Journal of Chromatography, 211 (1981) 290–294
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,784

Note

Separation and determination of thiamine-binding proteins in rats by high-performance liquid chromatography

MIEKO KIMURA* and YOSHINORI ITOKAWA
Department of Hygiene, Faculty of Medicine, Kyoto University, Kyoto (Japan)
(Received March 10th, 1981)

Ample evidence has accumulated to indicate that thiamine has a specific electrophysiological function in nervous tissues independent of its coenzyme function[1–5]. From several studies[4,6,7], we assume that thiamine-binding proteins play an important rôle in the former function. However, properties and actions of thiamine-binding proteins in animals are quite obscure because of their complexity.

Recently, high-speed gel filtration using high-performance liquid chromatography (HPLC) has been developed[8], and we have explored a new analytical method for the separation of thiamine-binding proteins that can be applicable for studies of thiamine-binding proteins in animals.

EXPERIMENTAL

Apparatus

The system consists of a LC-3A pump for liquid chromatography, a SIL-1A injector, a TSK-Gel G-3000 SW column, a SPD-2A UV detector, a PRR-2A proportioning pump, a RF-500 LCA spectrofluorophotometer and a strip chart recorder. The TSK-Gel column was purchased from Toyo Soda (Tokyo, Japan), and all other equipment was purchased from Shimadzu (Kyoto, Japan).

Preparation of samples

Male Wistar rats were killed and tissues removed. The tissues were homogenized with nine volumes of 0.1 *M* sodium acetate (pH 7.5) containing 1 % Triton X-100 and centrifuged at 44,000 *g* for 60 min. The supernatant was used as the sample.

Procedures

The HPLC system is schematically shown in Fig. 1. A 50–100-μl aliquot of sample was injected onto the column, after the zero time being marked, using 0.1 *M* sodium acetate (pH 7.5) as mobile phase at a flow-rate of 0.5 ml/min. The absorbance at 280 nm was monitored continuously with a UV detector. A solution containing 0.01 % potassium hexacyanoferrate(III) and 15 % sodium hydroxide was applied and mixed with the column eluate at a flow-rate of 0.5 ml/min with a proportioning pump to convert thiamine-binding proteins into fluorophores. The fluorescence was measured using a 12-μl flow cell with a spectrofluorimeter (excitation wavelength, 375 nm; emission maximum, 450 nm) and recorded graphically.

0021-9673/81/0000–0000/$02.50

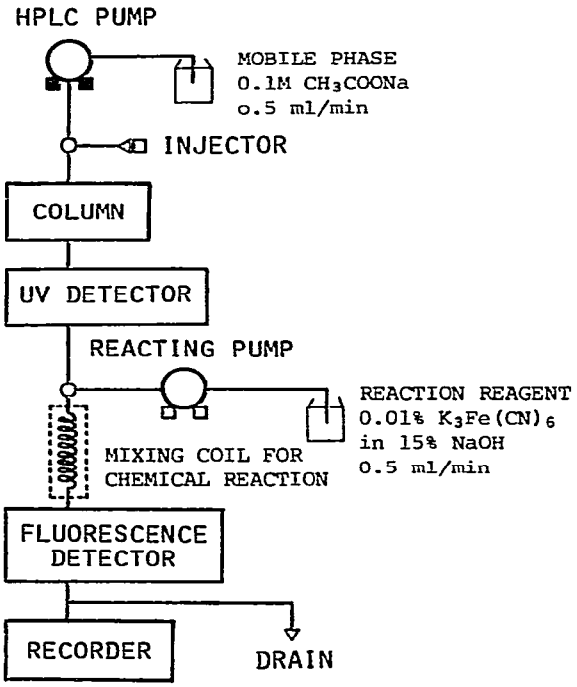

Fig. 1. Schematic diagram of the HPLC system.

Determination of thiamine and transketolase activity by conventional methods

Thiamine was determined by the thiochrome method of Fujiwara and Matsui[9]. Transketolase (E.C. 2.7.1.1) activity was assayed by the method described by Itokawa[10].

RESULTS AND DISCUSSION

Fig. 2 shows separation patterns of thiamine-binding proteins from rat brain. Nine peaks with UV absorption and three peaks with fluorescence were observed. A blank study was performed by adding a solution without hexacyanoferrate(III), and a small peak at the position of the first fluorescent peak (indicated by the broken line) was observed. When the free form of thiamine or each thiamine phosphate ester was applied on this system, the substance yielded was found in a fluorescent peak at the position corresponding to the third fluorescent peak of rat brain proteins.

Thiamine concentration and transketolase activity of pooled fractions of each peak were determined. Thiamine content in 50 μl of brain sample was 5.78, 6.00 and 6.09 ng in the first, second and third peak, respectively. Transketolase activity was detected only in the second fluorescent peak.

Elution patterns of thiamine-binding proteins from sciatic nerve are shown in Fig. 3. Similar to the case of brain proteins, three fluorescent peaks were observed. The height of the first peak increased and those of the second and third peaks decreased as compared with the pattern of brain proteins. As the distribution of

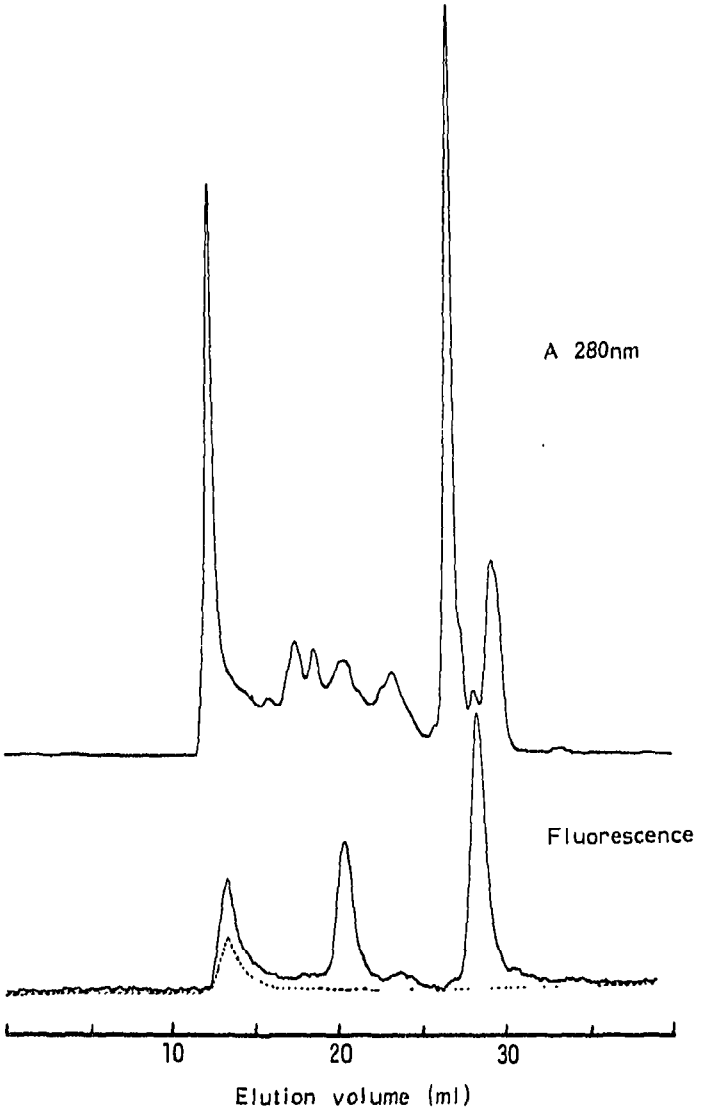

Fig. 2. Elution profiles of thiamine-binding proteins in rat brain.

