Site-specific modification of apolipoprotein B by advanced glycosylation end-products: implications for lipoprotein clearance and atherogenesis

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Abstract. An AGE-modified form of LDL (AGE-LDL) circulates in patients with diabetes mellitus or renal insufficiency and shows impaired plasma clearance kinetics when injected into transgenic mice that express the human LDL receptor. The advanced glycosylation inhibitor aminoguanidine decreases plasma LDL in diabetic patients, further suggesting that the AGE modification of LDL contributes significantly to increased LDL in vivo. We utilized AGE-specific antibodies to identify the major site(s) of AGE modification within apolipoprotein B (apoB), which impairs the binding of AGE-LDL by human fibroblast LDL receptors. Despite the large size of apoB (4536 amino acid residues) and its high content of potentially reactive lysines, the predominant site of AGE-immunoactivity was found to lie within a single 67 amino acid domain located 1791 residues N-terminal to the LDL receptor binding site. These data point to the high specificity and reactivity of this site toward AGE formation and to a significant structural interaction between this region of apoB and the LDL receptor binding domain. A low but detectable degree of AGE modification was found to affect this site in LDL which was isolated from normal, non-diabetic individuals, suggesting that advanced glycosylation may contribute to elevated LDL in the general population as well.

Key words: advanced glycosylation end-products; apolipoprotein B; atherogenesis; lipoprotein clearance; site-specific modification

Diabetes mellitus or renal insufficiency is associated with an increased risk of atherosclerosis that results in part from an abnormal elevation in the plasma concentration of the apolipoprotein B (apoB)-containing lipoproteins VLDL and LDL [1–4]. Diabetic or renally impaired patients also have been shown to exhibit increased circulating concentrations of protein-bound advanced glycosylation end-products or AGEs [5–7]. These products arise by the spontaneous conversion of glucose-derived Amadori products to more reactive species (i.e. AGEs) capable of covalently modifying and irreversibly cross-linking protein amino groups [8,9]. Although circulating AGE-modified proteins arise from the reaction of glucose with serum proteins, recent data suggest that a large portion of the AGEs present in the blood form in part from the catabolism of AGE-modified tissue proteins [5,7]. Thus, high circulating levels of reactive AGE-peptides occur under normoglycaemic conditions if plasma filtration is impaired by renal insufficiency. The precise structure(s) of the major cross-linking AGEs which form in vivo remain to be established. Nevertheless, an immunochemically cross-reactive AGE epitope has been found to exist in a number of proteins, such as collagen and basement membrane components [6], haemoglobin [10], lens proteins [11], β2-microglobulin [12] and the β-amyloid peptide [13]. In certain cases, the presence of the AGE modification has been shown to critically affect the functional properties of the native substrate.

We recently described an AGE-modified form of low-density lipoprotein (AGE-LDL) which circulates in elevated amounts in patients with diabetes or renal insufficiency and exhibits impaired plasma clearance kinetics when injected into transgenic mice expressing the human LDL receptor [14,15]. The formation of AGEs on LDL thus has been proposed to be an important mechanism for the dyslipidaemia and accelerated atherogenesis that occurs in patients with diabetes or renal insufficiency. This concept has been further substantiated by the observation that short-term administration of the advanced glycosylation inhibitor aminoguanidine to diabetic patients decreases circulating LDL by 28% [15].

Chemical modification of basic residues within apoB has been shown previously to interfere with the ability of LDL to undergo receptor-mediated uptake and
We considered that AGEs might act in a similar manner to covalently modify site(s) critical for the recognition of LDL by tissue LDL receptors. To approach this question experimentally, we used an AGE-specific antibody to identify the major peptides modified by advanced glycosylation contained within protease-digested preparations of AGE-modified LDL [6,18]. For these studies, native LDL first was modified by glucose to achieve a level of AGE-modification present in vivo in diabetic or renally impaired patients. AGE-LDL prepared in this manner showed diminished recognition and uptake when studied by competitive ligand binding in human fibroblasts. Of note, both AGE formation and defective LDL uptake were inhibited markedly by adding aminoguanidine during the in vitro preparation of AGE-LDL [18].

