Binding of Low Density Lipoprotein Particles to Human Aortic Proteoglycans in Thermo Scientific Enhanced Binding Microtiter Microplates

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Abstract
Binding of low density lipoprotein (LDL) to proteoglycans and modification of LDL are key processes in atherogenesis. In this application note we describe a method for studying LDL binding to proteoglycan in microplate-format.

Introduction
Accumulation of cholesterol in the inner layer of the arterial wall, the intima, leads to the development of atherosclerotic lesions, the lesional cholesterol being mostly derived from circulating low density lipoprotein (LDL) particles. Once LDL particles have entered the subendothelial space they are entrapped by the extracellular matrix, particularly by intimal proteoglycans, which form an organized, negatively charged, tight network. The retention of LDL particles by proteoglycans is considered to have key role in the development of atherosclerosis. In this application note we describe a method for studying the binding of LDL particles to human aortic proteoglycans.

Materials and methods
Isolation and modifications of LDL
Human LDL (d = 1.019–1.050 g/ml) was isolated from plasma of healthy volunteers by sequential ultracentrifugation in the presence of 3 mM EDTA (1, 2). The amounts of LDL are expressed in terms of their protein concentrations, which were determined by the method of Lowry et al. (3) with bovine serum albumin as a standard.

Preparation and characterization of aortic proteoglycans
Proteoglycans from the intima-media of human aortas were obtained at autopsy within 24 h of accidental death and were prepared essentially by the method of Hurt-Camejo et al. (4), as described previously (5). Glycosaminoglycans were quantified by the method of Bartold and Page (6), and the amounts of proteoglycans are expressed in terms of their glycosaminoglycan content.

Binding of LDL to human aortic proteoglycans
The wells of Thermo Scientific polystyrene 96-well microplates (Enhanced binding) were coated with 100 µl of human aortic proteoglycans (50 µg/ml in phosphate-buffered saline) by incubation at 4 °C overnight. Wells were blocked with 3% BSA, 1% fat-free milk powder, and 0.05% Tween 20 in phosphate-buffered saline for 1 h at 37 °C. Wells without proteoglycans served as controls.

100 µl of LDL (0.02–0.2 mg/ml) in a buffer containing 1% BSA, 140 mM NaCl, 2 mM CaCl2, 2 mM MgCl2 and 20 mM MES (pH 5.5) was incubated in the wells for 1 h at 37 °C. Wells without proteoglycans served as controls. 100 µl of LDL (0.02–0.2 mg/ml) in a buffer containing 1% BSA, 140 mM NaCl, 2 mM CaCl2, 2 mM MgCl2 and 20 mM MES (pH 5.5) was incubated in the wells for 1 h at 37 °C, unbound LDL was removed, the wells were washed with MES buffered saline for 1 h at 37 °C. Wells without proteoglycans served as controls.

Results and Discussion
The binding of LDL to human aortic proteoglycans was examined in microtiter well assays at pH 5.5. For this purpose, the wells were coated with the proteoglycans with BSA-coated wells serving as controls. LDL was incubated in the proteoglycan-or BSA-coated wells, the unbound LDL was removed, and the amount of bound LDL was measured. As shown in Fig. 1, LDL binds to aortic proteoglycans in the microplate assay. Results shown are the average ± SD of incubations performed in quadruplicate. For some data points, the sizes of symbols are larger than the error bars. A saturation level is observed at a concentration of 20 µg LDL / well.

Conclusions
The assay described here is suitable for studying binding of LDL to human aortic proteoglycans. The assay can be used for determining the effect of microenvironmental factors (like pH) or modifications of LDL on binding to proteoglycans.

Abbreviations
LDL: low density lipoprotein
BSA: bovine serum albumin
MES: 4-morpholineethanesulfonic acid
References
This application note is based on: