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

Yoshio Kato, Koji Nakamura and Shigeru Nakatani

Nanyo Research Laboratory, Tosoh Corporation, Yamaguchi, Japan

Introduction

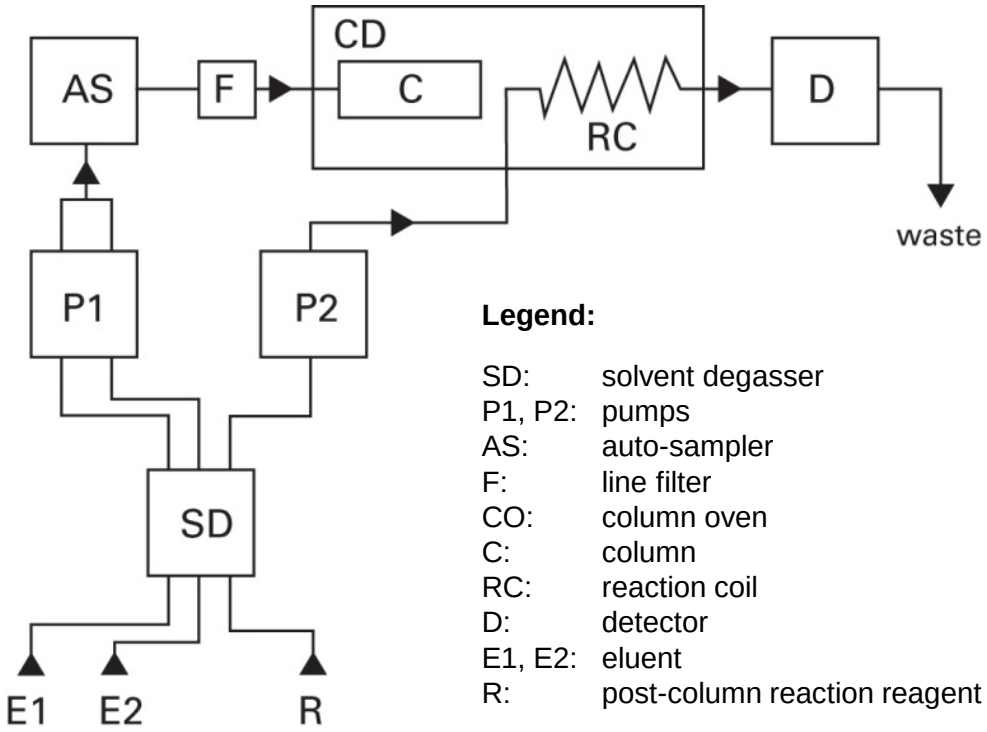
- Tosoh scientists developed a rapid, simple and precise method to analyze glycated albumin in human serum.
- Glycated (glycosilated) and non-glycated (non-glycosilated) proteins in human serum were separated by high performance boronate affinity chromatography.
- Amounts of albumin in both fractions were then determined by on-line post column derivatization with bromocresol purple.
- The method did not require any sample pretreatment, e.g., the separation of albumin from other serum proteins was not required.
- Although analysis results (the amount of glycated albumin) varied with mobile phase pH and temperature, the reproducibility of the method was high under rigorously controlled conditions.
- The analysis time was 4 minutes.
- This method will be useful for routine analyses of glycated human serum albumin, which is an index for the short-term level of glucose in blood.

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 often inaccurate.
- In this study, a rapid, simple and precise method to analyze glycated human serum albumin by high performance boronate affinity chromatography coupled with online post column reaction detection was developed.

