the Amadori product undergoes rearrangement and a cleavage reaction to give rise to advanced glycosylation end products (AGEs).[2] AGEs are a group of brown or fluorescent compounds such as FFI,[3] pyrraline[4], AFGP,[5] carboxymethyllysine[6] and pentosidine.[7] These compounds remain irreversibly bound to long-lived proteins such as collagen, and accumulate as a function of age.

The importance of AGEs in the pathogeneses of diabetic complications is primarily supported by the fact that AGEs accumulate in tissues affected by diabetic complications. AGEs accumulation has been demonstrated in lens crystalline of cataract patients,[8] in arterial wall collagen of patients with cardiovascular insufficiency,[9] and in the lesions of diabetic nephropathy and retinopathy.[10] Advanced glycosylation of low-density lipoproteins may induce hydroxyalkenal formation[11] and increase the serum advanced glycosylation end product, N-epsilon-(carboxymethyl) lysine concentration.[12] Both N-epsilon-(carboxymethyl) lysine and carboxymethy-ethanolamine have served as bio-markers of phospholipid modification during the Maillard reaction *in vivo*.[13] Consistently, administration of AGEs induces a similar dysfunction in animals suggesting that AGEs accumulation is important in diabetic and aging complications.[14]

Circulation low-molecular-weight AGEs (LMW-AGEs) have been shown to contribute to vascular complications. The *in vivo* formed circulating AGEs are found to have a striking chemical reactivity with tissue matrix proteins. The reactivity can be inhibited by aminoguanidine and is both AGE specific and covalent as demonstrated in animal studies.[15] Aminoguanidine is a nucleophilic hydrazine compound that has been shown to significantly reduce the adverse effects seen in diabetic patients including dysfunction in the retina, kidney, peripheral nerve, aorta, and albuminuria.[16,17] Early vascular structural changes in diabetes-induced vascular dysfunction are also significantly suppressed by treatment with aminoguanidine. Covalent reactivity of serum LMW-AGEs is not limited to tissue matrix proteins; LMW-AGEs can also react with plasma lipoproteins *in vitro* to form AGEs-modified LDL.[18,19] The AGEs-modified LDL leads both to the loss of recognition by cellular LDL receptors and to the preferential uptake of oxidized LDL by scavenger receptors.[20] It can be conjectured that LMW-AGEs are likely to be the molecular agent responsible for tissue damage by reattaching to vascular and plasma proteins.

Given that AGEs accumulation is closely related to diabetic complications, monitoring the AGEs level is important for diabetic control. AGEs were traditionally quantified by HPLC or gas chromatography-mass spectrometry (GC/MS). Yet, these methods are not appropriate for clinical use. Recently, advanced glycosylation end products in human serum have been monitored with on-line spectrophotometric and spectrofluorometric detection in a flow system.[21] However, the on-line spectrophotometric method lacks specificity and may be interfered with by a number of native components. Recently, a competitive enzyme-linked immunosorbent measurement has been used to detect circulation AGEs.[22] The antibodies used in the assay recognize AGEs epitopes derived from other proteins and have proven to be a useful reagent in characterizing any possible protein glycosylation end products.[22] However, this method may be affected by different antibodies' affinities, the amount of antigen coated, and many other factors often seen in competitive ELISA. Recently, a standardized method that can overcome these problems has been described.[23]

MATERIALS AND METHODS

Preparation of Advanced Glycosylation End Products

Bovine serum albumin (BSA) and other protein-derived advanced glycosylation end products (AGEs) were prepared by incubating 0.5 M glucose with 10 mg/mL BSA or other proteins in phosphate buffered saline (PBS) at pH 7.4 for 6 months or for the time periods indicated in the figure legends. The incubation was performed under sterile conditions in the dark at 37 °C. After incubation, unreacted sugars were removed before the assay by extensive dialysis against PBS. These solutions were stored frozen before assaying for fluorescence and AGEs. Fluorescent emission was detected at 410 nm with a Shimatsu spectrophotometer using BSA as a blank. AGEs were assayed using direct ELISA as described below.