

Interaction of prostaglandin E₁ with human high density lipoproteins

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Prostaglandin E₁ has been shown to interact with serum high density lipoproteins (HDL) in a manner resembling the interaction of a ligand with a high affinity binding site. The presence of 10^{-12} – 10^{-10} M prostaglandin E₁ induces a rearrangement of the HDL surface lipids and probably influences the biological functions of the lipoproteins.

Fluorescent phospholipid probe

High density lipoprotein

Prostaglandin E₁

1. INTRODUCTION

The antiatherogenic high density lipoproteins have attracted much attention due to their important role in lipid metabolism [1] and their inverse correlation with the risk of coronary artery disease [2]. The structure of HDL globules has been intensively studied and a fairly consistent model has emerged. Its main structural features are a core of cholesterol esters and triglycerides covered by a polar monolayer of apoproteins, phospholipids and free cholesterol (reviewed in [3]). The fine structure of the outer layer as revealed by in vitro studies may not be an accurate representation of the actual HDL structure in the bloodstream where a great number of factors may influence the molecular organization and function of the lipoproteins. One of such factors are the prostaglandins which are present in the blood in low concentration (10^{-11} – 10^{-9} M). Up to now nothing

is known about the influence of prostaglandins on the structure of serum lipoproteins. In this communication we examine the influence of prostaglandins E₁ and E₂ on the molecular organization of HDL phospholipids. To this end we employed a previously described fluorescence method using anthrylvinyl-labeled analogs of phosphatidylcholine (I) and sphingomyelin (II) which mimic the behaviour of the corresponding natural lipids on the HDL surface [4]. We demonstrate that PGE₁ binds in a highly specific manner to HDL and that the molecular organization of the latter changes in the presence of physiological concentration of PGE₁.

2. MATERIALS AND METHODS

HDL fractions (HDL₂ and HDL₃) were isolated from the blood of healthy donors according to [5]. Their purity was controlled by gel electrophoresis which revealed the absence of detectable amounts of albumin. The lipid-specific fluorescent probes I and II were synthesized according to [6,7]. Fluorescent measurements were performed as described in [4] using Hitachi 650-60 and Aminco SPF-1000 spectrofluorimeters. Prior to measurements, HDL suspensions were incubated with

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Abbreviations: HDL, high density lipoproteins; PG, prostaglandin; ASM, anthryl-labeled sphingomyelin; *P*, fluorescence polarization; *E*, parameter of tryptophanyl to probe resonance energy transfer

ethanolic solutions of probes I or II for 8 h at 36.5°C (probe/phospholipid ratio 0.01). Prostaglandins in ethanol were then added to the fluorescent labeled HDL suspensions. The total amount of ethanol in the suspension was less than 0.5 vol%. At such a concentration ethanol had no measurable influence on the fluorescent parameters of the probes.

3. RESULTS AND DISCUSSION

The anthrylvinyl-labeled phosphatidylcholine I and sphingomyelin II revealed similar fluorescence polarization (P) values when incorporated into HDL from donors with normal HDL cholesterol levels [4]. After addition of small amounts (3.7×10^{-9} M) of PGE₁ to HDL doped with the sphingomyelin probe II, P increased significantly, reaching a maximal value after 35–40 min (fig.1). At the same time the fluorescence polarization of HDL labeled with the phosphatidylcholine probe I was not changed (not shown). Neither were any changes of P observed when PGE₁ was added to fluorescent labeled lipid vesicles whose composition corresponded to that of the outer layer lipids of HDL.

