Deuterium labeling as a test of intramolecular hydride mechanisms in the fragmentation of 2-(1-hydroxybenzyl)-N1′-methylthiamin[1]

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Abstract: 2-(1-Hydroxybenzyl)-N1′-methylthiamin (**1b**) is a model for the addition intermediate in the thiamin catalyzed benzoin condensation. However, N-alkylation alters the reactivity of the compound: instead of undergoing base-catalyzed formation of benzaldehyde and N1′-methylthiamin, it rapidly forms trimethyl amino pyrimidine (**2b**) and phenylthiazole ketone (**3**). The base-catalyzed fragmentation process is faster than the analogous enzymic reaction (in benzoylformate decarboxylase) under the same conditions. One possible mechanism for the rapid fragmentation is an internal hydride transfer from α-C2 to the methylene bridge between the heterocycles. To test the hydride mechanism we prepared α-C2-deuterated **1b** and conducted the fragmentation reaction in normal water. Spectroscopic analysis revealed that the trimethyl aminopyrimidine product does not contain any deuterium, ruling out a hydride transfer mechanism. This supports a mechanism for fragmentation that proceeds instead via a proton transfer from α-C2. Since protonation (and hence, deprotonation) of that site is part of the normal catalytic cycle of benzoylformate decarboxylase, the enzyme must divert the reaction from the lowest energy pathway since it would share a common intermediate with the fragmentation process.

Key words: thiamin, fragmentation, benzoylformate decarboxylase, proton transfer, hydride shift.

Résumé : La 2-(1-hydroxybenzyl)-N1′-méthylthiamine (**1b**) est un modèle pour l'intermédiaire d'addition dans la condensation de la benzoïne catalysée par la thiamine. Toutefois, la N-alkylation altère la réactivité du composé qui, au lieu de subir une réaction catalysée par les bases conduisant à la formation de benzaldéhyde et de N1′-méthylthiamine conduit à la formation rapide de triméthylaminopyrimidine (**2b**) et phénylthiazole cétone (**3**). Le processus de fragmentation catalysé par les bases est plus rapide que la réaction enzymatique analogue (en présence de décarboxylase de benzoylformate) dans les mêmes conditions. Un mécanisme possible pour la fragmentation rapide implique un transfert interne d'hydrure de la position α-C2 vers le pont méthylène entre les hétérocycles. Afin de tenter de confirmer ce mécanisme impliquant un hydrure, le composé **1b** deutéré en α-C2 a été préparé et soumis à une fragmentation dans de l'eau normale. Une analyse spectroscopique a révélé que le produit triméthyl aminopyrimidine ne contient pas de deutérium, ce qui élimine le mécanisme par transfert d'hydrure, mais qui supporte un mécanisme de fragmentation qui se produirait plutôt par le biais d'un transfert de proton à partir de α-C2. Puisque la protonation (et, par voie de conséquence, la déprotonation) de ce site fait partie du cycle catalytique normal de la décarboxylase du benzoylformate, l'enzyme doit faire dévier la réaction de la voie impliquant l'énergie minimale puisqu'il doit partager un intermédiaire commun avec le processus de fragmentation.

Mots clés : thiamine, fragmentation, décarboxylase de benzoylformate, transfert de proton, déplacement d'hydrure.

[Traduit par la Rédaction]

Introduction

The base-catalyzed addition of thiamin to benzaldehyde produces 2-(1-hydroxybenzyl)thiamin (HBnT, **1a**), following the mechanism deduced by Breslow (1, 2).

HBnT also resembles HBnT diphosphate, an intermediate on the pathway of benzoylformate decarboxylase (BFD) (3–5). In the enzymic system, ionization of the hydroxyl group facilitates the elimination of benzaldehyde. However, the major products from decomposition of HBnT in solution are an aminopyrimidine (AP, **2a**) and a phenylthiazole ketone (PTK, **3**) (6). These products were originally reported by Oka et al. for different conditions (7). Base-catalyzed fragmentation overwhelms elimination, being over 10^3 times faster at pH 7 (8). To observe fragmentation without competition from elimination, the N1′-methylated derivative of HBnT (MHBT, **1b**) was prepared, an analogue of the BFD intermediate with N1′ protonated on the enzyme (5).

