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Note

Determination of blood transketolase by high-performance liquid chromatography (a preliminary note)

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The technique of high-performance liquid chromatography (HPLC) is utilized in various analytical methods for diagnostic purposes, because of its sensitivity and rapidity.

In order to diagnose the nutritional status of thiamin, total thiamin level in blood and erythrocyte transketolase activity are considered to be the most sensitive measures. Recently we have explored a liquid chromatographic method for the determination of the total thiamin content in blood, which evaluates the nutritional status of thiamin in clinical studies[1].

However, a liquid chromatographic method has not yet been utilized for the assay of transketolase activity in blood, another useful diagnostic measure for thiamin. Under these circumstances, we have contrived a new analytical method to determine the amount of transketolase in blood using HPLC.

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

Blood (100 μl) was put in a polypropylene centrifuge tube with 400 μl of 0.05 *M* sodium acetate (pH 7.5) and mixed vigorously with a vortex mixer. The sample was then centrifuged at 33,000 *g* for 60 min. The supernatant solution was used as the sample.

Procedures

Fig. 1 shows the schematic diagram of this system. The mobile phase (0.05 *M* sodium acetate (pH 7.5)) was pumped at a flow-rate of 0.5 ml/min into the HPLC column. An aliquot (200 μl) of the sample was injected onto the column. 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

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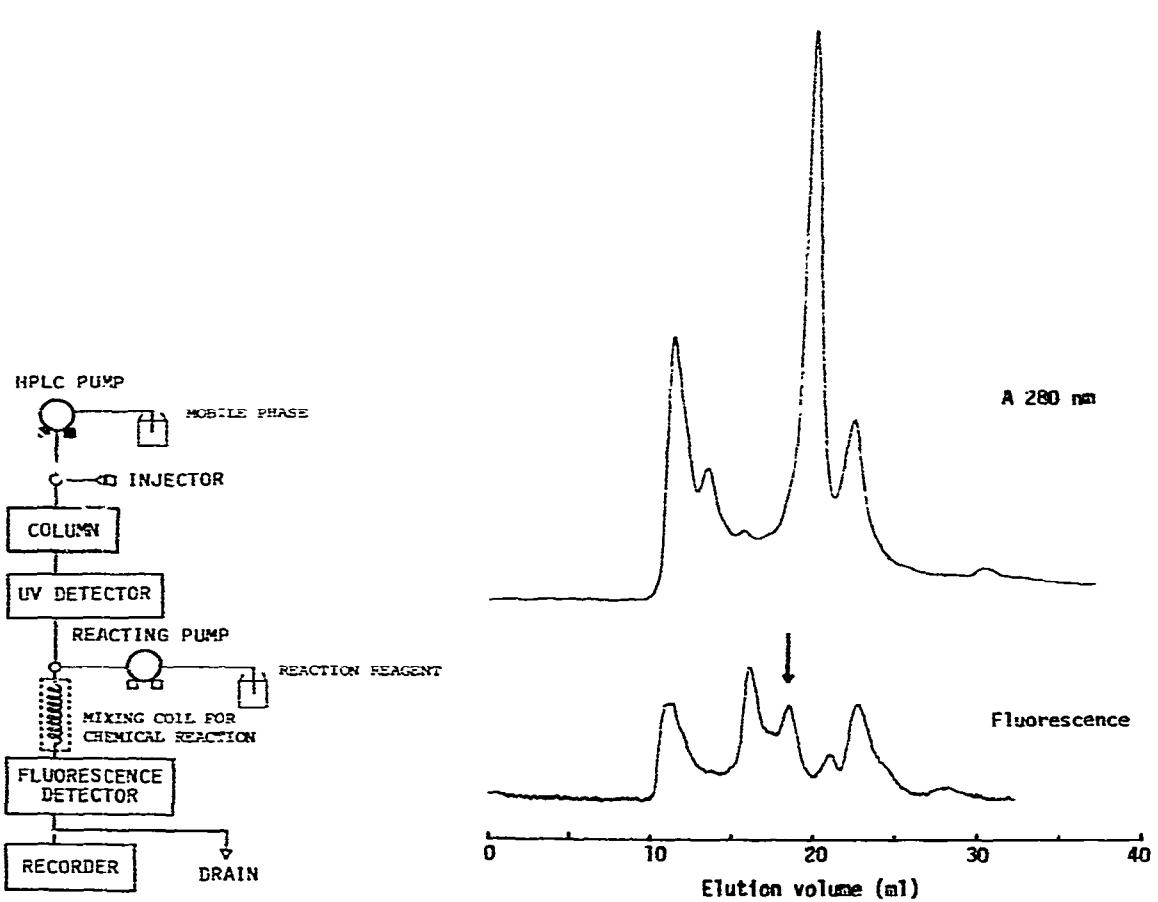

Fig. 1. Schematic diagram of transketolase analyzing system.

Fig. 2. Elution profiles of transketolase in rat blood.

and mixed with the column eluate at a flow-rate of 0.5 ml/min with a proportioning pump (thiochrome reaction). By this procedure, transketolase and other thiamin-binding proteins are converted into fluorophores. The fluorescence was measured using a 12-μl flow-cell with a spectrofluorimeter (excitation, 375 nm; emission, 450 nm) and recorded graphically.

RESULTS AND DISCUSSION

Fig. 2 shows elution profiles of transketolase in normal rat blood. Six peaks each were observed with UV absorption and with fluorescence. Each peak was collected fractionally before the thiochrome reaction and transketolase activity was assayed by the conventional method[2]. The transketolase activity was observed only in the third fluorescent peak.

Elution profiles of blood samples taken from a normal and a thiamin-deficient rat (fed a thiamin-deficient diet for 4 weeks) are shown in Fig. 3. In blood of the thiamin-deficient rat, the peak of transketolase was not detected.

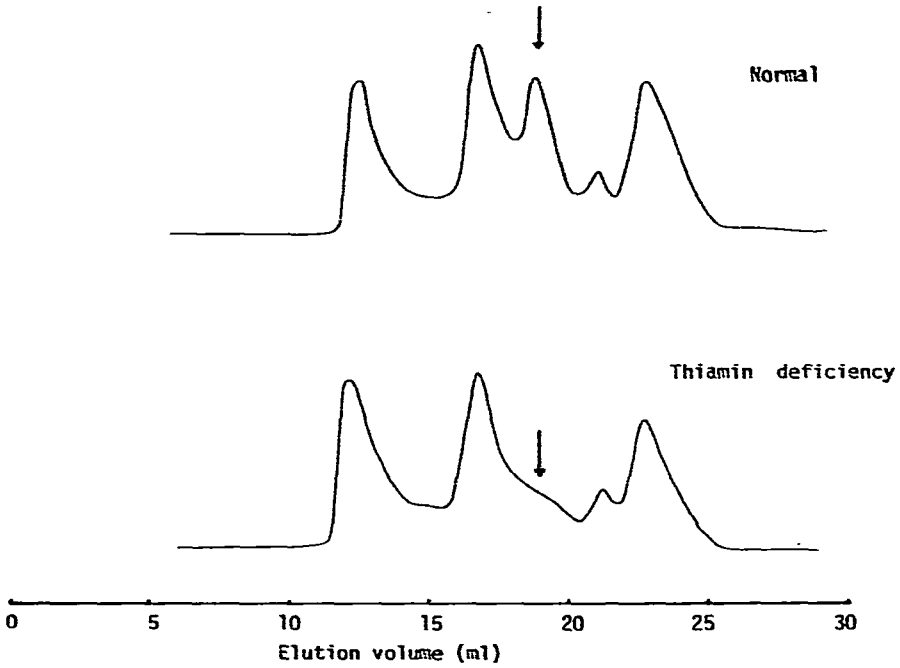

Fig. 3. Elution profiles of transketolase from blood in normal and thiamin-deficient rats.

Fig. 4 shows elution profiles of blood from a normal person and a beriberi patient with fluorescence. The decrease in the peak height of transketolase in the beriberi patient is significant when compared with the normal person. Erythrocyte transketolase activity in these persons determined by the conventional method[2] were

