INVESTIGATION OF THE SURFACE STRUCTURE OF THE VERY LOW DENSITY LIPOPROTEIN USING FLUORESCENCE PROBES

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Received 30 October 1978

1. Introduction

The metabolism of the serum lipoproteins may be a function of their composition and structure, which can be altered under different nutritional and pathological conditions. It has been shown that the association of VLDL and HDL apoproteins with lipid are dependent upon the fluidity of the lipid [1–5]. In addition, the ability of the hydrolytic enzyme lipoprotein lipase to hydrolyze its substrate, VLDL, may be dependent on the physical state of the surface lipid [6]. To elucidate the surface structure of VLDL, we have utilized the fluorescence probes β-parinaric acid (all *trans*-9,11,13,15-octadecatetraenoic acid) (β-P) and diphenylhexatriene (DPH), a new quenching agent trinitrophenylglycine (glycine-TNP), and a computer-centered spectrofluorimeter. The fluorescent probe molecules β-parinaric acid and 1,6-diphenyl-1,3,5-hexatriene were incorporated into the very low density lipoprotein (VLDL) enriched with oleate by perfusion of the isolated rat liver in vitro. The fluorescence emission of both probe molecules overlapped with the absorption spectrum of trinitrophenylglycine. Trinitrophenylglycine maximally quenched fluorescence of β-parinarate and diphenylhexatriene in VLDL, 94% and 20%, respectively. Thus, trinitrophenylglycine did not penetrate the surface of the VLDL and appeared to quench only those diphenylhexatriene molecules near the surface. In contrast, the almost complete quenching of β-parinarate indicated that the β-parinarate was exclusively located in the surface. The results were consistent with the existence of monolayer surrounding an interior core structure for VLDL.

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2. Materials and methods

The fluorescence probes β-parinaric acid and 1,6-diphenyl-1,3,5-hexatriene were obtained from Molecular Probes Inc., Roseville, MN, and from Aldrich Chemical Co., Milwaukee, WI. Trinitrophenylglycine (glycine-TNP) was synthesized from 2,4,6-trinitrobenzenesulfonate and glycine (Sigma Chemical Co., St Louis, MO) as in [7]. The very low density lipoprotein was isolated from the medium following perfusion of the rat liver in vitro. The liver was perfused with a blood-free medium containing purified bovine serum albumin bound to oleate as in [8,9].

The fluorescence probe molecule, β-parinaric acid was incorporated into the VLDL (50 μg lipid/ml phosphate-buffered 0.9% NaCl, pH 7.4) as in [10]. The 1,6-diphenyl-1,3,5-hexatriene (0.5 μg/ml) was incorporated into the VLDL (50 μg lipid/ml) resuspended in phosphate-buffered saline, (pH 7.4) as in [11]. The computer-centered spectrofluorimeter in [12,13] was used to measure absorbance, absorbance corrected fluorescence (corrected for the inner filter effect), relative fluorescence efficiency, corrected fluorescence emission, and light scattering corrections as in [14,15]. Excitation for β-parinaric acid and 1,6-diphenylhexatriene was measured at 313 nm and 362 nm, respectively, while emission was measured at 420 nm and 424 nm, respectively.

3. Results and discussion

Fluorescence probe molecules are used as reporter groups in membrane biology. However, the locations of probe molecules are understood in a qualitative sense only. Spin-labelled and fluorescent-labelled fatty acids are located in the surface of HDL, LDL or mammalian membranes with their carboxyl ends at the water–membrane lipid interface [16,17]. Thus, β-parinaric acid would be expected to orient in a similar fashion in VLDL. In contrast, 1,6-diphenyl-1,3,5-hexatriene has been used to measure 'core properties' of lipoproteins [11]. To determine the location ('surface' or 'core') of these probes we utilized a novel chemical quenching reagent glycine-TNP. As shown in fig. 1, trinitrobenzenesulfonate (TNBS) has an A_{340} max. Upon reaction with the free amino group of glycine, the A_{340} increases and a new A_{420} max appears. Since the latter absorption peak overlaps with the fluorescence emission of β-parinaric acid in the VLDL and since glycine-TNP itself does not fluoresce, this molecule should be an excellent quencher of β-parinaric acid fluorescence if the following conditions are also met:

(i) The β-parinaric acid is located close to the surface of the VLDL such that quenching can occur. Energy transfer quenching is proportional to r^{-6} where r represents the intermolecular distance [18].

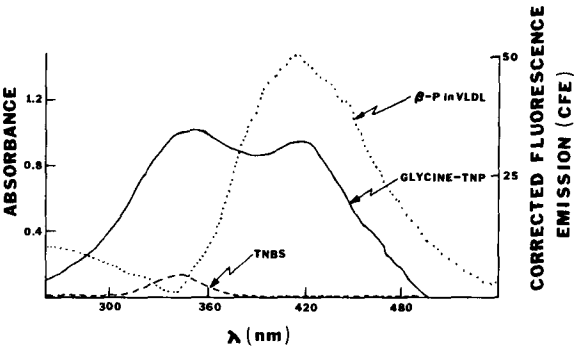

Fig.1. Overlap of glycine-TNP absorption with β-parinaric acid fluorescence emission. All conditions were as in section 2. (- - - -) is 4 mM 2,4,6-trinitrobenzenesulfonic acid in PBS; (——) is 72 μM glycine-TNP in PBS; (· · · · ·) is the absorption spectrum (260–340 nm) and corrected fluorescence emission spectrum (340–540 nm) of β-parinarate in VLDL, 0.38 μg/50 μg lipid/ml PBS.