To identify the sites on apoB modified by advanced glycosylation, V8 protease hydrolysates of AGE-modified apoB were subjected to polyacrylamide gel electrophoresis and Western blotting analyses using an AGE-specific polyclonal and an AGE-specific monoclonal antibody. Significant immunoreactivity was localized to two electrophoretically distinct peptide bands with apparent molecular weights of 28 and 15 kDa. Of note, low but detectable amounts of AGE-immunoreactivity were found to be present consistently in native LDL (not modified with glucose in vitro), verifying previous observations that measurable quantities of AGE-modified LDL exist in the circulation of normal, non-diabetic individuals [14,15].

Once immobilized to PVDF membranes, the AGE-immunoreactive peptide fragments were further hydrolysed in situ and subjected to gas phase microsequencing to determine the precise position of the 28 and 15 kDa AGE-modified sequences within apoB. Microsequencing analyses showed that both these peptides shared the same N-terminus, indicating that the 15 kDa peptide was derived from the larger 28 kDa species by an additional V8-protease cleavage. By comparing the overlapping regions of the two peptides, it thus was established that AGE modification involves (at least) one of nine lysine residues (Lys1388, Lys1401, Lys1408, Lys1414, Lys1422, Lys1446–1448, Lys1454) present within a 67 amino acid domain located between residues 1388 and 1454 of the apoB primary sequence (Figure 1).

Despite the high content of potentially reactive lysine residues in apoB (359 Lys), the fact that the predominant site of AGE-immunoreactivity was mapped to a single 67 amino acid domain attests to the highly reactive nature of this region toward AGE formation. This result was not completely unanticipated given the observation that circulating LDL exhibits measurable quantities of AGE modification despite having an average circulating half-life of only 36–48 h. The reason for the high susceptibility of this region of apoB to AGE formation is unknown at present but presumably reflects microenvironmental factors that favour the formation of the initial Amadori product or the subsequent rearrangement reactions which lead to an irreversibly bound AGE. The 67 amino acid AGE-reactive domain has been mapped to a lipid-associating region of the apoB sequence and advanced glycosylation at this site may be favoured by the fact that anhydrous, lipophilic environments can enhance the dehydration and rearrangement reactions leading to AGE formation [19].

The AGE-reactive domain within apoB lies 1791 residues N-terminal to the putative LDL receptor binding domain. An important question raised by these recent findings is the mechanism by which the modification of lysine residue(s) remote from the receptor-binding domain of apoB can have such a profound impact on LDL receptor binding. AGE modification presumably induces a conformational change within the LDL particle which is sufficient to perturb ligand–receptor interaction. This may be the result of a charge or other surface alteration in the
affected lysine, or possibly by the formation of metastable, AGE-cross-links with more remote regions of the apoB molecule. Recent biophysical studies indicate that an elaborate spacial geometry is involved in the binding of the LDL particle to its receptor. For example, the DNA mutation responsible for a familial syndrome of defective apolipoprotein B-100 produces a Glu→Arg substitution in codon 3500 and occurs C-terminal to the LDL receptor binding domains. NMR-based studies indicate that this mutation perturbs the microenvironment of six lysines, changing their pKa values on average from 8.9 to 10.5 [19]. Of note, several anti-apoB monoclonal antibodies have been described that effectively block LDL receptor binding but which map to epitopes spanning 1000 amino acids and which localize to regions lying well outside the LDL receptor binding domain [20].

These data provide the first structural basis for beginning to assess how advanced glycosylation can have such a profound impact on LDL clearance. These studies also provide important corroborative data for the importance of long-range interactions between the LDL receptor binding domain and remote regions of the apoB polypeptide. More detailed study of the three-dimensional changes induced in LDL by AGE formation together with the precise chemical nature of the apoB-AGE should ultimately assist in the rational design of specific pharmacological inhibitors of LDL-advanced glycosylation.

References

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