
SHORT REPORT

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DIFFERENT RESULTS ARISING FROM TWO TYPES OF THIOCHROME ASSAYS OF THIAMIN IN THE THIAMIN-TANNIC ACID REACTION

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Summary

Results from two different types of thiochrome assays showed that there was an underestimation of free thiamin during the early stages of the thiamin-tannic acid reaction caused by the interference of thiochrome formation by tannic acid present in the assay system. This interference was dependent on tannic acid concentration.

It was previously reported by us that thiamin reacted with tannic acid to give an extremely rapid initial phase and a much slower subsequent phase¹. A similar phenomenon was observed by Davis and Somogyi² in an earlier study on thiamin-polyphenol interactions and the rapid initial phase was later confirmed by Schaller and Höller³ in their thiamin-caffeic acid study. However, Murata and her colleagues⁴ did not see the so-called "biphasic" reaction rate. On inspecting the publications involved in the controversy, it was found that this latter group used a cation-exchange column to strip their reaction mixtures of polyphenolic substances prior to the thiochrome-producing oxidation step, while the others did not. It was felt that a reinvestigation of the effect of pre-treatment by a cation-exchange column on our thiamin-tannic acid system might help settle the situation.

In this brief report, the results obtained from two types of thiochrome assays are compared. In the DTT (Decalco-Treated-Thiochrome) assay⁵, the acidified reaction mixture was passed through a cation-exchange column (Decalco column) which preferentially retains positively charged thiamin over tannic acid. The eluted thiamin was then oxidized by alkalinized $K_3Fe(CN)_6$ to produce thiochrome. The NDT (No-Decalco-Thiochrome) assay followed the modified thiochrome method of Leveille⁶ in

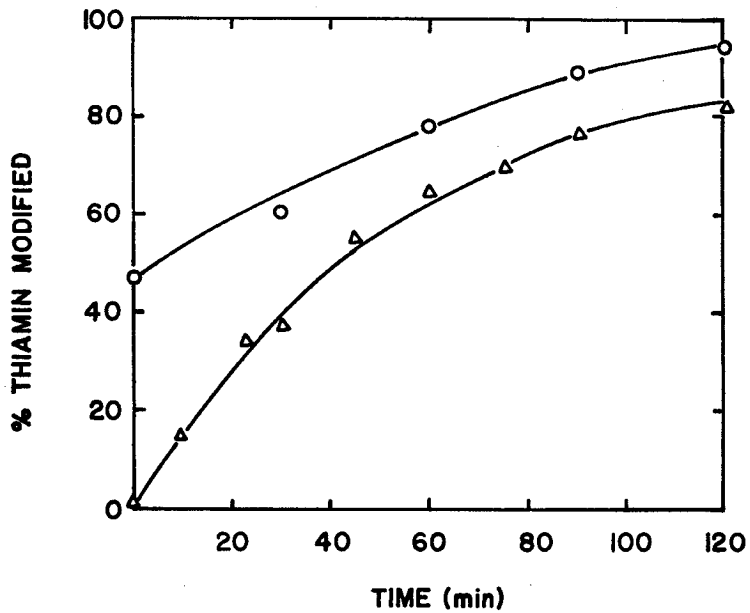


Fig. 1. Time-rate profiles of percent thiamin modification at tannic acid to thiamin molar ratio of 2:1, pH 7.5, 60°C. Upper curve by NDT assay and lower curve by DTT assay. Thiamin concentration was 37.8 μ M

which $K_3Fe(CN)_6$ oxidation step took place in the reaction mixture in the presence of tannic acid. Isobutanol was used for extracting the thiochrome in both cases. Fluorescence readings were done at the emission wavelength of 425 nm with the excitation at 365 nm.

Fig. 1 shows the results at tannic acid: thiamin molar ratio of 2:1, pH 7.5, 60°C. The time-rate profile from the NDT assay exhibits an extremely rapid initial phase followed by a much slower subsequent phase, whereas the DTT assay shows a profile with a rate comparable to the slower phase of the above. Also, the extent of thiamin modification is always lower in the case of DTT. We also found that at a constant thiamin concentration the initial-phase thiamin modification as determined by NDT assay increased with increasing concentrations of tannic acid present in the reaction mixture, whereas no change in initial phase thiamin modification was detected by DTT assay from tannic acid to thiamin molar ratio of 0:1 to 2:1. It can be concluded that the presence of tannic acid in the thiochrome assay system, i.e., during oxidation by $K_3Fe(CN)_6$ and extraction by isobutanol, results in lowered thiochrome fluorescence readings.

The effect of tannic acid may be due to its interference with thiochrome formation on oxidation of thiamin by $K_3Fe(CN)_6$ and/or its presence in the isobutanol which quenches the thiochrome fluorescence. It was found that tannic acid added to thiamin solutions after termination (by H_2O_2) of the ferricyanide oxidation step but before isobutanol extraction gave the same thiochrome readings as the thiamin controls without added tannic acid. Isobutanol extracts of tannic acid solutions oxidized by ferricyanide did not show any significant fluorescence at 425 nm. These isobutanol extracts did not give different fluorescence readings from controls when they were used to extract thiamin solutions after oxidation by $K_3Fe(CN)_6$, treatment by H_2O_2 and addition of tannic acid. Therefore tannic acid must affect the isobutanol-extracted thiochrome fluorescence by interfering with the oxidation-reduction involved in the formation of thiochrome and not by quenching the thiochrome fluorescence in the isobutanol extract. This interference is probably the cause of the observation of the extremely rapid initial rate and the higher estimation of thiamin modification as determined by the NDT assay over that by the DTT assay. Preliminary thiochrome-independent assays seem to support this conclusion^{1,7}.

We are currently studying the effects of ten antithiamin polyphenols on the formation of thiochrome and are exploring other non-thiochrome assays to provide independent results for comparison. We recognize that our findings may have implications on the mechanism of thiamin-polyphenol interactions, the true effectiveness of various polyphenolic compounds as antithiamin factors and the validity of comparing NDT determined with biologically determined free thiamin in the presence of polyphenols.

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References

1. Rungrungsak, K., Tosukhowong, P., Panijpan, B., Vimokesant, S.L. (1977) *Am. J. Clin. Nutr.* **30**, 1680.
2. Davis, J.S., and Somogyi, J.C. (1969) *Int. J. Vit. Nutr. Res.* **39**, 401.
3. Schaller, K. and Höller, H. (1976) *Int. J. Vit. Nutr. Res.* **46** 143.
4. Murata, K., Tanaka, R., and Yamaoka, M. (1974) *J. Nutr. Sci. Vitaminol.* **20**, 351.
5. Pearson, W.N. (1967) in *The Vitamins VII* (György, P., and Pearson, W.N., eds.) 2nd ed., Academic Press, New York, p. 60.
6. Leveille, G.A. (1972) *Am. J. Clin. Nutr.* **25**, 273.
7. Kositawattanakul, T., Tosukhowong, P., Vimokesant, S.L. and Panijpan, B. (1977) *Am. J. Clin. Nutr.* **30**, 1686.

บทคัดย่อ

จากการเปรียบเทียบผลการหาความเข้มข้นของ thiamin โดยวิธีไรโอโครม (thiochrome) 2 วิธี สรุปได้ว่า กรดแทนนิกในสารละลายทำให้เกิดไรโอโครมได้น้อยลง ความสามารถในการลดปริมาณไรโอโครมขึ้นอยู่กับความเข้มข้นของกรดแทนนิกด้วย