Interaction of prostaglandin E_1 with human high density lipoproteins

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Prostaglandin E_1 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 E_1 induces a rearrangement of the HDL surface lipids and probably influences the biological functions of the lipoproteins.

Fluorescent phospholipid probe *High density lipoprotein* *Prostaglandin E_1*

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 E_1 and E_2 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 PGE_1 binds in a highly specific manner to HDL and that the molecular organization of the latter changes in the presence of physiological concentration of PGE_1.

2. MATERIALS AND METHODS

HDL fractions (HDL_2 and HDL_3) 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

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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 PGE_1 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 PGE_1 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 PGE_1 to ASM-labeled HDL resulted also in an increase of tryptophanyl to probe resonance energy transfer (E). The PGE_1 concentration dependence of P and E were similar (fig.2). The sensitivity of the fluorescence parameters of ASM-labeled HDL to the action of PGE_1 was extremely high: the minimal PGE_1 concentration inducing measurable changes of P and E was $\sim 10^{-12}$ M (fig.2). Such a concentration corresponds to one PGE_1 molecule per 10^3–10^4 HDL globules. The saturation concentration, i.e., the PGE_1 concentration inducing maximal changes of P and E, was $\sim 10^{-10}$ M. The stability of the PGE_1–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 PGE_1 appeared to be reversible: after separation of PGE_1 from the lipoproteins the fluorescence polarization returned almost to the initial level and after addition of a new portion of PGE_1 the P value increased again (fig.3). The effect of PGE_1 on the fluorescence parameters of ASM-labeled HDL could be reversed also by addition of human serum albumin

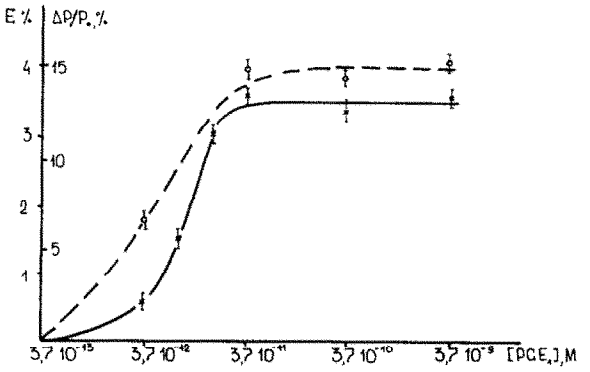

Fig.2. Dependence of $\Delta P/P_o$ (×) and E (○) of ASM labeled HDL_2 upon the concentration of PGE_1. $E = (F_n^{PG} - F_n^o)/F_n^o$, where F_n^o and F_n^{PG} are parameters of resonance energy transfer before and after addition of PGE_1, respectively (for definition of F_n, see [4]). In all cases incubation time after addition of the PGE_1 was 30 min. For other experimental conditions see legend to fig.1.

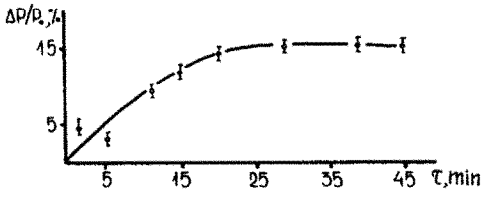

Fig.1. Time dependence of the PGE_1-induced fluorescence polarization changes of ASM-labeled HDL_2 at 36.5°C. Protein concentration 1.54 mg/ml; sample volume 0.6 ml; PGE_1 concentration 3.7×10^{-9} M; $\Delta P = P_{PG} - P_o$, where P_o and P_{PG} are the fluorescence polarization values before and after addition of PGE_1, respectively. Averaged results of three experiments are shown.

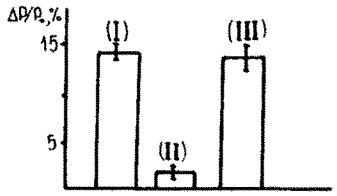

Fig.3. Changes of the fluorescence polarization of ASM-labeled HDL_2. I, 4×10^{-11} M PGE_1; II, sample I after dialysis against 0.9% NaCl, solution pH = 7.4 (20 h, 36.5°C); III, sample II after addition of 4×10^{-11} M PGE_1. Averaged results of three experiments are shown.

which effectively binds prostaglandins [8]. Interestingly, removal of PGE_1 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 PGE_1 on the fluorescence of ASM-labeled HDL is highly specific. Addition of PGE_2 to such HDL induced no changes in the fluorescence parameters and did not interfere with the action of PGE_1 (fig.5).

Recently it was shown that PGE_1 does not readily enter the lipid bilayer [9]. The high-selective sensitivity of the HDL structure towards PGE_1 and the absence of any effect of PGE_1 on the fluorescence of probes I and II incorporated into lipid vesicles indicate that PGE_1 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 PGE_1. 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 PGE_1 remains to be shown. $ApoA_1$ seems to be a likely candidate because of the following: (i) Addition of PGE_1 to fluorescent

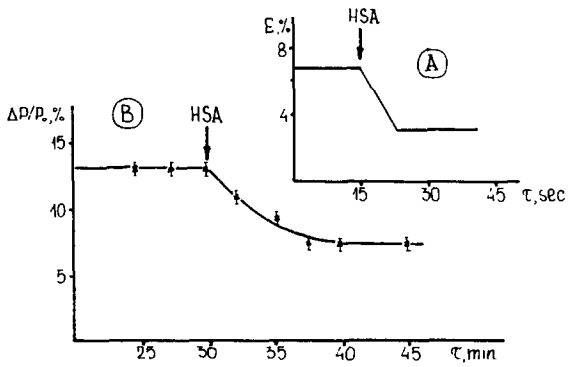

Fig.4. Influence of human serum albumin (HSA) (~1 × 10^{-6} M) on the fluorescence parameters of ASM-labeled HDL_2 after addition of 2×10^{-9} M PGE_1. A, resonance energy transfer from tryptophanyls of the apoproteins to the ASM. B, fluorescence polarization of the probe.

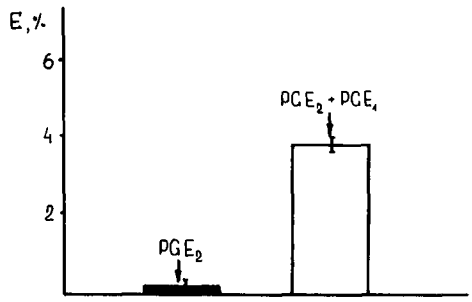

Fig.5. Influence of PGE_1 and PGE_2 on the E values of ASM-labeled HDL_2. I, PGE_2 (9.3×10^{-9} M) was added to the sample and the mixture was incubated 30 min at 36.5°C; II, sample I after addition of the same concentration of PGE_1 and 30 min incubation.

labeled HDL induced an increase of resonance energy transfer from apoprotein tryptophanyls 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 $apoA_1$ [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 PGE_1 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 PGE_1 under these conditions could be explained by the fact that upon dilution $apoA_1$ dissociates from the HDL surface [10–12].

In summary, the results of the present study demonstrate for the first time that prostaglandin E_1 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 PGE_1 on HDL differs from classical ligand–receptor interactions because one PGE_1 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 PGE_1–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 PGE_1 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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