CHEMICAL BEHAVIOR OF ISOLATED THIAMIN YLIDE IN NEUTRAL AQUEOUS SOLUTION — A NEW MECHANISM OF INTERCONVERSION OF THIAMIN AND THIAMIN THIOLATE

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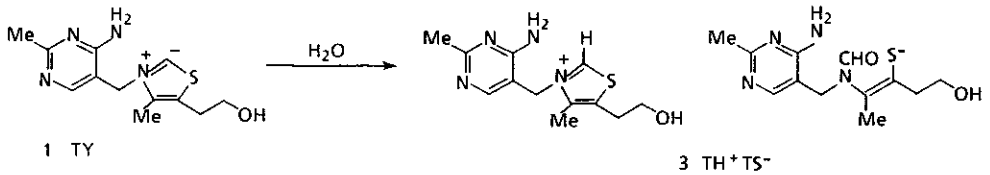
Abstract — The isolated thiamin ylide (TY) was found to be converted into thiamin thiolate (TH^+TS^-, 3) in neutral aqueous solution. The formation of the ion-pair can best be explained by the disproportionation of the ylide into the yellow form (TH^+YF^-, 4) followed by ring-opening by the attack of water to pyrimido[4,5-d]pyrimidine ring of the yellow form. These results suggested a new mechanism for the interconversion between thiamin (TH^+Cl^-) and thiamin thiolate (Na^+TS^-).

In order to account for the enzymatic and non-enzymatic reactivity of thiamin 2a, a number of hypothetical intermediates have been assumed to exist but none had been definitely isolated. We offered the first proof of their existence with the isolation and characterization of thiamin ylide 1 which was easily isolated as a mixture with NaCl by neutralization of 2a with an equivalent molar amount of NaOEt in EtOH and remained stable under nitrogen storage.[1]

In this study, we examined the chemical properties of the isolated 1 under neutral condition to try to understand more about the nature of thiamin itself. Formal protonation of 1 was demonstrated by the reaction with weak acids, that is, when 1 was treated with p-nitrobenzoic acid or p-nitrothiophenol in DMSO under neutral condition, it reverted to thiamin p-nitrobenzoate 2b or p-nitrothiophenolate 2c, respectively. Here, we report the chemical behavior of the isolated thiamin ylide in H_2O under neutral condition, which is closely related to the alkaline-induced transformation of 2a.

The generally accepted mechanism starts from the nucleophilic attack of hydroxide anion toward thiazolium C2 carbon of 2a to form pseudo B_1. We thought that studying the behavior of the isolated 1 in neutral water would provide us with some hint as to how protonation of 1 by H_2O and subsequent



(1)

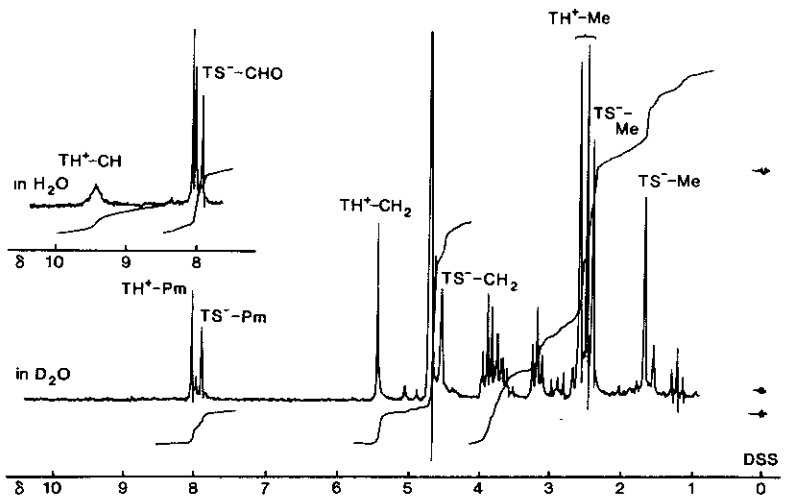
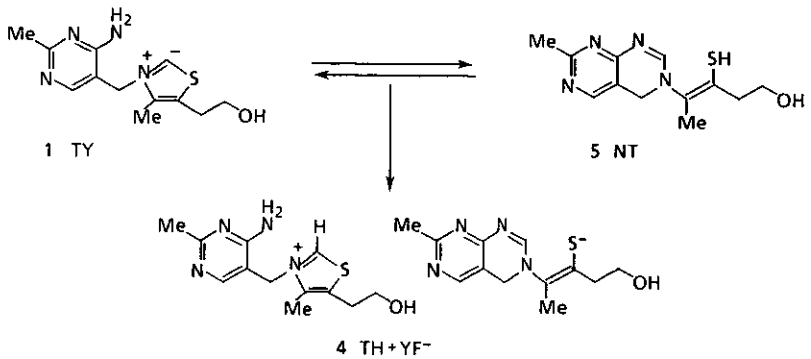


Fig 1. NMR spectra of TY in H_2O or D_2O

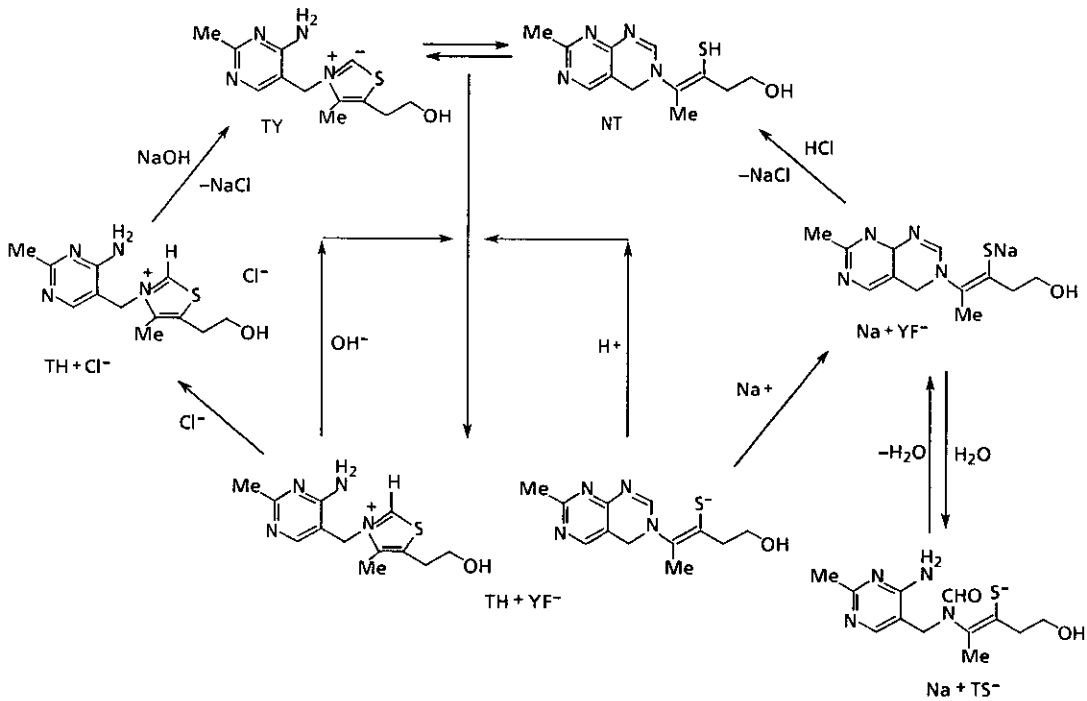
intramolecular nucleophilic attack of hydroxide anion can generate pseudo B_1 or its ring-opened free form of thiamin thiolate (H^+TS^-), however, this was not the case. When 1 was dissolved in neutral water, the transient yellow solution that formed turned into a colorless solution in a few minutes. The nmr spectrum of the colorless solution in H_2O or D_2O clearly showed the formation of the ion-pair 3 (TH^+TS^-, (1) and Fig. 1). No further chemical changes occurred when the mixture was left standing overnight. Thus, 1 was converted into thiamin thiolate 3 in water even under neutral condition. Product isolation was not attempted. The yellow coloring, whose λ_{max} was identical with that of yellow form of B_1, indicates that thiamin thiolate 3 was formed via the yellow form 4 with the gegen cation being thiamin (TH^+).

Discussion: Thiamin ylide was found to change into thiazolium thiolate 3 via the yellow form 4 in neutral water. Water first provides a solvent effect in which ylide 1 disproportionates into 4 and then causes ring-opening of pyrimido[4,5-d]pyrimidine moiety of 4 to give the stable ion-pair 3. Disproportionation of 1 into 4 can be explained by assuming an equilibrium or acid or base independent isomerization between ylide 1 and neothiamin (NT) 5 (2).



(2)

Neothiamin 5 itself has not been proved to occur during the transformation of 2, although, some derivatives have been synthesized.[2] Based upon our finding on the chemical nature of 1 in neutral aqueous solution and the assumption of interconversion between 1 and 5, we propose a new mechanism of transformation between thiamin 2 and thiamin thiolate (Scheme 1).



Scheme 1. A simplified mechanism for base- or acid-induced transformation of TH^+Cl^- or Na^+YF^-.

Hopmann et al.[3] characterized the alkali-induced transformation of TH^+Cl^- based on kinetic studies and reached the following conclusions. (1) Two moles of OH^- (NaOH) are needed to generate Na^+YF^-, with TY as an intermediate. (2) The decay of YF^- (the gegen cation is not specified) is not mechanistically clear. (3)

Z^+YF^- (Z = gegen cation) can change directly into Z^+TS^-. Horman[4] recently confirmed this reaction by nmr spectroscopy which gave the same results. (4) No mechanism was found for the regeneration of TH^+Cl^- from Na^+TS^- under acidic condition. (5) Two moles of H^+ (HCl) are needed for transformation from Na^+TS^- to TH^+Cl^-. (6) Positively charged and negatively charged species are included in the transformation of $TH^+Cl^- \leftrightarrows Na^+TS^-$. Hopmann et al. assumed that, in addition to TY, dihydrothiochrome (TC) would act as an intermediate in the process from TH^+Cl^- to Na^+TS^- and pseudo B_1 for the reverse reaction. However, they emphasized the need for the more experimental facts to understand the total aspects of thiamin kinetics. Dubois et al.[5] also studied thiamin kinetics and reported that an intermediate, which was generated by the reaction of TH^+Cl^- with one mole of NaOH, existed in aqueous solution and assigned it as being pseudo B_1 based on its UV spectrum. However, its reported UV spectrum closely resembles that of our isolated TY.

The characteristic features of our proposed mechanism are: (1) OH^- acts as a base at any pH region to yield TY from TH^+Cl^-;[6] (2) Z^+TS^- is generated by attack of H_2O to Z^+YF^- and thus mechanistic discussion can be simplified by considering only interconversion between TH^+Cl^- and Na^+YF^-; (3) Neutralization of TH^+Cl^- or Na^+YF^- first with a mole of OH^- (NaOH) or H^+ (HCl), respectively, gives a neutral intermediate TY or NT, respectively. They disproportionate into an ion-pair, TH^+YF^-, provided that a fast and acid- or alkali-independent interconversion takes place between TY and NT.[7] This ion-pair intermediate reacts with a second mole of OH^- for reaction of TH^+Cl^- to Na^+YF^- or H^+ for the reverse reaction, respectively, to regenerate TY and afford Na^+YF^- for the forward reaction or NT and TH^+Cl^- for the reverse reaction, respectively. Thus, we conclude that there are two distinct intermediates, a neutral species (TY, which might be chemically equivalent with NT) and an ionic one (TH^+YF^-) between thiamin (TH^+Cl^-) and the yellow form (Na^+YF^-). These mechanistic features completely satisfy Hopmann's kinetic demands.

