FORMATION OF THIAMINE DISULFIDE BY FERRICYANIDE AND ITS DEPENDENCY ON PH OF THE REACTION MEDIA

CHIKATARO KAWASAKI, TAKATOMO HORIO AND ISAMU DAIRA¹

The School of Pharmacy, Osaka University, Toyonaka¹

(Received February 24, 1964)

It was reported in the previous papers (1, 2) that the oxidation of thiamine by alkaline ferricyanide was a reaction always yielding thiamine disulfide (TDS) as a byproduct and the effect of oxygen dissolved in the medium on the production of TDS and thiochrome was investigated. It was further found that thiochrome was poorly produced even when thiamine was immediately oxidized by ferricyanide reagent in an alkaline medium at pH 11 to 12, whereby a marked production of TDS was detected by paper chromatography (3). When the production of TDS was determined through the adsorption of TDS on Amberlite IRC-50 by ferricyanide oxidation of thiamine solution at alkaline pH levels, the production of TDS reached its maximum at pH 11.6. Under these conditions, TDS was produced from thiamine in a high yield, which seemed to be of interest as a synthetic method of TDS.

EXPERIMENTAL AND RESULTS

1. *Effect of pH on Formation of TDS*

Buffer solutions, pH 8.7, 9.6, 10.6, 11.6 and 13.1, were prepared as given in the preceding paper (4).

Immediate Addition of Ferricyanide after pH Adjustment — To 5 ml of thiamine solution (2 mg/ml), 5 ml of the buffer was added and mixed. Immediately 3 ml of 1% ferricyanide was added. After a definite interval, 1.3 ml each of the solution was pipetted out and dilute HCl and water were added up to 100 ml and pH 4.8. To 2 ml of the diluted solution was added 8 ml of water and the mixture was passed through an Amberlite IRC-50 column. Thus the amount of TDS was determined as described in the preceding paper. The results were shown in Fig. 1.

Delayed Addition of Ferricyanide after pH Adjustment — To 5 ml of thiamine solution (2 mg/ml), 5 ml each of the buffer of various pH levels was added and the whole was allowed to stand for various periods. To 1 ml of the solution was added 0.3 ml of 1% ferricyanide and the volume was adjusted with dilute HCl and water up to 100 ml and pH 4.8. The amounts of TDS and thiochrome of this solution were determined with the results shown in Fig. 2.

¹ 川崎近太郎, 堀尾嘉友, 大良 勇.

FIG. 1 *Formation of TDS from Alkaline Thiamine by Ferricyanide Reagent*
Ferricyanide was added immediately after the solution was made alkaline. The mixture was made acidic and after dilution, the amount of TDS or thiochrome was determined.

—, TDS produced; A, immediately; B, after 2 min; C, after 5 min; D, after 30 min; ----, thiochrome produced.

FIG. 2 *TDS Formation from Alkaline Thiamine by Delayed Addition of Ferricyanide Reagent*

Ferricyanide was added after thiamine had been left standing for certain periods in an alkaline solution. The mixture was made acidic and after dilution TDS or thiochrome produced was determined.

—, TDS produced; A, immediately; B, after 5 min; C, after 10 min; D, after 30 min; E, after 60 min; ----, thiochrome produced; F, after 5 min; G, after 10 min; H, after 30 min; I, after 60 min.

2. Production of TDS by Ferricyanide at pH 11 to 12

Ten grams of thiamine hydrochloride was dissolved in 70 ml of water and the pH was adjusted to 11.7–11.8 by addition of 10% NaOH. To this solution a ferricyanide solution (11 g $K_3Fe(CN)_6$ was dissolved in 50 ml water) was slowly added, whereby the solution became turbid and the temperature rose to about 30°. After standing at room temperature for 3 hours, a yellow, oily precipitate appeared at the bottom of the vessel. The supernatant fluid was decanted and the yellow oily residue was dissolved in about two volumes of ethanol, followed by filtration. About 20 volumes of acetone was added to the filtrate and the vessel wall was rubbed with a glass rod, whereby white rhombic crystals separated out. After standing overnight in the cold, about 7.6 g crystals melting at 85 to 92° were obtained. Analysis for $TDS \cdot (CH_3)_2CO \cdot H_2O$, *i.e.*, $C_{27}H_{42}N_8S_2O_6$. Calcd.: C, 50.92%; H, 6.65%; N, 17.60. Found: C, 51.14%; H, 6.62%; N, 17.67%. After drying in a desiccator with calcium chloride, the melting-point of the crystals rose further, reaching finally 134° after seven days. By drying the raw crystals in a P_2O_5 -desiccator at 50°, the melting-point rose to 177–178° (decomp.). Three grams of the crude crystals were dissolved in 2.5 ml of water, to which 50 ml of ethanol was added. To the filtrate 200 ml of ether was added and the solution was placed overnight in a refrigerator. The crystals thus separated gave a melting-point of 57–60°, which rose to 177–178° (decomp.) after drying *in vacuo* on P_2O_5 . Analysis for TDS; $C_{24}H_{34}N_8O_4N_2$: Calculated C, 50.22%; H, 6.09%; N, 19.91%. Found:

C, 50.91 %; H, 6.07 %; N, 19.61 %. The infrared spectra of the raw and purified crystals showed a good coincidence with that of the authentic sample.