Experimental

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



Legend:

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

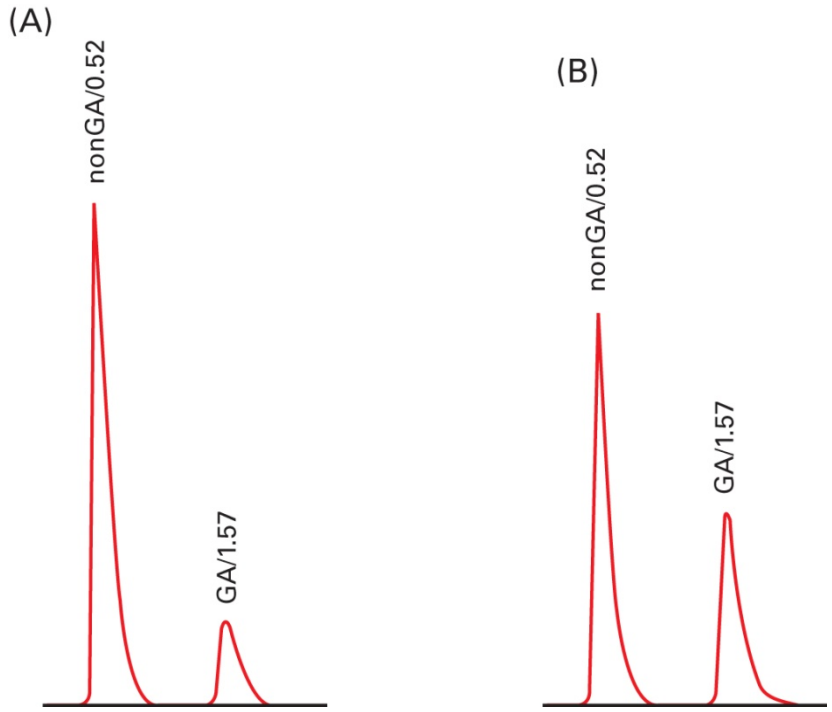
Boronate affinity chromatography coupled with an online post-column reaction detection was performed with the system shown in Figure 1. The system was operated automatically through a system controller.

Experimental

- Glycated and non-glycated proteins in human serum were analyzed using a TSKgel Boronate-5PW column, 4.6mm ID x 10cm, at a flow rate of 0.8mL/min at 37°C with a step gradient elution of sorbitol from 0 to 200mmol/L in a mobile phase containing 50mmol/L glycine-NaOH buffer, pH 7.5, 200mmol/L magnesium chloride and 0.05% sodium azide.
- One microliter (1µL) human serum was injected after a 2 minute equilibration of the column with the starting eluent. The starting eluent was delivered for 1 minute to elute unbound non-glycated proteins.
- The eluent containing sorbitol was then delivered for 1 minute to elute glycated proteins bound on the column.
- Post-column, the mobile phase was mixed with 500mmol/L succinate buffer, pH 5.5, containing 0.1mmol/L bromocresol purple, 0.05% Brij-35 and 0.1% sodium azide, which was delivered at a flow rate of 1.5mL/min.
- The mixture was passed through a reaction coil of 0.6mm ID x 3m at 37°C and albumin was monitored using a UV/Visible detector at 620nm. No attempts were made to reduce band broadening by air-segmentation.
- Pooled serum samples from normal adults and diabetic patients were used.

Results and Discussion

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



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 minutes as sharp peaks, and were well separated.

Reproducibility of Analyses

Table 1: Reproducibility of analyses of glycated human serum albumin

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		

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 this method is high.

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

- The effect of eluent pH, concentrations of glycine, magnesium chloride, and sorbitol, as well as variations in temperature and equilibration time were studied.
- Similar values for the amount of glycated albumin were obtained when eluent pH was varied between 7.5 and 8.0, although lower values were observed at pH values below 7.5 and above 8.0.
- The effect of varying concentrations of glycine, magnesium chloride and sorbitol were found to be minimum at 50-200, 50-250 and 100-300mmol/L, respectively. At lower concentrations of these components, slightly lower values were observed for the amount of glycated albumin.
- The amount of glycated albumin steadily increased at higher temperature between 20 and 45°C. It is important, therefore, to rigorously control temperature to obtain reproducible values.
- Equilibration times with initial eluent longer than 2 minutes did not affect glycated albumin values, although the amount of glycated albumin decreased at shorter equilibration times.

Conclusions

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