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Decrease in the particle size of low-density lipoprotein (LDL) by oxidation

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Abstract—A radical reaction of low-density lipoprotein (LDL) causes fragmentation and cross-link of apolipoprotein B-100 (apoB). LDL (50 μg/ml) was subjected to the well-studied oxidation with Cu^{2+} (1.67 μM). The concentration of α-tocopherol decreased to 10% of the initial level during the first 30 min. After this lag time, the conjugated diene content, as measured by absorption at 234 nm, started increasing and the residual apoB at 512 kDa determined by immunoblot after SDS-PAGE (sodium dodecylsulfate-polyacrylamide gel electrophoresis) was also decreased. The particle size of LDL determined by nondenaturing gradient gel electrophoresis decreased steadily during the initial 120 min, when residual native apoB was only 30% of the initial level. Plasma was also oxidized with Cu^{2+} (400 μM). Under this condition, a clear lag time was not observed and α-tocopherol content, apoB, and the LDL particle size were decreased simultaneously. Based on these experiments, we propose that an oxidation reaction is involved in the formation of small dense LDL.

1. Introduction

The role of oxidized low-density lipoprotein (LDL) in atherogenesis has been well studied.[1,2] Although LDL is composed of lipids, protein, and sugar chains, studies on the oxidation of LDL have focused mainly on lipid peroxidation[3] and the resulting modification of apolipoprotein B-100 (molecular mass of 512 kDa) (apoB) by aldehydes,[4,5] hydroperoxide,[6,7] and oxidized phosphatidylcholine[8] produced. Palinski et al.[2] reported that malondialdehyde- or 4-hydroxynonenal-modified LDL was detected immunochemically in the atherosclerotic lesions of the Watanabe heritable hyperlipidemic rabbit aorta, and in human sera using antibodies against LDL modified with these aldehydes.

The protein part of LDL, apoB, is also reactive to oxidation. Radical reaction of LDL induced by Cu^{2+} causes cleavage of peptide bonds and cross-link of apoB.[9–18] This reaction was inhibited by radical scavengers, demonstrating that the oxidation by Cu^{2+} caused radical reaction.[19] Recently, we reported that an immunoblot assay using anti-human apoB antiserum after SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was an effective method to follow radical reaction of LDL in isolated solutions, as well as in plasma.[19] This approach allowed us to show that the radical reaction of serum caused by Cu^{2+} gave a characteristic pattern of fragmented apoB.[19]

Among plasma proteins, apoB is unusually reactive to radical reactions compared to albumin and transferring,[20] and its reactivity is almost the same as α-tocopherol.[20] In normal human serum, both fragmented and cross-linked apoB proteins were present and these oxidation reaction products of LDL tended to increase with age.[19] In addition, we reported[21] that the sum of fragmented and conjugated apoB proteins determined by the immunoblot assay showed significant positive correlation with IMT (intima-media thickness of the carotid artery) and LDL cholesterol and a negative correlation with high-density lipoprotein (HDL) cholesterol and

Keywords: Apolipoprotein B-100; Atherosclerosis; LDL; Oxidation; Particle size; Protein degradation; Radical reaction; Small dense LDL.
Abbreviations: apoB, apolipoprotein B-100; apoE, apolipoprotein E; HDL, high-density lipoprotein; IMT, intima-media thickness of the carotid artery; LDL, low-density lipoprotein; PBS, phosphate buffered saline; PVDF, polyvinylidene difluoride; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis.
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plasma vitamin C, which was an outstanding anti-oxidant in plasma.[22]

Oxidation of LDL should produce many species and normal human LDL consists of multiple subclasses.[23–25] In the present study, we investigated whether a radical reaction is a possible mechanism to generate small dense LDL because a radical reaction of LDL has been assumed as the initial event in atherogenesis,[1,2] and the role of oxidation reactions has never been examined in the formation of small dense LDL, which is associated with the incidence of atherosclerosis.[24,25]

2. Results

2.1. Oxidation of LDL

LDL (50 μg/ml) was subjected to the well-studied oxidation[27] with Cu^{2+} (1.67 μM). LDL samples from three individuals were used and the reaction profiles closely resembled each other. Therefore, a typical example is shown in Figures 1 and 2. The concentration of α-tocopherol decreased to 10% of the initial level during the first 30 min (Fig. 1). After this initiation period (often designated as a lag time), the conjugated diene content as measured by absorption at 234 nm started increasing, while the residual apoB at 512 kDa determined by immunoblot after SDS-PAGE was also decreased in a similar manner to that reported previously[19] (Fig. 1).