Besides this, two kinds of crystals having melting-points of 50–60° and 128–134°, respectively, were simultaneously obtained. Under some conditions, no oily substance appeared. In this case, pale yellow crystals, scarcely soluble in water, having the melting-point of 134°, were obtained after the concentrated solution was allowed to stand overnight. The crystals, after dissolution in a large amount of hot water, could be converted into readily water-soluble crystals, having a melting-point of 50–60°, which were dehydrated into anhydrous crystals by further purification. The R_F values of all these samples (detected by Dragendorff reagent) on the paper chromatogram were found to be 0.56 (Toyo Filter paper No. 50, ascending method, at room temperature, developed with water-saturated *n*-butanol.)

3. Ferricyanide Oxidation of Thiamine Homologs at pH 11 to 12

Synthesis of O-Benzoylthiamine Disulfide from O-Benzoylthiamine Hydrochloride—(a) Three grams of *O*-benzoylthiamine hydrochloride was dissolved in 280 ml water. The pH of the solution was adjusted to 11.7 with *N* NaOH, to which a mixture of 3 ml of ferricyanide solution and 30 ml of water was slowly added, whereby pale yellow or white needles appeared. The crystals were separated by filtration, and dissolved in CHCl_3 . After evaporation of the solvent, they were recrystallized from ethyl acetate to give pure crystals, melting at 146° (decomp.). Mixed melting point with authentic *O*-benzoylthiamine disulfide (BTDS), showed no depression and by paper chromatography they gave a R_F value of 0.87, as detected by Dragendorff reagent (Toyo Filter Paper No. 50, ascending method, room temperature, developed with water-saturated *n*-butanol). The yield was 1.7 g. (b) 5 g of *O*-Benzoylthiamine hydrochloride was dissolved in 25 ml of water and the pH of the solution was adjusted to 11.8 with 1 *N* NaOH. 50 ml of ferricyanide solution (10 g per 100 ml) was slowly added to the solution, whereby yellowish resinous substance was separated. After extraction with CHCl_3 , the substance was recrystallized from ethanol, giving 3.7 g of the crystals, melting at 146–7° (decomp.). Analysis for BTDS; $\text{C}_{38}\text{H}_{42}\text{N}_8\text{O}_6\text{S}_2$. Calcd: C, 59.18 %; H, 5.49 %. Found: C, 58.84 %; H, 5.70 %.

Synthesis of O-Methylthiamine Disulfide from O-Methylthiamine Hydrochloride—Ten grams of crude *O*-methylthiamine hydrochloride (mp 226–8°) (5, 6) was dissolved in 50 ml of water and the pH was adjusted to 11.6 with *N* NaOH. After addition of 50 ml of 20 % ferricyanide solution, the solution was allowed to stand, whereby needles were separated. After standing for three hours, the crystals were collected, dissolved in ethanol and after addition of acetone they were allowed to cool, whereby 3.7 g of the crystals melting at 95–97° were obtained with a yield of 48 %. Analysis for *O*-methylthiamine disulfide, $\text{C}_{26}\text{H}_{38}\text{N}_8\text{O}_4\text{S}_2 \cdot \text{H}_2\text{O}$. Calcd: C, 51.27 %; H, 6.63 %; N, 14.42 %; Found: C, 50.97 %; H, 6.96 %; N, 14.47 %.

Deoxythiamine Disulfide from Deoxythiamine Hydrochloride—Five grams of deoxythiamine hydrochloride (mp 256°, decomp.) was dissolved in 40 ml of water and the pH was adjusted to 11.7 by addition of 10 % NaOH. To this solution 25 ml of 20 % ferricyanide solution was slowly added, whereby yellowish resinous

substance appeared. After standing overnight at room temperature, the supernatant was discarded and 30 ml of ethanol was added to the residue. The filtrate was concentrated *in vacuo* to one-half volume. Colorless needles were separated by the addition of 100 ml acetone. After standing overnight in the cold, 3.2 g of the crystals (mp 166—7°) was obtained with a yield of 77.5 %. The crystals obtained by recrystallization from alcohol melted at 157°, but after drying on P_2O_5 *in vacuo*, they melted at 166°. Analysis for deoxythiamine disulfide, $C_{24}H_{34}N_8O_2S_2$. Calcd: C, 54.31 %; H, 6.46 %; N, 21.11 %. Found: C, 54.23 %; H, 6.40 % N, 20.98 %. Paper chromatography: R_F 0.80—0.81 (water-saturated *n*-butanol); 0.52 (acetic acid-*n*-butanol-water, 1:4:5). Infrared spectra of *O*-methylthiamine disulfide and deoxythiamine disulfide were shown in Fig. 3.

FIG. 3 Infrared Spectra of TDS (I), *O*-Methylthiamine Disulfide (II) and Deoxythiamine Disulfide (III) (in Nujol)

DISCUSSION

It has been reported that the disulfide derivative of benzothiazole or *N*-benzylthiazolium can be synthesized by oxidation with ferricyanide, H_2O_2 , or iodine after the thiazole ring of the compounds had been opened by treatment with NaOH (7) and that the disulfide of 4-hydroxythiamine or of *N*-methylthiamine can also be synthesized by oxidation with alkaline ferricyanide (8). TDS can also be synthesized by adding one mole ferricyanide reagent to a mixture of 1 mole thiamine and 3 moles NaOH (9). As described in the preceding paper, thiochrome was poorly produced at pH 11 and 12 when the ferricyanide reagent was added immediately after the solution was made alkaline. TDS was predominantly produced and the production of thiochrome was increased beyond that pH range. As shown in Fig.

1, the production of TDS was 62 % immediately after addition of ferricyanide and 82 % after 5 minutes in contrast to low production at other pH levels. If the rise in TDS production at various pH levels is compared between immediately and after 30 minutes, the production was highest between pH 11 to 12. That the production of TDS is low at pH above 13, is due to greater production of thiochrome than at any other pH levels. The fluorescent substance produced at pH 13 was identified as thiochrome not only by ultraviolet absorption spectrum, but also by isolation of the pure crystals of thiochrome.

It has already been reported that thiamine, having been left standing in an alkaline solution, produces thiamine disulfide by following addition of ferricyanide (8—10). As seen in Fig. 2, the formation of TDS reaches its maximum at pH 11.6, whereas at other pH levels the formation of thiochrome increased. But the amount of thiochrome produced was reduced with the lapse of minutes at pH above 13, and at the same time TDS production was increased. Finally, the formation of TDS became the main reaction. Hence, it is clear that the thiamine solution, which has been left standing at pH above 11, exclusively produces TDS by addition of ferricyanide. Employing such conditions under which TDS is easily formed from thiamine, the disulfide can be quantitatively obtained even at high concentrations. The disulfide compounds thus synthesized by ferricyanide oxidation are almost colorless in crude crystals and can be obtained with high yield in comparison with the preparation by alkaline iodine oxidation. It can therefore be said to be an excellent synthetic method of thiamine disulfide. The main reaction products of ferricyanide oxidation of thiamine are thiochrome and TDS. The ratio of both the compounds was found to be dependent on pH, oxygen dissolved in the medium and the lapse of minutes after the solution was made alkaline.

SUMMARY

Thiamine disulfide produced from thiamine in an alkaline medium immediately after adding ferricyanide solution or after allowing to stand for some time was determined by adsorption on and elution from Amberlite IRC-50. The formation was maximal at pH around 11.6, decreasing at other pH levels. Addition of ferricyanide after the solution was left standing for some time in an alkaline medium, resulted in production of less thiochrome and more thiamine disulfide with the lapse of minutes. The oxidation of thiamine with ferricyanide produces both thiochrome and thiamine disulfide simultaneously, but at pH 11 to 12, thiamine disulfide was predominantly produced and the reaction can be used as a practical synthetic method of the disulfide.

ACKNOWLEDGEMENT

The authors wish to express their gratitude to Dr. Kiyoshi Tsutsui, director of the Central Laboratory of Dai-Nippon Seiyaku Co. Ltd., and to Dr. Jo Fukuda and of our university for elementary analyses.

REFERENCES

1. Horio, T., *Vitamins* **21**, 517 (1960).
2. Horio, T., *ibid.* **24**, 88 (1961).
3. Horio, T., *ibid.* **24**, 79 (1961).
4. Horio, T., *ibid.* **21**, 520 (1960).
5. Clarke, H. T., and Gurin, S. T., *Amer. Chem. Soc.* **57**, 1876 (1935).
6. Yurugi, S., and Fushimi, T., *Yakugaku Zasshi* **77**, 264 (1957).
7. Mils, W.H., and Clarke, L.M., and Aeschliman, J.A., *J. Chem. Soc.* **1923**, 2353.
8. Nesbitt, P., and Sykes, p., *ibid.* **1954**, 4585.
9. Wornat, K., *U.S.P.* **2**, 458, 453 (1949).
10. Matsukawa, T., and Hirano, H. *Vitamins* **13**, 362 (1957).