Journal of Chromatography, 488 (1989) 503–505
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4588

Letter to the Editor

Stability of serum γ-glutamyltranspeptidase fractionated by gel permeation using high-performance liquid chromatography and fast protein liquid chromatography

Sir,

Serum γ-glutamyltranspeptidase (GT) has been shown by gel permeation using Sephadex to exhibit heterogeneity in its molecular size [1,2]. As separation of serum GT by Sephadex G-200 is ineffective and laborious, establishment of a method for rapid and effective separation is desirable. Nishii et al. [3] reported that when serum GT was fractionated by gel permeation using a TSK-G3000 SW column attached to a high-performance liquid chromatographic (HPLC) system, excellent separation was obtained in a short time. However, when this method was employed, the activities of the GT species obtained after gel permeation were labile, and therefore assays of the enzyme activity in the various fractions had to be carried out immediately after chromatography.

In order to obtain more stable GT fractions, we have devised a gel-permeation system employing a TSK-G3000 SW column attached to a fast protein liquid chromatographic (FPLC) system, and compared the stabilities of the GT fractions isolated by this method with those obtained by the HPLC method.

Two TSK-G3000 SW columns (Toso 60 cm × 7.8 mm I.D.) were connected and used with the HPLC (HLC 803A, Toso) and FPLC (Pharmacia) systems. Each column was equilibrated with 0.1 mol/l phosphate buffer (pH 7.0), and 0.1 ml of serum was applied to the column.

Chromatography was carried out at a flow-rate of 1.0 ml/min for the HPLC system and of 0.5 ml/min for the FPLC system. Fractions of 0.75 ml were collected, and the GT activity in each fraction was assayed using γ-glutamyl-P-N-ethyl-N-hydroxyethylaminoanilide as the substrate (γ-GTP-C-test, Wako).

No essential difference was found between the elution patterns of serum protein fractionated by the HPLC and FPLC systems. Human serum GT obtained from patients with hepatobiliary diseases was fractionated into three fractions: a high-molecular-mass fraction (GT-1) detected in the void volume, an intermediate-molecular-mass fraction (GT-2) detected between the 19S and 7S serum protein fractions, and a low-molecular-mass fraction (GT-3) detected between the 7S and 4S serum protein fractions (Fig. 1).

0378-4347/89/$03.50

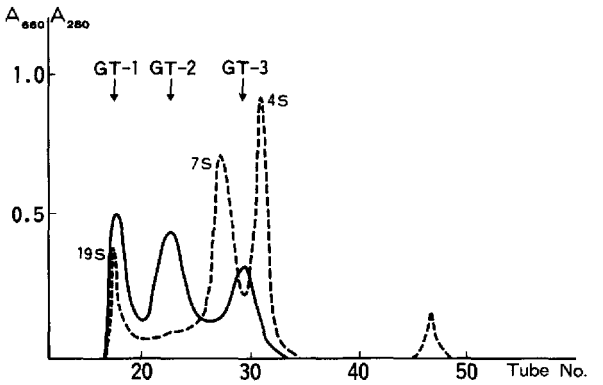

Fig. 1. Elution pattern of serum protein and GT separated by a TSK-G3000 SW column attached to an HPLC system. ——=GT activity (A_{660}); - - - -=serum protein (A_{280}).

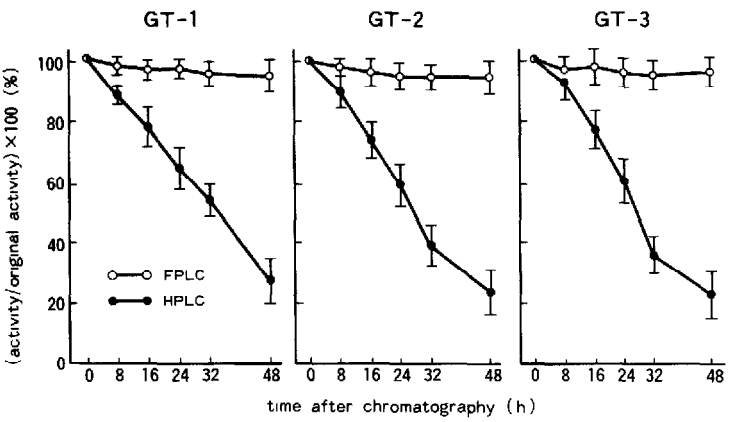

Fig. 2. Stability of serum GT-1, GT-2 and GT-3 fractionated by a TSK-G3000 SW column attached to both HPLC (●) and FPLC (○) systems. Activities are expressed as percentages (mean ± S.D.) of the activities assayed at each time-point relative to those assayed at zero time.

The elution patterns of serum GT obtained by the HPLC and FPLC methods using the same sample were almost identical immediately after chromatography. Fractions GT-1, GT-2 and GT-3 were stored in a refrigerator and the activities of the fractions were assayed at 0, 8, 16, 24, 36 and 48 h after chromatography.

The activities of GT fractionated by HPLC were unstable and those by the FPLC system were more stable, as shown in Fig. 2. The reason for this difference in stability is not clear. The tube and connecting parts of the HLC 803A are made of metal, but those of the FPLC system are made of metal-free materials. It is known that some enzymes become labile during chromatography, especially when metal parts are used. There is no conclusive evidence for an inhibitory effect of metal on GT activity, but the most probable reason for the difference in stability seems to be this difference in the line materials used. The TSK-G3000 column was originally produced for gel permeation by HPLC, and its excellent separation in a short time is well known. This newly devised method using a TSK-G3000

SW column attached to an FPLC system required ca. 2 h to achieve the separation because of the lower flow-rate employed, thus giving excellent separation as well as stable GT activity.

Department of Laboratory Medicine,
Tokyo Medical and Dental University,
5-45, Yushima 1-chome,
Bunkyo-ku, Tokyo 113 (Japan)

SEIJIN HOSAKI*
JUNKO SAWAMOTO
MICHIO HAGIWARA
TAKAMI KUSANO

Third Department of Internal Medicine,
Tokyo Medical and Dental University,
5-45, Yushima 1-chome,
Bunkyo-ku, Tokyo 113 (Japan)

HIROYUKI ODA

Department of Laboratory Medicine,
Tokyo Medical and Dental University,
5-45 Yushima 1-chome,
Bunkyo-ku, Tokyo 113 (Japan)

SHIN-ICHI SHIINA

1 M. Orlowski and A. Szczeklık, Clin. Chim. Acta, 15 (1967) 387.
2 P.R. Wenham, C.P. Price and H.G. Sammons, J. Clin. Pathol., 32 (1979) 902.
3 K. Nishii, H Oda, K. Kamisaka and S. Hosaki, Gastroenterol. Jpn., 23 (1988) 435.

(First received July 28th, 1988; revised manuscript received November 3rd, 1988)