Addition of $\sim 10^{-9}$ M PGE₁ to ASM-labeled HDL resulted also in an increase of tryptophanyl to probe resonance energy transfer (E). The PGE₁ concentration dependence of P and E were similar (fig.2). The sensitivity of the fluorescence parameters of ASM-labeled HDL to the action of PGE₁ was extremely high: the minimal PGE₁ concentration inducing measurable changes of P and E was $\sim 10^{-12}$ M (fig.2). Such a concentration cor-

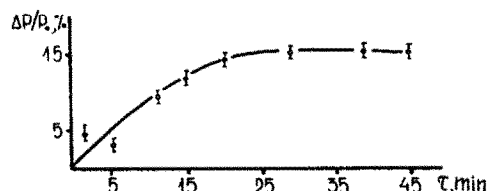


Fig.1. Time dependence of the PGE₁-induced fluorescence polarization changes of ASM-labeled HDL₂ at 36.5°C. Protein concentration 1.54 mg/ml; sample volume 0.6 ml; PGE₁ concentration 3.7×10^{-9} M; $\Delta P = P_{PG} - P_0$, where P_0 and P_{PG} are the fluorescence polarization values before and after addition of PGE₁, respectively. Averaged results of three experiments are shown.

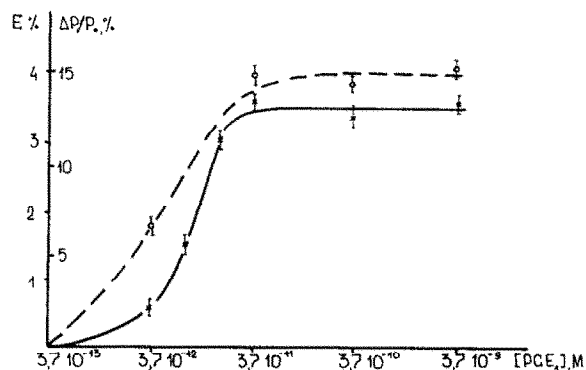


Fig.2. Dependence of $\Delta P/P_0$ (x) and E (o) of ASM labeled HDL₂ upon the concentration of PGE₁. $E = (F_n^{PG} - F_n^0)/F_n^0$, where F_n^0 and F_n^{PG} are parameters of resonance energy transfer before and after addition of PGE₁, respectively (for definition of F_n , see [4]). In all cases incubation time after addition of the PGE₁ was 30 min. For other experimental conditions see legend to fig.1.

responds to one PGE₁ molecule per 10^3 – 10^4 HDL globules. The saturation concentration, i.e., the PGE₁ concentration inducing maximal changes of P and E , was $\sim 10^{-10}$ M. The stability of the PGE₁–HDL complex appeared to be low: the prostaglandins added to the HDL suspension were separated from the lipoproteins almost completely upon dialysis or gel filtration over Sephadex G-75. The structural changes induced by PGE₁ appeared to be reversible: after separation of PGE₁ from the lipoproteins the fluorescence polarization returned almost to the initial level and after addition of a new portion of PGE₁ the P value increased again (fig.3). The effect of PGE₁ on the fluorescence parameters of ASM-labeled HDL could be reversed also by addition of human serum albumin

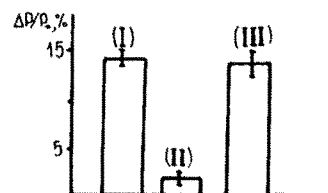


Fig.3. Changes of the fluorescence polarization of ASM-labeled HDL₂. I, 4×10^{-11} M PGE₁; II, sample I after dialysis against 0.9% NaCl, solution pH = 7.4 (20 h, 36.5°C); III, sample II after addition of 4×10^{-11} M PGE₁. Averaged results of three experiments are shown.

which effectively binds prostaglandins [8]. Interestingly, removal of PGE₁ from the HDL surface by addition of albumin resulted in a relatively fast decrease of *E* and a much slower relaxation of *P* (fig.4). This difference may be explained by the fact that energy transfer occurs only to probe molecules which are in the nearest vicinity of the apoprotein tryptophans, whereas changes in *P* involve the cooperative interaction of many lipid molecules.

The influence of PGE₁ on the fluorescence of ASM-labeled HDL is highly specific. Addition of PGE₂ to such HDL induced no changes in the fluorescence parameters and did not interfere with the action of PGE₁ (fig.5).

