centage of HbA_{1c} at the end of the assay.

Statistics

Data in figures are means ± S.E.M. Statistical analyses were performed by use of Student's and unpaired *t*-tests as appropriate. A value of $p < 0.05$ was considered statistically significant. Correlation coefficient was calculated by the best-fit linear regression of least squares statistics using Sigma Plot computer software.

RESULTS

Fluorescence Intensity of Advanced Glycosylation End Products

Serum proteins were modified by sugars via a non-enzymatic reaction. This reaction proceeds from reversible Schiff bases to a stable covalently bounded Amadori rearrangement and leads to a group of fluorescent compounds or "advanced glycosylation end products" (AGEs). As shown in Fig. 1, the magnitude of fluorescence intensities increased as the incubation time was prolonged. The fluorescent substances formed after a 6-month incubation were dialyzed against phosphate-buffered saline (PBS) and used as the coating antigen for both direct and indirect ELISA.

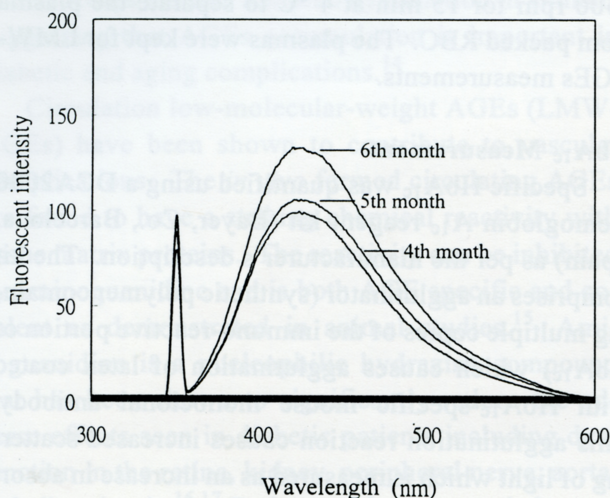

Fig. 1. Fluorescent spectrum produced by RNase-AGEs. RNase (l0 mg/mL) was incubated with glucose for different periods of time. Aliquots of the reaction mixture were dialyzed against PBS and diluted to 1 mg/mL. The fluorescent intensities at different spectrums were measured at an excitation wavelength of 350 nm. Data represent a typical spectrum which has been reproduced at least 3 times.

Antibodies Raised against BSA-AGEs Recognition of RNase and Polv-L-lysine-derived AGEs

Glucose-derived BSA-AGEs-specific antibodies were used to develop a direct enzyme-linked immunosorbent assay (ELISA). The immuno-absorbed anti-BSA-AGEs recognized RNase-AGEs but not RNase, suggesting that anti-BSA-AGEs antibodies recognized AGEs epitopes in RNase-AGEs (Fig. 2). When the immuno-absorbed anti-BSA-AGEs antibodies (1:500) were used for competitive ELISA, the antibodies could be neutralized by addition of AGEs derived from different proteins. As shown in Fig. 3, the RNase-AGEs, poly-L-lysine-AGEs, and Hb-AGEs but not RNase, poly-L-lysine, or Hb competed with RNase-AGEs in competitive ELISA. The amount of protein-AGEs required for 50% competition was 0.0005 mg/mL, 0.005 mg/mL, and 0.01 mg/mL for RNase-AGEs, poly-L-lysine-AGEs, and Hb-AGEs,

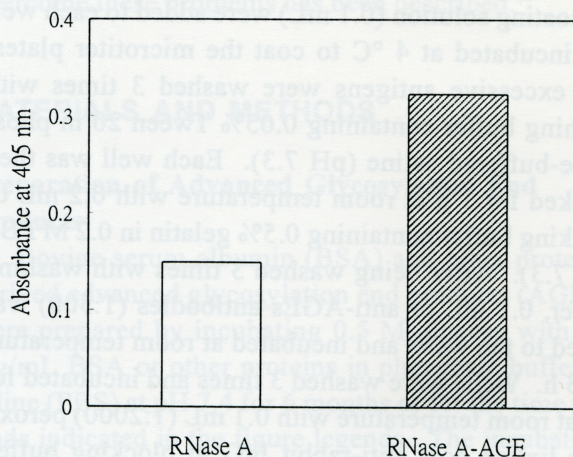

Fig. 2. Immunoreactivity between anti-BSA-AGEs antibody and RNase or RNase-AGEs. A direct ELISA was performed on both the RNase-coated (0.01 μg/well) or RNase- AGEs-coated (0.01 μg/well) plates. Data are expressed as means S.E.M. of optical densities measured at 405 nm from 3 independent experiments, each done in duplicate.