With regard to the fast interconversion between TY and NT, there has been no report on the isolation of neothiamin, although, the isolation of Na^+YF^- as a trihydrate has been reported,[8] neutralization with an equimolar quantity of acid has not yet been carried out. If the neutralization product of Na^+YF^- is chemically and spectroscopically equivalent to TY,[9] then it might be possible to unify TY and NT into a single neutral intermediate of mp 127-129°C.[10] To understand more about TY or NT, the electronic states of both compounds need to be studied from the theoretical standpoint.[13]

REFERENCES AND NOTES

1. H. Sugimoto and K. Hirai, Tetrahedron Lett., 1985, **26**, 883.
2. (a) Preparation of neocyanothiamin: S. Kasahara, Chem. Pharm. Bull., 1960, **8**, 340. (b) Isolation of neothiamin disulfide: G. E. Risinger, P. N. Parker, and H. H. Hsieh, Experientia, 1965, 434.
3. (a) R. F. W. Hopmann, G. P. Brugnoni, and B. Fol, J. Am. Chem. Soc., 1982, **104**, 1341. (b) R. F. W. Hopmann, Anal. N.Y. Acad. Sci., 1982, **378**, 32.
4. I. Horman, Helv. Chim. Acta, 1984, **67**, 1478.
5. J.-M. E. H. Chahine and J.-E. Dubois, J. Am. Chem. Soc., 1983, **105**, 2335.
6. Nucleophilic addition and the ring-opening reaction of azole compound by OH^- are well documented, but the acid-base reaction seems to kinetically favored for thiamin transformation.
7. Participation of the amino group in the pyrimidine ring has been considered for the initial state taking into consideration the basicity or nucleophilicity of the group. In our mechanism, the amino group takes part in the isomerization between 1 and 5, that is, its role is substantial to thiamin chemistry.
8. O. Zima and R. R. Williams, Ber., 1940, **73**, 941.
9. We have observed the formation of the yellow form (Z^+TF^-) when NaOEt in EtOH was added to a suspension of TH^+Cl^- in EtOH (under excess thiamin condition) which was followed by decoloring to yield TY. This indicates occurrence of the reaction $Z^+YF^- + TH^+Cl^-$ (as an acid) $\rightarrow$ 2 TY + Z^+Cl^- ($Z^+ = Na^+$ or TH^+).
10. Maier and Metzler[11] isolated the neutralized product of TH^+Cl^- to which the TC structure was assigned and they described its disproportionation into "neutral and yellow forms of thiamin" in water. As we discussed in our previous paper,[1] comparing this product spectroscopically and chemically with the related ortho-ester type compound ruled out the possibility of it being tricyclic. Recently, Doughty et al.[12] synthesized two derivatives of the ortho-ester type 2,3-dihydrothiazole and found them to be stable under alkaline condition. Their results support our idea that tricyclic compound such as TC is stable toward base.
11. (a) G. D. Maier and D. E. Metzler, J. Am. Chem. Soc., 1957, **79**, 4386, 6583. (b) D. E. Metzler and G. D. Maier, Ann. N.Y. Acad. Sci., 1962, **98**, 495.
12. (a) M. B. Doughty and D. S. Lawrence, J. Chem. Soc., Chem. Commun., 1985, 484. (b) M. B. Doughty and G. E. Risinger, *ibid.*, 1985, 1551.
13. Akiba et al. reported a bond switch reaction which seemed to be suggestive of an equilibrium between TY and NT: K. Akiba, K. Kashiwagi, Y. Ohyama, Y. Yamamoto, and K. Ohkata, J. Am. Chem. Soc., 1985, **107**, 2721.

Received, 8th August, 1986