GLYCATION OF HUMAN SERUM ALBUMIN IN LONG-TERM INCUBATION WITH LOW AND HIGH CONCENTRATIONS OF GLUCOSE

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Glycated albumin levels of incubation mixtures with high (25.8 mM) and low (8.2 mM) concentrations of glucose when measured by the nitroblue tetrazolium (NBT) reducing method, showed similar values at day 20-25. But when tested by thiobarbituric acid (TBA) colorimetry, the levels with high concentrations of glucose were about twice that with low concentration of glucose.

KEYWORDS human serum albumin; glycation; glycated albumin; nitroblue tetrazilium (NBT) reducing method; thiobarbituric acid (TBA) colorimetry; hyperglycemia; diabetes mellitus

We reported a method using agarose gel electrophoresis with nitroblue tetrazolium (NBT) coloration (NBT-reducing activity) to determine lipoproteins and glycated albumin in serum from diabetics.1) Conventional methods based on thiobarbituric acid (TBA),2) furosine,3) affinity chromatography4) and immunological methods5) gave higher levels (1.6-2.0-fold) of glycated albumin in hyperglycemics than that in euglycemics. However, in our method which is based on essentially on an NBT reducing method, the glycated albumin did not increase in diabetics in proportion to the hyperglycemia.1)

To explain this unexpected finding, we compared changes in the glycated albumin levels in the incubation mixture determined by the NBT reducing method6) and TBA colorimetry.2) The solution (66 mM phosphate buffer, pH 7.4) containing human serum albumin (fraction V, fatty acid-free, 40 g/l), glucose (25.8 or 8.2 mM) and sodium azide (3 mM) was incubated at 37°C for 25 days. A portion of the incubation mixture was taken to measure the NBT-reducing activity (fructosamine value, glycated albumin) and TBA coloration (OD 443) on the days indicated in Fig. 1, followed by dialysis against the phosphate buffer as described above at 4°C for 48 h.

As shown in Fig. 1, the TBA coloration of both incubation mixtures (with low (8.2 mM) and high (25.8 mM) concentrations of glucose) increased linearly with incubation time. The TBA coloration of the incubation mixture with 25.8 mM glucose was 1.7-1.9-fold of that with 8.2 mM glucose at day 20-25. But the NBT-reducing activity of the incubation mixture with 25.8 mM glucose had risen rapidly by day 5, then remained at a constant level (about 3 mM) until day 25, while the NBT-reducing activity of the incubation mixture with 8.2 mM glucose increased slowly up to about 3 mM at day 20-25.

The glycated albumin levels at day 20-25 can be compared to those in sera from euglycemics and hyperglycemics because the half-life of circulation serum albumin is 12-20 days.7) Thus, the fructosamine values (glycated albumin levels) of incubation mixtures with high and low concentrations of glucose, which were measured by the NBT-reducing method, showed similar values at day 20-25. Hyperglycemia-induced glycated albumin may change to advanced Maillard products,8,9) which does not show NBT-reducing activity but does show TBA coloration when heated with acid, in long-term incubation.

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Schleicher et al. demonstrated that the steady-state production of glycated albumin which was determined by the furosine method was reached at day 40. In the present study, the production of glycated albumin having NBT-reducing activity seems to reach the steady-state at by day 5 in the hyperglycemics. A higher fructosamine value (4-5 mM), which is clinically observed in serum from poorly controlled diabetic patients, may be caused by the glycation of serum proteins other than albumin, e.g., lipoproteins which are known to increase in diabetes mellitus.

![Graph showing the glycation of human serum albumin and the effect of glucose concentration.](image)

**Fig. 1. Glycation of Human Serum Albumin**

---, 25.8 mM glucose; ----, 8.2 mM glucose. Each point is the mean of two separate experiments. Fructosamine value (mM) is expressed in terms of 1-deoxy-1-morpholino fructose (DMF)

**REFERENCES AND NOTES**


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