The fragmentation pathway of HBnT is consistent with the first step being the loss of a proton from α-C2 to generate the delocalized conjugate base, which undergoes the fragmentation reaction as shown (Fig. 1, mechanism 1). The carbanion can also be accessed by decarboxylation (9, 10).

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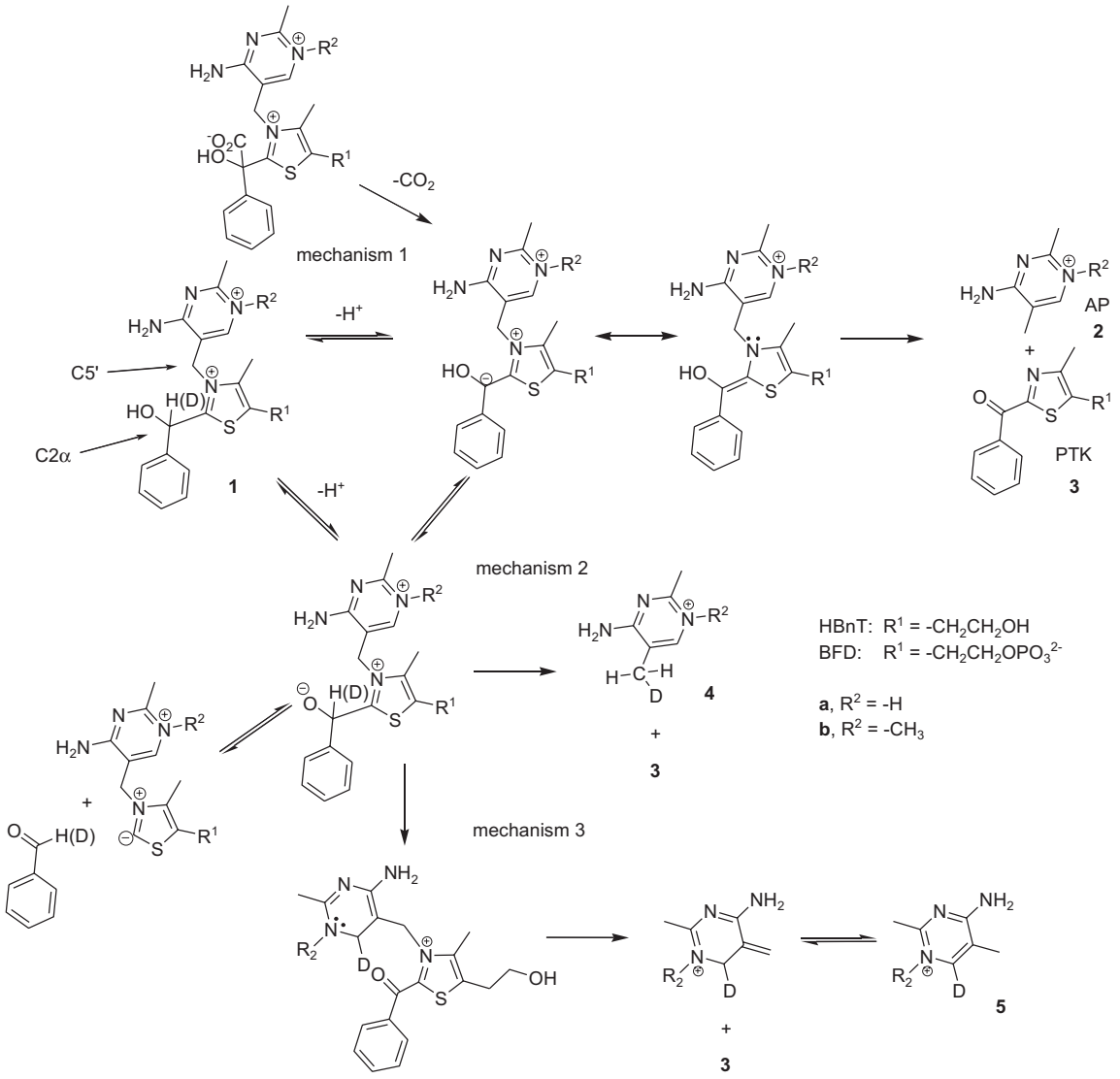
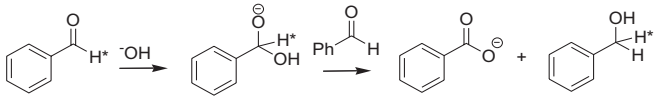
Fig. 1. Mechanisms and deuterium tracing in the fragmentation of HBnT.



