Bioorganic Chemistry 57 (2014) 251–262

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Progress in the experimental observation of thiamin diphosphate-bound intermediates on enzymes and mechanistic information derived from these observations

Frank Jordan *, Natalia S. Nemeria *

Department of Chemistry, Rutgers University, Newark, NJ 07102, USA

ARTICLE INFO

Article history:
Available online 3 September 2014

Keywords:
Thiamin diphosphate
C2-ylide/C2-carbanion/C2-carbene
C2α-carbanion or enamine
Circular dichroism
1′,4′-imino tautomer
Pyruvate dehydrogenase complex
2-oxoglutarate dehydrogenase complex
Yeast pyruvate decarboxylase
Benzaldehyde lyase
Glyoxylate carboligase
1-Deoxy-D-xylulose-5 phosphate synthase
Benzoylformate decarboxylase
C2α-hydroxyethylthiamin diphosphate
C2α-lactylthiamin diphosphate

ABSTRACT

Thiamin diphosphate (ThDP), the vitamin B1 coenzyme is an excellent representative of coenzymes, which carry out electrophilic catalysis by forming a covalent complex with their substrates. The function of ThDP is to greatly increase the acidity of two carbon acids by stabilizing their conjugate bases, the ylide/carbene/C2-carbanion of the thiazolium ring and the C2α-carbanion/enamine, once the substrate binds to ThDP. In recent years, several ThDP-bound intermediates on such pathways have been characterized by both solution and solid-state methods. Prominent among these advances are X-ray crystallographic results identifying both oxidative and non-oxidative intermediates, rapid chemical quench followed by NMR detection of several intermediates which are stable under acidic conditions, solid-state NMR and circular dichroism detection of the states of ionization and tautomerization of the 4′-aminopyrimidine moiety of ThDP in some of the intermediates. These methods also enabled in some cases determination of the rate-limiting step in the complex series of steps. This review is an update of a review with the same title published by the authors in 2005 in this Journal. Much progress has been made in the intervening decade in the identification of the intermediates and their application to gain additional mechanistic insight.

© 2014 Elsevier Inc. All rights reserved.

1. Introduction

Among the coenzymes derived from water-soluble vitamins, thiamin diphosphate (ThDP) and pyridoxal phosphate are frequently used as examples of cofactors, which carry out electrophilic catalysis by forming covalent intermediates with their substrates. Pyridoxal phosphate gives rise to a variety of chromophoric intermediates with its substrates, which have been characterized over the years. Due to a fortuitous acid stability of several of the key ThDP-bound covalent intermediates, which enable both their synthesis and chemical trapping (acid quench) for eventual detection, ThDP enzymes provide both an opportunity and a challenge to the enzymologist wishing to observe and to study the catalytic competence of such intermediates. In addition, due to the special

Abbreviations: ThC, thiamin; ThDP, thiamin diphosphate; YPDC, yeast pyruvate decarboxylase from *Saccharomyces cerevisiae*; PDHc, pyruvate dehydrogenase complex; OGDHc, 2-oxoglutarate dehydrogenase complex; TK, transketolase; POX, pyruvate oxidase from *Lactobacillus plantarum*; AHAS, acetohydroxyacid synthase; ALS, acetolactate synthase; GCL, glyoxylate carboligase; BFDC, benzoylformate decarboxylase; BAL, benzaldehyde lyase; PFOR, pyruvate ferredoxin oxidoreductase; DXPS, 1-deoxy-D-xylulose 5-phosphate synthase; E1p-ec, the first component of the *E. coli* PDHc; E1p-h, the first component of the human PDHc; E1o-ec, the first component of *E. coli* OGDHc; E1o-h, the first component of human OGDHc; PDA, photodiode array; Sf, stopped-flow; CD, circular dichroism; IP, the 1′,4′-iminopyrimidine tautomer of ThDP or its C2-substituted derivatives; AP, the canonical 4-aminopyrimidine tautomer of ThDP or its C2-substituted derivatives; APH^+, the N1-protonated 4-aminopyrimidinium form of ThDP or its C2-substituted derivatives; Yl, the C2-carbanion/ylide/carbene form conjugate base of ThDP; HEThDP, C2α-hydroxyethylThDP, the adduct of acetaldehyde and ThDP; HBThDP, C2α-hydroxybenzylThDP, the adduct of benzaldehyde and ThDP; LThDP, C2α-lactylThDP, the adduct of pyruvic acid and ThDP; MThDP, C2α-mandelylThDP, the adduct of benzoylformic acid and ThDP; MAP, acetylphosphonic acid monomethyl ester; MBP, benzoylphosphonic acid monomethyl ester; PMThDP, C2α-phosphonomandelylThDP, the adduct of MBP and ThDP; PLThDP, C2α-phosphonolactylThDP, the adduct of MAP and ThDP; 3-PKB, (*E*)-4-(pyridin-3-yl)-2-oxo-3-butenoic acid; AcThDP, 2-acetylThDP, the oxidized form of HEThDP; PDC, pyruvate decarboxylase; E1p, the first (ThDP-dependent) component of PDHc; E1o, the first (ThDP-dependent) component of OGDHc; -ec, *E. coli*, *Escherichia coli* enzyme; -h, human enzyme.

