# THE LOCATION OF APOPROTEIN IN PLASMA HIGH-DENSITY LIPOPROTEINS: PHOTOCHEMICAL LABELLING STUDIES

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## 1. Introduction

In a convincing model [1] of human high-density lipoprotein (HDL) structure, it is proposed that the apoproteins have a predominantly surface location. While supporting evidence for such an arrangement exists [2], models involving a more extensive penetration of the lipid core of HDL have been described [3]. If photochemical labelling experiments from within the lipid core [4] of HDL could be conducted, a minimal degree of labelling of apoprotein would be expected if the model described in [1] proved realistic. We have carried out such experiments using $^3$H-labelled phenylnitrene photo-generated from phenylazide as a non-specific reagent. The results obtained, taken in conjuction with parallel data obtained with membrane systems where penetration of the lipid region by protein is well documented, point to a predominantly surface location of apoproteins in both human and bovine plasma HDL.

## 2. Materials and methods

Human and bovine HDL were prepared by flotation centrifugation of sera at densities of 1.125 g ml$^{-1}$, adjusted with NaBr. Purification was achieved by recentrifugation. Disc-gel/electrophoresis showed no contamination with plasma albumin or other lipoprotein fractions, while the sub-unit structure, established by disc-gel electrophoresis in the presence of SDS, was in agreement with published data [5,6]. Lipid:protein ratios, determined on a dry weight basis after a standard delipidation procedure [7], were 49:51 and 68:32 for human and bovine HDL respectively. Erythrocyte ghosts and sarcoplasmic reticulum residues were prepared as described [8,9].

[$^3$H]Phenyl azide was prepared from 2,3,4,5,6-[$^3$H]aniline (Radiochemical Centre) specific activity 150 mCi/mM [$^3$H]aniline (5 mCi), diluted with [$^1$H]aniline (93 mg) was dissolved in 1 M HCl (50 ml) and diazotised with $NaNO_2$ (80 mg) at 4°C. $NaN_3$ (100 mg) was the added with stirring at 4°C. After 30 min, [$^3$H]phenyl azide was extracted into ether, and purified by TLC (Silica Gel G, $CHCl_3$ solvent). The product, obtained in 95% yield, had specific activity $1.35 \times 10^{12}$ dpm M$^{-1}$, was radiochemically pure (TLC), and gave ultraviolet and infrared spectra identical to those of authentic phenylazide. A stock solution ($1.4 \times 10^{-1}$ M in ethanol) was stable indefinitely at 4°C in the dark.

HDL and membrane preparations in 0.05 M phosphate, pH 7.3, were pre-incubated in the dark at 23°C with phenylazide ($3 \times 10^{-3}$ M) for at least 4 days. The protein concentration in all samples was adjusted to 5 mg ml$^{-1}$. Excess phenylazide was removed by dialysis against excess phosphate buffer in the dark for at least 24 h, and the radioactivity remaining was taken to represent phenylazide incorporated into the lipid 'core' of the samples.

Irradiations were conducted with a Hanovia 500 W medium pressure mercury lamp. Samples were flushed with $N_2$, and irradiated for 30 min in stoppered Pyrex tubes 10 cm from the light source. Control experiments showed complete decomposition of phenylazide in this time interval.

After exhaustive dialysis, lipoproteins were fractionated into lipid and protein as described [7], while the membrane preparations were extracted with chloroform/methanol (2:1;v/v). Aliquots of lipid and protein fractions were assayed for radioactivity using a dioxane-based scintillation mixture.

## 3. Results and discussion

The relative incorporation of $^3H$ into total lipid, and protein fractions after irradiation is indicated in table 1. Figures in brackets indicate the relative labelling obtained without the preliminary dialysis procedure to remove excess phenylazide: under these condtitons phenyl nitrene would also be generated in the aqueous phase. All samples were dialysed exhaustively before fractionation: the protein fractions retained radioactivity even after exhaustive treatment with the delipidation solvents. Saponification of the total lipid extracts showed up to 60% of radioactivity in the fatty acid fraction. The final figures thus refer to covalently incorporated label. This incorporation was strictly light-dependent, and the observed ratios proved highly reproducible.

Table 1

| Sample | Total radioactivity (dpm) incorporated into | |
|---|---|---|
| | Protein | Lipid |
| Human HDL | $4.8 \times 10^3$ | $2.3 \times 10^5$ |
| | $(5.4 \times 10^5)$ | $(5.6 \times 10^5)$ |
| Bovine HDL | $4.4 \times 10^3$ | $1.05 \times 10^5$ |
| | $(3.0 \times 10^5)$ | $(7.0 \times 10^5)$ |
| Erythrocyte ghosts | $2.0 \times 10^4$ | $8.0 \times 10^4)$ |
| | $(7.0 \times 10^4)$ | $(1.3 \times 10^5)$ |
| Sacroplasmic reticulum | $2.0 \times 10^4$ | $8.5 \times 10^4$ |
| | $(6.0 \times 10^4)$ | $(1.2 \times 10^5)$ |

Samples were equilibrated with [$^3$H]phenyl azide, subjected to the dialysis-irradiation-dialysis sequence described in the text, and finally fractionated into lipid and protein. Bracketed values refer to experiments in which the first dialysis step was omitted, and thus contain excess phenyl azide during irradiation. All values represent means of 4 separate irradiation experiments.

Both erythrocyte ghosts and sarcoplasmic reticulum vesicles are known to contain protein(s) which penetrate the lipid bilayer [10,11]. Table 1 indicates the (~20%) significant partitioning of phenylnitrene towards protein in these systems. A similar result has been reported with sarcoplasmic reticulum [4]. Both lipoproteins, however show minimal labelling of apoprotein by phenylnitrene generated within the lipid cores. That the apoproteins can indeed react with phenylnitrene is shown by the heavy labelling obtained when exess phenyl azide is not removed prior to irradiation. Our results thus suggest a predominantly surface location of the HDL apoproteins: a significant penetration of the lipid core by these proteins would have resulted in their labelling with phenylnitrene. This conclusion is in line with results obtained in this laboratory on hydrogen-tritium exchange rates for bovine HDL: the exchange-in and exchange-out times for apparently complete exchange, are of the order of 5 min. This result would point to the surface location of the majority of exchangeable protons in HDL.

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