An alternative pathway involves the kinetically equivalent oxyanion, obtained from rapid proton transfer from oxygen to carbon that may derive additional stabilization from the positively charged pyrimidine. Fragmentation then would result from an internal Cannizzaro-type reaction (11) involving a hydride shift from α-C2 (Fig. 1, mechanisms 2 and 3). If the hydride mechanism was responsible for the fragmentation, it might involve a manifold of intermediates that are clearly distinct from those formed in the enzymic process. In these mechanisms, the conjugate base of MHBT would react in analogy to the conjugate base of the hydrate of benzaldehyde (Fig. 2). The distinction of hydride and proton transfer mechanisms is reminiscent of the alternatives that were considered as possibilities in the normal catalytic process of glyoxylase I (12–14), which were resolved by the work of Jordan, Kozarich, and their co-workers (13, 14).

The hydride transfer mechanism must give a direct transfer to the acceptor site while a proton transfer mechanism leads to equilibration with the solvent. Thus, we produced the α-C2 deuterated analogue of MHBT (MHBT-*d*) by addition of thiamin addition to deuterobenzaldehyde, and then

Fig. 2. The hydroxide induced Cannizzaro reaction as a model for hydride transfer in MHBT.



conducted the reaction in normal water. The α-C2 carbon acid exchanges very slowly under the conditions of our studies of the fragmentation reaction, so loss of the deuteron will not occur prior to the fragmentation process. The hydride mechanisms require incorporation of the deuterium into the methyl or aryl groups of the pyrimidine product, and the relative rates assure us that this would be detected. Furthermore, proton–deuterium exchange does not occur into the products, so that any loss of deuterium comes from the initial reaction.

The proposed mechanism could potentially give C5′-deuterated AP from the fragmentation of MHBT-*d* in water if the aromatization step occurred with internal deuterium transfer. However, tautomerization would be subject to an

isotope effect and could be solvent-mediated. Therefore, only partial incorporation of deuterium into the C5′ hydrogen is possible in this mechanism.

In the case of BFD, a carbanion must be generated from loss of carbon dioxide from the intermediate generated by addition of thiamin diphosphate to benzoylformate. The same carbanion would be generated from the loss of a proton from the conjugate of thiamin diphosphate and benzaldehyde (HBnT diphosphate). If the fragmentation reaction occurs by a hydride shift mechanism, then this would compete with elimination of the TDP ylide in the enzyme reaction. The enzyme could promote formation of benzaldehyde by controlling the direction of elimination in formation of the carbonyl group, forming the aldehyde rather than the ketone.

Experimental section

The syntheses of MHBT and MHBT-*d* have been previously reported (8, 15). The bisperchlorate of MHBT-*d* (0.3 mmol) was dissolved in 10 mL of water at 25 °C. The solution acidity was maintained at pH 8.0 by addition of 0.1 mol/L potassium hydroxide by an automated burette. After 2 h, the reaction was stopped by addition of hydrochloric acid (1 mol/L) to give an acid concentration in the sample of 0.01 mol/L. The product was purified by extraction with two portions of dichloromethane. Lyophilization of the aqueous layer yielded a mixture of AP and potassium chloride (6–8).

