

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 ($d = 1.019\text{--}1.050$ 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 μ l of human aortic proteoglycans (50 μ g/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 μ l 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 50 mM saline, and the amounts of bound LDL were determined using the Amplex Red cholesterol kit (Molecular Probes, Eugene, Oregon). The amount of bound LDL is expressed as μ g of LDL protein/well, which was calculated from the amounts of cholesterol (2.7 nmol of cholesterol/ μ g of LDL protein).

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 \pm 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 μ g 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:

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1. Mahley R W, Weisgraber K H and Innerarity T L. 1979. *Biochim. Biophys. Acta* 575, 81–91.
2. Radding C M and Steinberg D. 1960. *J. Clin. Invest.* 39, 1560–1569.
3. Lowry O H, Rosebrough N J, Farr A L and Randall R J. 1951 *J. Biol. Chem.* 193, 265–275.
4. Hurt-Camejo E, Camejo G, Rosengren B, Lopez F, Wiklund O and Bondjers G. 1990. *J. Lipid Res.* 31, 1387–1398.
5. Öörni K, Pentikäinen M O, Annala A and Kovanen P T. 1997. *J. Biol. Chem.* 272, 21303–21311.
6. Bartold P M and Page R C .1985. *Anal. Biochem.* 150, 320–324.

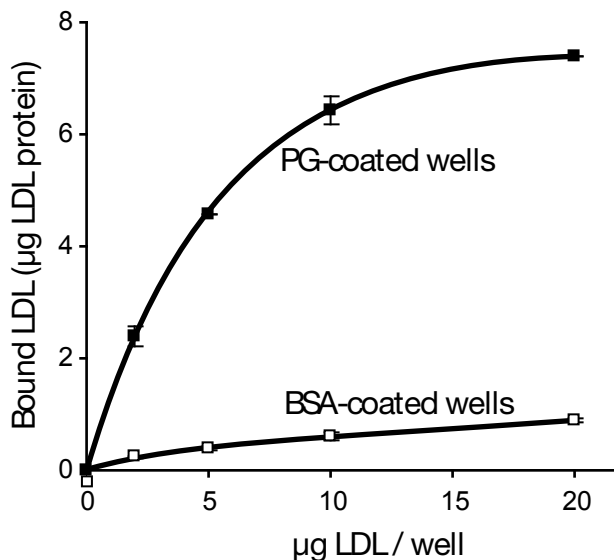


Figure 1. Saturation curves of native LDL in proteoglycan (PG)- and BSA-coated wells.

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