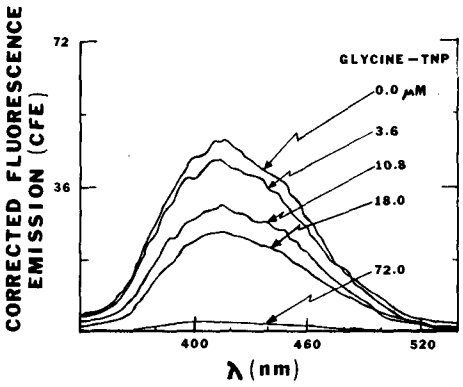

Fig.2. Effect of glycine-TNP on shape of β-parinarate corrected fluorescence emission spectrum. All curves represent β-parinarate fluorescence emission in VLDL in the presence of increasing concentrations of glycine-TNP.

(ii) The quenching is non-trivial; that is, it must not be reabsorption of emitted light.

(iii) Glycine-TNP does not penetrate into the VLDL 'interior core'.

Condition (ii) can easily be satisfied as follows. Figure 1 indicates that if non-trivial quenching occurs, then the left side of the β-parinarate emission peak should be quenched more than the right side. However, as shown in fig.2 the shape of the fluorescence curve does not change with increasing concentration of quencher. Condition (i) is met as shown in fig.2,3.

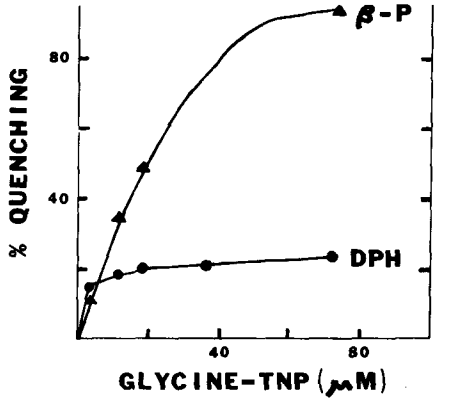

Fig.3. Quenching of β-parinaric acid (β-P) 1,6-diphenyl-1,3,5-hexatriene fluorescence (DPH) in VLDL by glycine-TNP. β-P or DPH were incorporated into VLDL as in section 2. Quenching of corrected fluorescence emission was measured in the presence of increasing concentrations of glycine-TNP.

The fluorescence of β-parinarate is maximally quenched at 72 M glycine-TNP (94% quench). Thus, all of the β-parinarate must be located in one monolayer of a bilayer surrounding the VLDL triglyceride core or the surface of the VLDL may be a monolayer. The former possibility is unlikely since if the quencher is located only on one side of a bilayer membrane (LM cell plasma membrane) then only 45% of the β-parinarate fluorescence is quenched (F. S. (1979) Nature, in press). This would indicate approximately equal partitioning of β-parinarate across both monolayers of a bilayer, a process that occurs within 1–3 min [19]. The $C_{1/2}$ (the concentration of quencher needed for 50% decrease in fluorescence yield) of glycine-TNP for β-parinarate in VLDL is ~20 μM. Condition (iii) is met by the fact that at equivalent concentrations of glycine-TNP (72 μM), the fluorescence of 1,6-diphenyl-1,3,5-hexatriene in VLDL is maximally quenched only ~20%. If either β-parinarate or 1,6-diphenyl-1,3,5-hexatriene are dissolved in ethanol and increasing amounts of glycine-TNP are added, then fluorescence was quenched ~95% in both cases. This indicates that the 20% maximal quench of 1,6-diphenyl-1,3,5-hexatriene fluorescence in the VLDL is not due to lower inherent quenching efficiency of 1,6-diphenyl-1,3,5-hexatriene by glycine-TNP. 1,6-Diphenylhexatriene is located in the interior core of lipoproteins [11] and only a fraction would be expected to be near the surface and available for quenching by glycine-TNP if the quencher did not penetrate into the interior. However, if glycine-TNP penetrated into the interior it should quench with high efficiency. Thus since it does not quench 1,6-diphenyl-1,3,5-hexatriene fluorescence more than 20%, the glycine-TNP appears not to penetrate the VLDL surface. Therefore, the results obtained here with β-parinaric acid and 1,6-diphenyl-1,3,5-hexatriene quenching are more consistent with a monolayer surface structure of the VLDL. A monolayer structure for the VLDL surface was first proposed on the basis of chemical composition [20,21]. The results presented herein represent experimental verification consistent with the theoretical calculations. Such a monolayer would be composed of sterols, apoproteins, free fatty acids and phospholipid. Similar findings obtained by electron microscopy of chylomicrons also indicate a surface monolayer structure for chylomicrons [22,23].

Acknowledgements

This work was supported by a grant from the American Heart Association-Missouri Affiliate, the American Heart Association (AHA78-734), and by a grant from the National Institutes of Health (AM18125). We wish to also express our gratitude to Dr J. F. Holland of Michigan State University for the use of his computer-centered spectrofluorimeter.

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