 3-benzyl-4-methyl thiazolium bromide exchanged one proton for deuterium when dissolved in $D_2O$ at room temperature. The exchange occurred at the $C_2$ position of the thiazolium ring. Thiamin exchanged five protons, four on the OH and $\overset{\oplus}{N}H_3$ groups and the fifth with the hydrogen at the $C_2$ position of the thiazolium ring. The exchange reaction is depicted



(1) (2)

Breslow suggested the switterion (2) might be related to the cyanide ion, and therefore would catalyze benzoin formation as does the cyanide ion. Breslow [8] and Yount and Metzler [9] tested a number of thiazolium compounds for their ability to catalyze acetoin formation under the conditions employed by Mizuhara [3,4]. Thiamin was the most effective catalyst, although those thiazolium salts substituted with an aromatic group on the quatenary nitrogen of the thiazolium ring and unsubstituted at the $C_2$ position were active. A methyl-group substitution at the $C_2$ position rendered the thiazolium ion inactive as a catalyst for acetoin formation. It is interesting that Bergel and Todd [10] several years ago found that a methyl group substitution at position two of the thiazolium ring of thiamin resulted in complete loss of vitamin activity. One wonders why those of us interested in the mode of action of thiamin did not recognize the importance of the $C_2$ position much sooner.

As a result of these findings, Breslow [8,11] proposed the following sequence of reactions to explain the role of thiamin in the catalysis of benzoin formation from benzaldehyde, as illustrated.



An attack by the zwitterion (2) on benzaldehyde formed the benzaldehyde adduct of the thiazolium ion. After carbanion formation at the alpha carbon of the adduct, zwitterion (4) condensed with a second mole of benzaldehyde, forming the benzion adduct which formed benzoin and the original zwitterion (2). In an attempt to verify the proposal, Breslow [8] attempted to synthesize the benzaldehyde adduct with 3,4-dimethyl thiazolium bromide for the purpose of testing it as an intermediate in the reaction sequence but found it too unstable for isolation.

The reaction sequence which Breslow proposed for the reactions involving thiamin, pyruvate, and acetaldehyde for the formation of acetoin was: