Journal of Chromatography, 428 (1988) 415–418
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4197

Letter to the Editor

Use of a post-column enzymatic reaction for detection of macroamylasemia by high-performance liquid chromatography

Sir,

Macroamylase, a high-molecular-mass complex of serum α-amylases and immunoglobulins, other plasma proteins or polysaccharides that cannot pass through glomerular membranes, accumulates in the blood. This conditions, which is called macroamylasemia [1–6], results in increased α-amylase activity in serum. When accompanied by abdominal pain, the high α-amylase activity may be mistakenly thought to be due to acute pancreatitis.

Gel-permeation chromatography can be used to differentiate between hyperamylasemia caused by acute pancreatitis and by accumulation of this complex, because any kind of macroamylase can be separated easily from α-amylase of normal size. Fourmy et al. [7] used high-performance gel-permeation chromatography to detect macroamylase in serum. The elution was monitored by measuring the absorbance at 280 nm, and the enzyme activities of the eluates collected by a fraction collector were measured by the method of Bernfeld [8].

Continuous monitoring of the elution for enzyme activity makes high-performance liquid chromatography (HPLC) more effective and valuable for detection of macroamylase. This report describes a rapid method for identification of macroamylasemia by HPLC with continuous monitoring of α-amylase activity.

EXPERIMENTAL

High-performance liquid chromatography

The HPLC apparatus included a Waters Assoc. Model M-45 pump, a Rheodyne Model 7125 sample injector, a Toyo Soda G-3000 SW (300 mm $\times$ 7.5 mm I.D.) column, a post-column enzymatic reaction system with a mixing tee, a Hitachi Model 655 pump, a pulse-damping device, a reaction coil consisting of a PTFE tube (35 m $\times$ 0.5 mm I.D.), a Hitachi wavelength-tunable effluent monitor and a

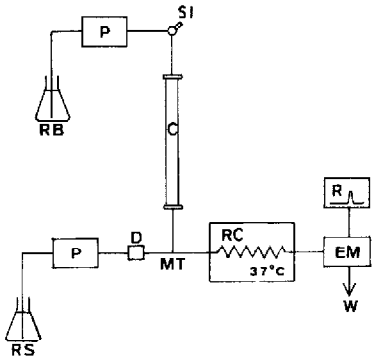

Fig. 1. Flow diagram of high-performance gel-permeation chromatography. RB = Reservoir of elution buffer; P = pump; SI = sample injector; RS = reservoir of the mixture of FG5P and coupled enzymes; C = column; D = damper; MT = mixing tee, RC = reaction coil; EM = effluent monitor; R = recorder; W = waste.

Hitachi Model 056 recorder (Fig. 1). The column was eluted with 0.15 *M* sodium chloride and 0.01 *M* N,N-bis(2-hydroxyethyl)-2-aminoethanesulphonic acid (BES)–sodium hydroxide buffer (pH 6.8) at a flow-rate of 0.6 ml/min. The eluate from the column was mixed with a solution of 0.67 m*M* FG5P (*p*-nitrophenyl O-6-deoxy-6-[(2-pyridyl)amino]-α-D-glucopyranosyl-(1→4)-O-α-D-glucopyranosyl-(1→4)-O-α-D-glucopyranosyl-(1→4)-O-α-D-glucopyranosyl-(1→4)-α-D-glucopyranoside) [9], 1.3 mg/ml glucoamylase (EC 3.2.1.3) from *Rhizopus delemar* and 0.28 mg/ml α-glucosidase (EC 3.2.1.20) from *Saccharomyces carlsbergensis* in 0.05 *M* BES–sodium hydroxide buffer (pH 7.5) containing 8 m*M* calcium chloride and 0.02% BRIJ 30 at a flow-rate of 0.2 ml/min in the mixing tee. The post-column enzymatic reaction was performed at 37 °C for 8.6 min in the reaction coil. The absorbance of the enzymatic reaction mixture from the reaction coil was continuously monitored at 400 nm.

RESULTS AND DISCUSSION

FG5P is hydrolysed by human pancreatic and salivary α-amylases in serum to give O-6-deoxy-6-[(2-pyridyl)amino]-α-D-glucopyranosyl-(1→4)-O-α-D-glucopyranosyl-(1→4)-D-glucose (FG3) and *p*-nitrophenyl α-maltoside, or to O-6-deoxy-6-[(2-pyridyl)amino]-α-D-glucopyranosyl-(1→4)-O-α-D-glucopyranosyl-(1→4)-O-α-D-glucopyranosyl-(1→4)-D-glucose (FG4) and *p*-nitrophenyl α-glucoside [9]. The coupled enzymes, glucoamylase and α-glucosidase, do not act on FG5P, FG3 and FG4 because of the pyridylamino group of the non-reducing-end glucose residue [9]. However, they liberate *p*-nitrophenol from *p*-nitrophenyl α-maltoside and *p*-nitrophenyl α-glucoside produced by α-amylases. The rate of the liberation of *p*-nitrophenol corresponds to the α-amylase activity in the presence of a large amount of the coupled enzymes. Thus, FG5P and the coupled enzymes can be used for continuous monitoring of α-amylase activity of the eluate from the column.

Sera from patients with macroamylasemia and normal serum were analysed by the HPLC system. Macroamylase was separated from the normal size α-amylase,

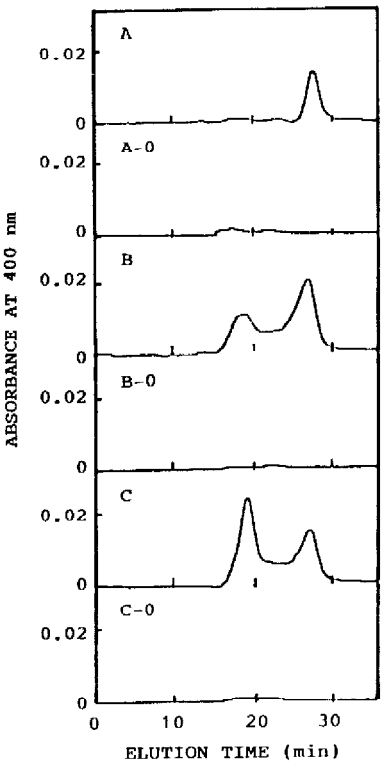

Fig. 2. Gel-permeation chromatography of human serum. A 10-μl volume of human serum was injected onto the column and chromatographed as described in Experimental. (A) Normal serum, (B and C) macroamylasemia sera; (A-0, B-0 and C-0) controls of A, B, and C, respectively (without FG5P in substrate delivery system)

as shown in Fig. 2. The results indicate that HPLC with this continuous monitoring system of α-amylase activity can be used in the clinical diagnosis of macroamylasemia.

ACKNOWLEDGEMENT

We thank Prof. Minoru Yakata, Niigata University School of Medicine, for his generous supply of macroamylasemia sera.

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(First received December 16th, 1987; revised manuscript received February 29th, 1988)