TOSOH

Analyses of glycated human serum albumin by high-performance boronate affinity chromatography coupled with on-line post column reaction detection

TOSOH CORPORATION
3-8-2, Shiba, Minato-ku,
Tokyo 105-8623, Japan
Tel: +81 3 5427-5118
Fax: +81 3 5427-5198
infoline@tosoh.co.jp
www.tosoh.com

Yoshio Kato, Koji Nakamura and Shigeru Nakatani
Nanyo Research Laboratory, Tosoh Corporation, Yamaguchi, Japan

Summary

We developed a rapid, simple and precise method to analyze glycated albumin in human serum. Glycated (glycosilated) and non-glycated (non-glycosilated) proteins in human serum were separated by high-performance boronate affinity chromato-graphy. Then, amounts of albumin in both fractions were determined by on-line post column reaction with bromocresol purple. The method did not require any pretreatment of samples like separation of albumin from other serum proteins. Although the result of analysis (the amount of glycated albumin) depended on chromatographic conditions such as eluent pH and temperature, the reproducibility was quite high under rigorously controlled conditions. It took only 4 minutes to analyze a sample. Therefore, the method should be useful for routine analyses of glycated human serum albumin, which is an index for short-term blood glucose level.

Introduction

The amount of glycated human serum albumin provides useful information on short term blood glucose control in diabetic patients. Although some methods to analyze glycated human serum albumin have been reported, they are time-consuming and not so accurate. In this paper we try to develop a rapid, simple and precise method to analyze glycated human serum albumin by high-performance boronate affinity chromatography coupled with on-line post column reaction detection.

Experimental

Boronate affinity chromatography coupled with on-line post column reaction detection was performed with a system illustrated in Fig. 1. The system was operated automatically by using system controller. Glycated and non-glycated proteins in human serum were separated on TSKgel Boronate-5PW column (10 x 4.6 mm I.D.) at a flow rate of 0.8 ml/min at 37 °C with a step gradient elution of sorbitol from 0 to 200 mM in 50 mM glycine-NaOH buffer (pH 7.5) containing 200 mM magnesium chloride and 0.05 % sodium azide. Human serum of 1 µl was injected after 2 min equilibration of the column with the initial eluent. The initial eluent was delivered for 1 min to elute unbound non-glycated proteins. Then, the eluent containing sorbitor was delivered for 1 min to elute glycated proteins bound on the column. The column effluent was mixed with 500 mM succinate buffer (pH 5.5) containing 0.1 mM bromocresol purple, 0.05 % Brij-35 and 0.1 % sodium azide, which was delivered at a flow rate of 1.5 ml/min. The mixture was passed through reaction coil of 3 m x 0.6 mm I.D. at 37 °C, and albumin was monitored by UV/Visible detector at 620 nm. Pooled serum samples from normal adults and diabetic patients were used.

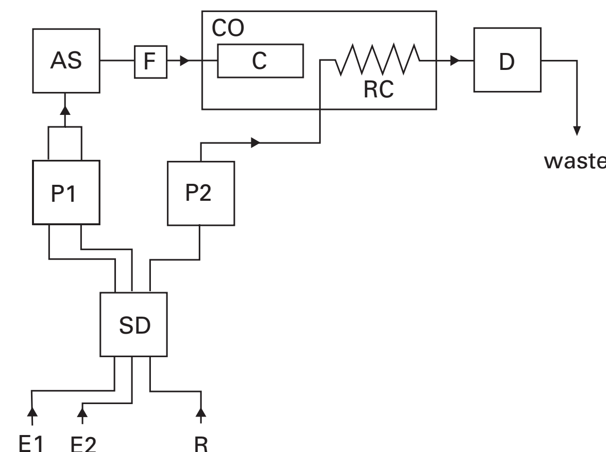

Figure 1: Schematic diagram of the system for boronate affinity chromatography coupled with on-line post column reaction detection

SD: solvent degassor; P1, P2: pump; AS: auto-sampler; F: line filter; CO: column oven; C: column; RC: reaction coil; D: detector; E1, E2: eluent; R: post column reaction reagent

Results and Discussion

Separation of glycated and non-glycated albumin

Fig. 2 shows examples of separations between glycated and non-glycated albumin. A and B are chromatograms of pooled serum samples from normal adults and diabetic patients, respectively. Non-glycated and glycated albumin were eluted at 0.52 and 1.57 min as sharp peaks, and were separated well.

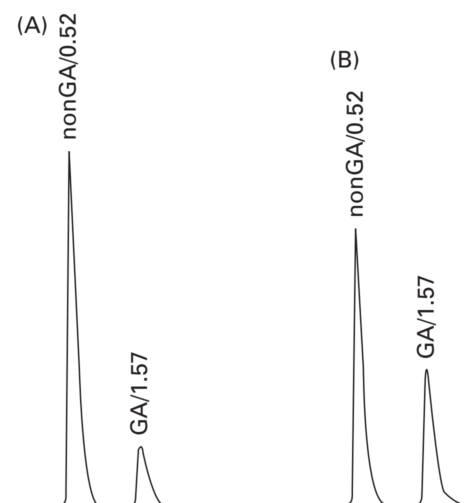

Figure 2: Chromatograms of pooled serum samples from normal adults (A) and diabetic patients (B) obtained from boronate affinity chromatography coupled with on-line post column reaction detection

Reproducibility of analyses

Results of tests for reproducibility are summarized in Table 1. Four pooled serum samples (two from normal adults and two from diabetic patients) were analyzed 20 times, respectively. Relative standard deviations of observed values for the amounts of glycated albumin were less than 3 % in all cases, indicating that the reproducibility of presented method is high.

Sample [a]	Mean Value [b] (standard deviation) (%)	Relative standard deviation (%)
A	13.36 (0.15)	1.12
B	15.16 (0.39)	2.57
C	27.52 (0.59)	2.14
D	29.81 (0.49)	1.64

a) A, B: pooled serum samples from normal adults
C, D: pooled serum samples from diabetic patients
b) mean value of percentage of glycated albumin in total albumin

Table 1: Reproducibility of analyses of glycated human serum albumin

Effects of chromatographic conditions on the amount of glycated human serum albumin

Effects of eluent pH, concentrations of glycine, magnesium chloride, and sorbitol, temperature and equilibration time with initial eluent were studied.
Almost constant values were obtained for the amount of glycated albumin at eluent pH between 7.5 and 8.0 although lower values were observed at pH below 7.5 and above 8.0. The effects of concentrations of glycine, magnesium chloride and sorbitor were little at 50-200, 50-250 and 100-300 mM, respectively. At lower concentrations of these components, slightly lower values were observed for the amount of glycated albumin. The amount of glycated albumin gradually increased at higher temperature between 20 and 45 °C. Therefore, it is important to rigorously control temperature to obtain reproducible values. No effect of equilibration time with initial eluent was observed when it was longer than 2 minutes although the amount of glycated albumin decreased at shorter equilibration time.

Conclusion

High-performance boronate affinity chromatography coupled with on-line post column reaction detection is useful for routine analyses of glycated human serum albumin. It takes only 4 minutes to analyze a sample. The method is simple and reproducible.