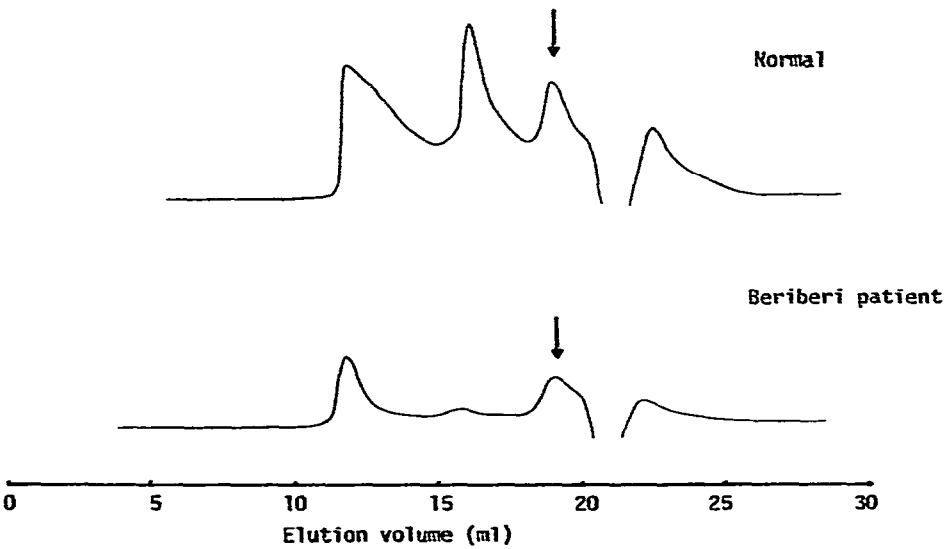

Fig. 4. Elution profiles of transketolase from blood in normal person and beriberi patient.

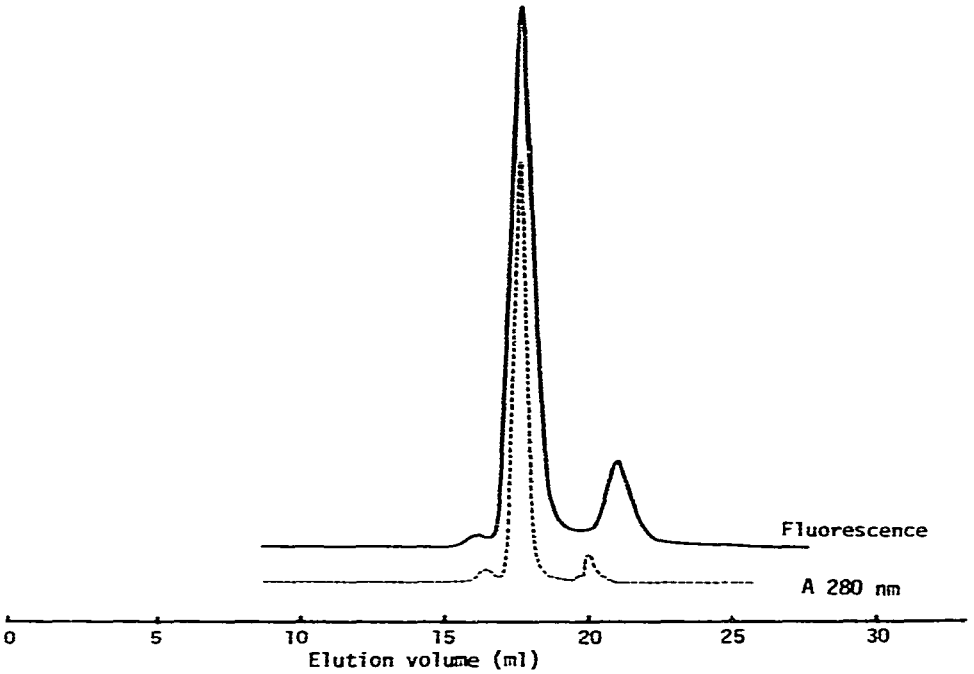

Fig. 5. Elution profiles of crystalline transketolase from baker's yeast.

found to be 656 (μg sedoheptulose produced per ml erythrocytes per h) for the normal person and 324 for the beriberi patient.

Fig. 5 shows the elution profiles of crystalline transketolase from baker's yeast (Sigma, St. Louis, MO, U.S.A.) determined by UV absorption and fluorescence. The elution volume in crystalline transketolase was the same as in blood transketolase.

Although this method is convenient for evaluating the transketolase content of blood, further studies to determine possible discrepancies between this and the conventional method are necessary before utilizing this method in clinical studies. Note that this method does not determine the activity of transketolase but rather the amount of this enzyme. The relationship between the amount and activity of transketolase remains to be clarified.

REFERENCES

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