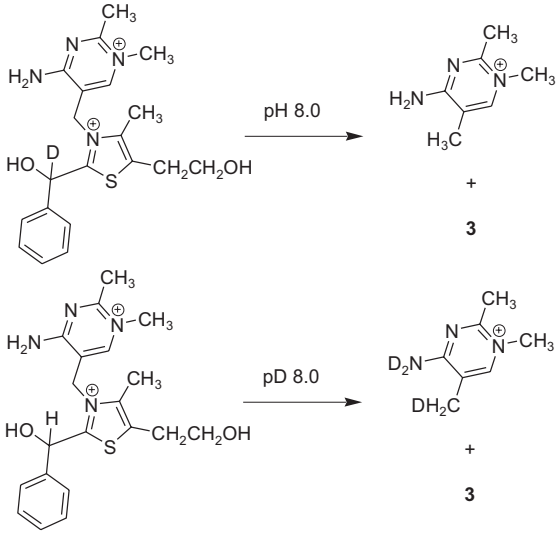
A similar procedure was used for the reaction of MHBT in deuterium oxide. We converted pH meter readings to those for pD by adding 0.4 to the displayed results (16). Fully deuterated acid–base reagents were used. This produced the C5′-monodeuterated analog of AP. ^{1}H NMR (500 MHz, CD_3OD) δ: 8.0 (s, 1H), 3.8 (s, 3H), 2.6 (s, 3H), 2.1 (dt, 3H). EI-MS (high resolution) calcd. for $[N_7H_{10}DN_3]^+$: 138.1016; found: 138.1018.

Results and discussion

No deuterium incorporation occurs into AP from the fragmentation of MHBT-*d* in water (^{1}H NMR and EI-MS (high resolution) calcd. for $[N_7H_{11}N_3]^+$: 137.0953; found: 137.0953). As a control, AP was characterized from the fragmentation of MHBT in deuterium oxide. EI-MS indicates the incorporation of a single deuterium. The location of the deuteron was determined by ^{1}H NMR to be at the C5′ position, which is derived from the methylene bridge between the two heterocycles. The signal for the C5′-methyl group initially consisted of a doublet of triplets ($^2J_{H\text{-}D}$ = 2.1 Hz, $^4J_{H\text{-}H}$ = 0.9 Hz, CD_3OD) from coupling to the geminal deuteron and neighbouring C6′ proton. After confirming the latter assignment by ^{1}H–^{1}H COSY, signal decoupling produced the characteristic 1:1:1 triplet of the monodeuterated methyl group. As the protons of AP do not exchange in deuterium oxide, deuterium incorporation in water would have been detectable under these conditions.

Owing to the absence of deuterated AP products **4** and **5** from the fragmentation of MHBT-*d* in water, we can rule out hydride transfer mechanisms (Fig. 3). Deuterium would be scrambled into solvent from deprotonation of the weak carbon acid to generate the carbanion that is consistent with

Fig. 3. Summary of the results from the deuterium labeling experiments in water and deuterium oxide.



mechanism 1. Monodeuteration of AP from the fragmentation in deuterium oxide is also consistent with mechanism 1 in which C—N bond cleavage occurs with the ionization of C5′ followed by solvent-mediated protonation. Regiospecific deuterium incorporation demonstrates that the isotope comes from solvent and is not transferred internally. The fact that proton–deuterium exchange is not observed in AP indicates that the C5′ methyl group is only weakly acidic, which is consistent with the fragmentation being highly exothermic.

The evidence against an internal hydride transfer supports a mechanism involving the conjugate base at carbon (α-C2). If fragmentation involves the delocalized conjugate base of MHBT, C—N bond cleavage, oxidation of the hydroxyl, and protonation of the C5′ carbon must occur, in steps or in concert. The surprisingly low barrier associated with fragmentation (10^4 s^{-1}) (15) suggests that the transition state of the C—N cleavage process has special stabilization. We note that BFD avoids fragmentation of a similar carbanionic intermediate that is generated by decarboxylation of the addition intermediate from benzoylformate and thiamin diphosphate. The enzyme's normal catalytic route has a higher barrier (k_{cat} = 10^2 s^{-1}) than the fragmentation reaction (17). Since the enzyme-catalyzed reaction necessarily involves a carbanionic structure upon loss of carbon dioxide, the mechanism of fragmentation avoidance is clearly a result of the effects of the enzyme environment (10). The protein manifold of the active site must increase the barrier to fragmentation while lowering that for protonation since the analogous substrates readily undergo fragmentation in solution.

Acknowledgements

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