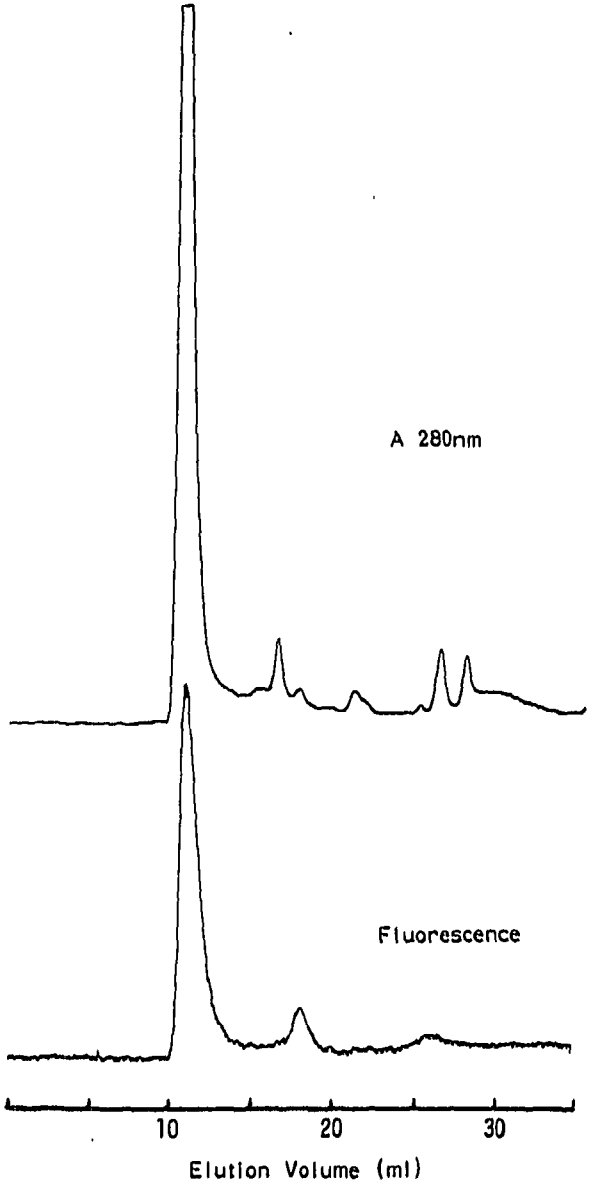

Fig. 3. Elution profiles of thiamine-binding proteins in rat sciatic nerve.