Recently it was shown that PGE₁ does not readily enter the lipid bilayer [9]. The high-selective sensitivity of the HDL structure towards PGE₁ and the absence of any effect of PGE₁ on the fluorescence of probes I and II incorporated into lipid vesicles indicate that PGE₁ binds to HDL through the apoproteins. At the same time, however, neither the CD nor the inherent fluorescence of unlabeled HDL changed upon addition of PGE₁. Apparently the fluorescence parameters of lipid-specific probes are more sensitive to certain changes of the apoproteins than CD or inherent protein fluorescence.

Which of the apoproteins is responsible for binding of PGE₁ remains to be shown. ApoA₁ seems to be a likely candidate because of the following: (i) Addition of PGE₁ to fluorescent

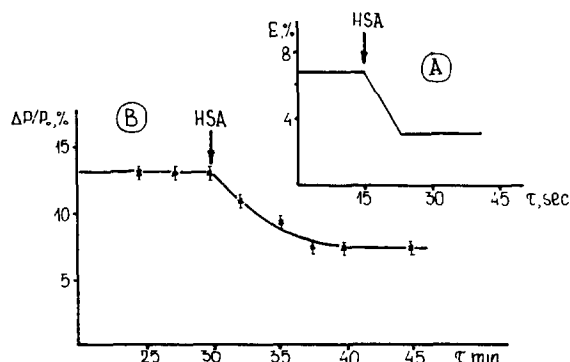


Fig.4. Influence of human serum albumin (HSA) ($\sim 1 \times 10^{-6}$ M) on the fluorescence parameters of ASM-labeled HDL₂ after addition of 2×10^{-9} M PGE₁. A, resonance energy transfer from tryptophanys of the apoproteins to the ASM. B, fluorescence polarization of the probe.

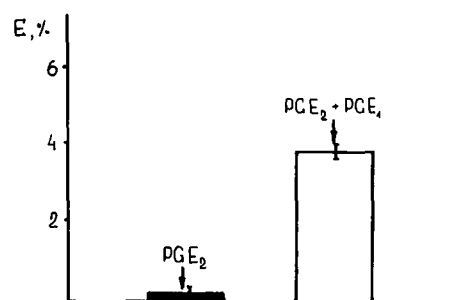


Fig.5. Influence of PGE₁ and PGE₂ on the *E* values of ASM-labeled HDL₂. I, PGE₂ (9.3×10^{-9} M) was added to the sample and the mixture was incubated 30 min at 36.5°C; II, sample I after addition of the same concentration of PGE₁ and 30 min incubation.

labeled HDL induced an increase of resonance energy transfer from apoprotein tryptophanys to the sphingomyelin probe II, but not to the fluorescent phosphatidylcholine I. It has been shown previously that the most probable donor in this energy transfer was apoA₁ [4]. (ii) After dilution of the ASM labeled HDL suspension (down to 0.01 mg/ml) no tryptophanyl-to-probe energy transfer is observed and addition of PGE₁ caused no change in the *P* value of the sample; at the same time the fluorescence intensity of the probe per mg protein was not altered upon dilution of the sample.

The disappearance of the effect of PGE₁ under these conditions could be explained by the fact that upon dilution apoA₁ dissociates from the HDL surface [10–12].

In summary, the results of the present study demonstrate for the first time that prostaglandin E₁ interacts with high density lipoproteins in a manner which resembles the interaction of a ligand with a high affinity binding site, because this interaction is specific, saturable, apparently reversible, time and temperature dependent. However, the action of PGE₁ on HDL differs from classical ligand–receptor interactions because one PGE₁ molecule induces structural changes in a large number of lipoprotein globules. At the moment, we have no fully adequate explanation for this unusual phenomenon, but we believe it to be related to the short lifetime of the PGE₁–HDL complex and relatively long relaxation times of the structural changes in the molecular organization of the lipids on the HDL surface (fig.4).

The physiological and pathological implications of the observations of the present study currently remain uncertain. It is tempting to speculate that the structural reorganization of HDL induced by PGE₁ may materially alter their biological function, specifically their influence on the activity of lecithin-cholesterol-acyltransferase in vitro. The latter possibility is currently under investigation.

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