* Address: 73 Warren St., Newark, NJ 07102, USA. Fax: +1 973 353 1264.
E-mail addresses: frjordan@rutgers.edu (F. Jordan), nemeria@andromeda.rutgers.edu (N.S. Nemeria).

http://dx.doi.org/10.1016/j.bioorg.2014.08.002
0045-2068/© 2014 Elsevier Inc. All rights reserved.

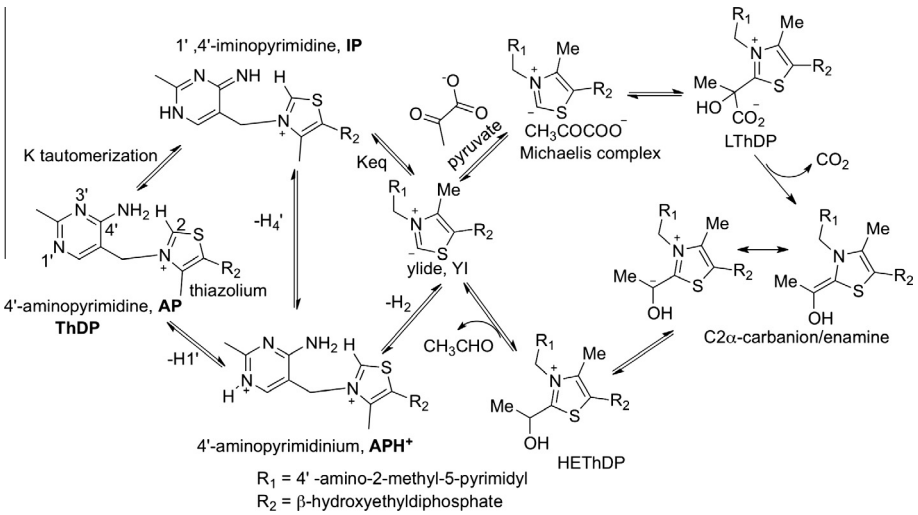
environment on the enzyme, several of these ThDP-derived intermediates give rise to circular dichroism (CD) signals, not seen in the absence of the enzymes. The authors' laboratory has during the past decades worked on 12 representatives of this large family of enzymes, starting with yeast pyruvate decarboxylase (YPDC, Scheme 1), and culminating with the ThDP-dependent first E1 components of both the human and *Escherichia coli* pyruvate dehydrogenase (E1p-h and E1p-ec) and 2-oxoglutarate (also known as α-ketoglutarate) dehydrogenase (E1o-h and E1o-ec) multienzyme complexes (Scheme 2 for the mechanism of the 2-oxoglutarate multienzyme complex). The goal of this review is to summarize data mostly from the authors' experience on these enzymes, and the authors apologize to those whose contributions have been overlooked, it was unintentional.

There are reviews appearing continuously on the enzymology of thiamin diphosphate (ThDP, the vitamin B1 coenzyme- see Fig. 1 for structures of small molecules mentioned in the review) [1–15], the purpose of this review is to summarize the current understanding of the ThDP-related intermediates on enzymes, including a fascinating, and perhaps unique aspect of thiamin enzymology, the existence of the rare 1′,4′-iminopyrimidinyl tautomeric form. This issue has been made more important by some of the X-ray crystal structure determinations of ThDP enzymes. The question raised by the finding is related to the conundrum that any plausible mechanism one can suggest for ThDP-dependent enzymes, whether they are 2-oxo acid decarboxylases or carboligases [see Schemes 1–3 for examples of a non-oxidative decarboxylase yeast pyruvate decarboxylase (YPDC; EC 4.1.1.1), an oxidative decarboxylase the 2-oxoglutarate dehydrogenase complex (OGDHc; EC 1.2.4.2), and a carboligase 1-deoxy-D-xylulose 5-phosphate synthase (DXPS; 2.2.1.7), respectively], requires some proton transfer steps. On the basis of accumulated understanding of enzyme mechanisms, such proton transfers are likely mediated by general acid–base catalysts, such as His, Asp, Glu, perhaps, Cys, Lys and Tyr, with the understanding that the enzyme active center could modulate the aqueous pK_a of these side chains, as needed.

Several groups including the authors [16] spent considerable time trying to assign acid–base functions to such residues on ThDP enzymes – with limited success. More recently, McLeish and coworkers have been carrying out saturation mutagenesis experiments [17] probing the function of two active center histidine residues (His70 and His281) on benzoylformate decarboxylase (BFDC), long believed to participate in acid–base reactions [18]. Surprisingly, their results indicated that hydrophobic residues could replace the His281 with little penalty, and even with His70 there was only a 70-fold penalty on k_{cat}/K_m. In the authors' laboratory a somewhat similar behavior emerged on the E1o-ec, where of the two His residues (260 and 298) assigned (on the basis of the X-ray structure) to bind the 5-carboxyl group of the 2-oxoglutarate (OG) substrate, residue 260 could not, while residue 298 could be substituted with other amino acids, with little loss of activity according to saturation mutagenesis studies [19]. A reasonable question in the interpretation of such results is what is an appropriate contribution from His or Asp or Glu to reflect general acid–base reactivity on the enzyme? The authors prefer to use two well-explored examples which could provide benchmark values for acid–base catalysis on enzymes, although precise interpretation of these numbers is not only risky, but also depends on the particular substitution used to arrive at them [20]: (a) Serine proteases, where substitution of either His (a presumed general acid–base catalyst) or Ser (a nucleophilic catalyst) by Ala in the well-characterized Asp-His-Ser catalytic triad of subtilisin leads to ca. 2×10^6 reduction in k_{cat} [21]; and (b) Ketosteroid isomerase (EC 5.3.3.1), where substitution of the catalytic Asp38 by Asn leads to $10^{5.6}$ decrease in k_{cat} [22], while substitution of the same residue by Ala only reduced the k_{cat} by 140 [23].

Complicating this issue on ThDP enzymes is that the pH dependence of the steady state kinetic parameters does not give clear evidence for participation of such residues in the rate-limiting step(s). For example, all potential active center acid–base residues were substituted on YPDC [16], with little perturbation of the pH dependence of such plots, perhaps with the exception of the substitution at the conserved glutamate. Therefore, the 100–500-fold reduction in steady state kinetic constants could not be unequivocally attributed to acid–base function, while such numbers are certainly consistent with hydrogen bonding interactions.

The X-ray structure of two unusual ThDP-dependent enzymes was solved. The enzyme benzaldehyde lyase (BAL, EC 4.1.2.38) carries out reversible decomposition of (*R*)-benzoin to two molecules of benzaldehyde; in the reverse direction the enzyme is a carboligase. The X-ray structure of BAL contained only two acid–base residues surrounding the ThDP at the active center [24–26]: a highly conserved Glu50 within hydrogen bonding distance of the N1′ atom of the 4′-aminopyrimidine ring and a His29 residue. The residue His29 is too far from the thiazolium C2 atom to be of value in the first steps of the reaction and was suggested to have a function in removing the β-hydroxyl proton of the ThDP-bound benzoin to assist in releasing the first benzaldehyde molecule. In the authors'



Scheme 1. Mechanism of yeast pyruvate decarboxylase [81].

Download English Version:

https://daneshyari.com/en/article/1355932

Download Persian Version:

https://daneshyari.com/article/1355932

Daneshyari.com