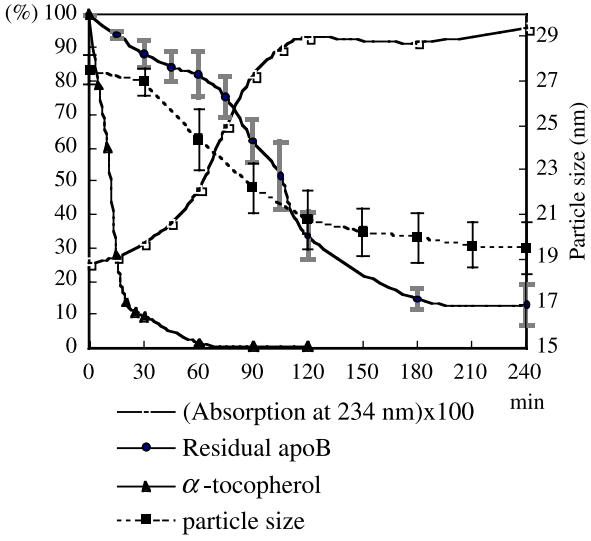

Figure 1. Reaction profile of the oxidation of LDL with Cu^{2+}. LDL (50 μg/ml) was treated with 1.67 μM Cu^{2+} at 37 °C. Samples were withdrawn at the indicated times after the addition of Cu^{2+} up to 4 h. The residual contents of α-tocopherol and apoB (% shown in the left vertical axis), and absorption at 234 nm (absorbance × 100 corresponds to the % value shown in the left vertical axis), residual apoB, and the LDL particle size (unit shown in the right vertical axis) were measured as described in the text. Each point was the mean ± SE of four independent determinations. Where no bars are shown, SE was smaller than the symbol.

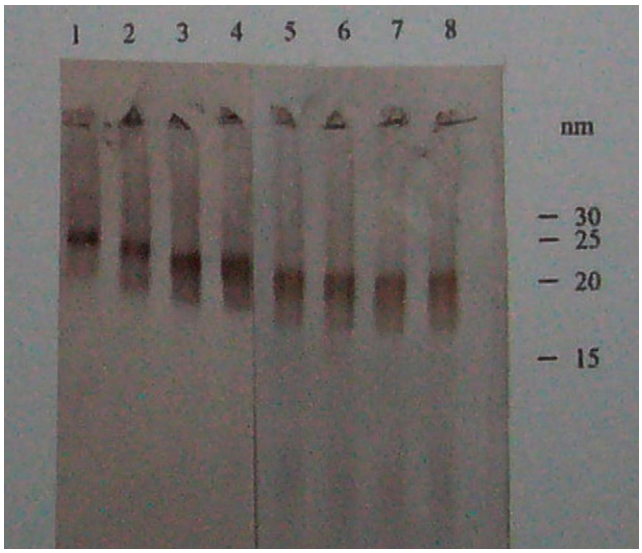

Figure 2. Change in the particle size of LDL during the oxidation of LDL. LDL (50 μg/ml) was treated with 1.67 μM Cu^{2+} at 37 °C. Samples in lanes 1–8 were withdrawn at 0, 60, 90, 120, 150, 180, 210, and 240 min, respectively. After electrophoresis, immunoblotting using anti-human apoB serum was performed and the LDL particle size was calibrated as described in the text.

The particle size of LDL was determined by nondenaturing gradient gel electrophoresis as described.[29] At the start time, LDL was detected as one broad band with the mean value of 27.5 nm. The LDL particle size as measured by the maximal, minimal, as well as the mean value decreased steadily during the initial 120 min (Figs. 1 and 2), when residual native apoB was only 30% of the initial level (Fig. 1). It was reported that LDL particles with a diameter smaller than 25.5 nm showed the strongest association with the risk of ischemic heart disease.[30] At 240 min, native apoB at 512 kDa disappeared almost totally, but the LDL particle was still detected as a broad band at approximately 19.5 nm (Fig. 2). These results demonstrate that LDL particles were retained instead of extensive cleavages in the apoB protein and that the LDL particle size was reduced by oxidation.

2.2. Oxidation of plasma

Human plasma diluted 4-fold was subjected to a radical reaction initiated by Cu^{2+} (400 μM) as previously described.[19,20] Plasma samples from three individuals were used. The reaction profiles closely resembled each other. Therefore, typical results from the same subject for LDL are shown in Figure 3. Under this condition, α-tocopherol content and the residual apoB steadily decreased in a similar manner to a previous study[20] (Fig. 3).

To determine LDL particle size in plasma, blot analysis using anti-apoB serum was used. Since apoB is a component of LDL, IDL, and VLDL, LDL must be identified first. To distinguish LDL from other apoB-containing particles, an immunoblot assay using anti-apoE antiserum was performed (Fig. 4). Comparing these blots, LDL was detected as two bands indicated below