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Note

Separation of thiamine and its derivatives on a Sephadex column

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The separation of thiamine on a Decalso column[1] is currently employed for the isolation of this vitamin from biological substrates. A Dowex ion-exchange material may also be employed[2]. The oxidation products of thiamine and its derivatives may be separated on a Sephadex gel column[3].

During a study of the interaction of the fungicide folpet with thiamine[4], a useful separation of the interaction products was obtained on a Sephadex G15 column. Thus, the possibilities of the separation of thiamine and its derivatives on a column of this type have been studied in this paper.

EXPERIMENTAL

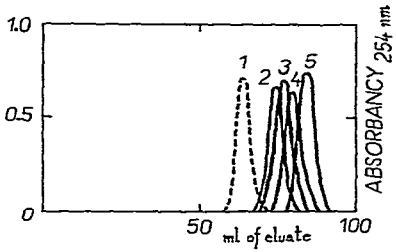
0.5–1.0 ml of a 10^{-3} *M* solution of thiamine or one of its derivatives (thiamine monophosphate, thiamine diphosphate, thiamine disulphide and thiochrome, respectively) were placed on a Sephadex G10 column; a mixture of all of these compounds (each 10^{-3} *M*) was placed on the column in the same way. The samples were eluted with water and/or 0.01 *M* hydrochloric acid. (The column was equilibrated with 500 ml of HCl prior to separation.) Further experiments were carried out by use of a stepwise elution (100 ml of water, then 200 ml of 0.01 *M* HCl) and gradient elution (a linearly increasing amount of 0.01 *M* HCl in water).

Thiamine and its derivatives were identified in the separated fractions after gel chromatography by using thin-layer chromatography (TLC)[5,6]. The separated thiamine was determined after oxidation with potassium ferricyanide by the thiochrome method[1]; thiamine diphosphate was determined in the same way after enzyme hydrolysis.

RESULTS AND DISCUSSION

The elution of thiamine and its derivatives by water and 0.01 *M* HCl, respectively, is shown in Fig. 1. Thiamine diphosphate is the only derivative separated by water elution; thiamine and the other derivatives (thiamine monophosphate, thiamine disulphide and thiochrome) are retained on the column and must be eluted with 0.01 *M* HCl.

The stepwise elution with water and 0.01 *M* HCl resulted in the separation of



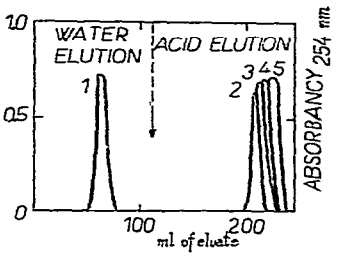


Fig. 1. Separation of thiamine and its derivatives on the Sephadex G10 column. For procedure see text. — — —, Elution with water; ———, elution with 0.01 *M* HCl. Peaks: 1 = thiamine diphosphate; 2 = thiamine monophosphate; 3 = thiamine disulphide; 4 = thiamine; 5 = thiochrome. Column, 400 × 26 mm (V_0 = 63 ml, V_t = 105 ml).

Fig. 2. Stepwise elution of thiamine and its derivatives from the Sephadex G10 column. For procedure see text. Other details as in Fig. 1.

thiamine diphosphate from the mixture of the other substances (Fig. 2). Gradient elution by water containing an increasing amount of 0.01 *M* HCl brings about the separation of thiamine diphosphate from thiamine monophosphate and the mixture of thiamine, thiamine disulphide and thiochrome. The last three compounds are incompletely separated (Fig. 3).

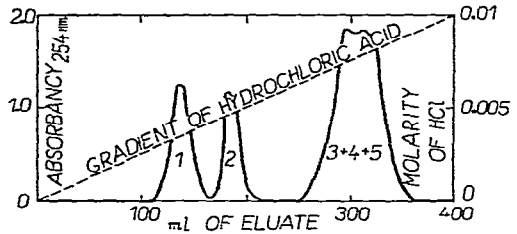


Fig. 3. Linear gradient elution of thiamine and its derivatives from the Sephadex G10 column. For procedure see text. Column, 700 × 26 mm (V_0 = 121 ml, V_t = 253 ml). Other details as in Fig. 1.

The determination of thiamine and thiamine diphosphate in the eluate after gel chromatography demonstrates the suitability of this separation technique. Thus, even if the separation on the Sephadex column requires more time in comparison to an ion-exchange column, there is no loss whatsoever during the whole procedure.

The elution pattern of thiamine and its derivatives does not change when the sample contains 0.08 *M* sodium sulphate. (Sodium sulphate would be formed in the neutralization of an acid extract of a biological sample prior to column separation.)

The method is suitable for the separation of thiamine diphosphate from thiamine monophosphate and from the mixture of thiamine, thiamine disulphide and thiochrome, and the subsequent determination by the thiochrome method. Even if the separation of thiamine, thiamine disulphide and thiochrome is incomplete, these compounds can be determined in the following way. One portion of the separated fraction is used to determine thiochrome fluorimetrically; a second portion is used to estimate the sum of thiamine and thiochrome after oxidation with potassium ferri-

cyanide and a third portion is used to determine all three components after primary reduction of thiamine disulphide by sodium thiosulphate[4,7] and subsequent application of the thiochrome method.

REFERENCES

1 W. Horowitz (Editor), *Official Methods of Analysis of the Association of Official Analytical Chemists*, Association of Official Analytical Chemists, Washington, 12th ed., 1975, p. 823.
2 G. Rindi and L. de Giuseppe, *Biochem. J.*, 78 (1961) 602.
3 T. Nishimune, M. Abe and R. Hayashi, *Biochim. Biophys. Acta*, 279 (1972) 527.
4 J. Seifert and J. Davidek, *Pest. Biochem. Physiol.*, 7 (1977) 56.
5 C. Levorato and L. Cima, *J. Chromatogr.*, 32 (1968) 771.
6 G. Katsui, in K. Macek (Editor), *Pharmaceutical Applications of Thin-Layer and Paper Chromatography*, Elsevier, Amsterdam, London, New York, 1972, p. 491.
7 I. Utsumi, K. Harada and K. Kohno, *J. Vitaminol.*, 9 (1963) 50.