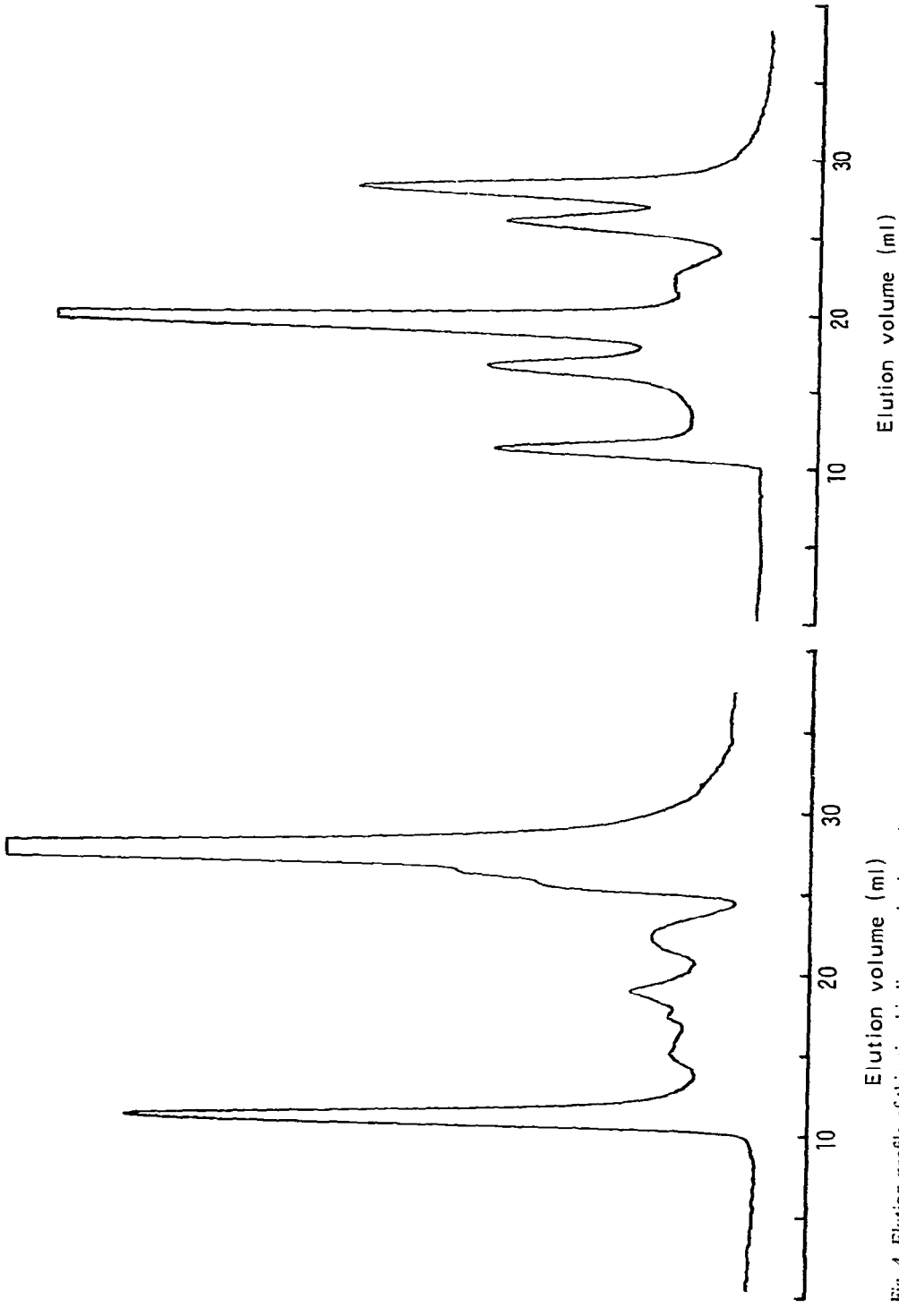

Fig. 4. Elution profile of thiamine-binding proteins in rat heart monitored by fluorescence.
Fig. 5. Elution profile of thiamine-binding proteins in rat liver monitored by fluorescence.

thiamine-binding proteins in membrane structure seemed to be higher in sciatic nerve rather than in brain, we presumed that the first fluorescent peak included thiamine-binding proteins in membranes.

The elution profiles of thiamine-binding proteins of heart and liver monitored by fluorescence are shown in Figs. 4 and 5, respectively. In both cases, six fluorescent peaks were observed. The third and highest peak of liver contains transketolase activity.

From these elution patterns of samples from various tissues, we can assume that the first peak (high-molecular-weight substances) contains thiamine-binding proteins in membrane structure, the middle peak includes transketolase and the last peak (low-molecular-weight material) corresponds to free forms of thiamine phosphate esters.

Although this study is at the preliminary stage at present, HPLC seems to be one of the most useful tools to identify the various thiamine-binding proteins in animal tissues since this method is sensitive and rapid.

ACKNOWLEDGEMENTS

We thank Dr. Y. Kojima, associate professor in the Department of Hygiene, Faculty of Medicine, Kyoto University and Mr. O. Ishikawa, Toyo Soda Manufacturing Co., for their valuable advice.

REFERENCES

1 A. von Muralt, *Exp. Cell Res., Suppl.*, 5 (1958) 72.
2 Y. Itokawa and J. R. Cooper, *Science*, 166 (1969) 759.
3 Y. Itokawa, R. A. Schulz and J. R. Cooper, *Biochim. Biophys. Acta*, 266 (1972) 293.
4 Y. Itokawa, *J. Nutr. Sci. Vitaminol.*, 22 (Suppl.) (1976) 17.
5 J. M. Fox and W. Duppel, *Brain Res.*, 89 (1975) 287.
6 M. Kimura and Y. Itokawa, *J. Neurochem.*, 28 (1977) 389.
7 T. Takenaka, T. Yoshioka, H. Inoue, K. Inomata and H. Horie, *Proc. Jap. Acad.*, 45B (1978) 316.
8 Y. Kato, K. Komiya, H. Sasaki and T. Hashimoto, *J. Chromatogr.*, 193 (1980) 29.
9 M. Fujiwara and K. Matsui, *Anal. Chem.*, 25 (1953) 810.
10 Y. Itokawa, *Brain Res.*, 94 (1975) 475.