ISOLATION OF APOLIPOPROTEIN B FROM THE PLASMA OF THE DOGFISH, SQUALUS ACANTHIAS

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Lipid transport and metabolism is a dominant feature of elasmobranch physiology yet few studies have focused on this aspect of elasmobranch function. In mammalian studies, Brown and Goldstein (Science 232:34, 1986) established that the main protein component of very low density and low density lipoprotein (VLDL, LDL) fractions is apoprotein B-100, a 450 kD protein. This protein functions in the transport of the hydrophobic lipid moieties and in ligand-receptor interactions essential for lipid entry into cells. Non-mammalian species which are heavily dependant upon lipid metabolism and in which the process of yolk precursor synthesis is a major hepatic metabolic pathway, such as elasmobranchs, provide excellent models for the study of hormone regulated lipoprotein metabolism. In an earlier study Mills et al (Biochem J. 163:455, 1977) conducted a careful examination of the lipids of a shark (Centrophorus squamosus) and tentatively identified a large protein moiety of VLDL and LDL as the homologue of mammalian apolipoprotein B. As part of a research program aimed at understanding the hormonal regulation of lipoprotein synthesis and function in non-mammalian species, we sought to verify and extend the observations of Mills et al (ibid, 1977) using a readily available species, Squalus acanthias.

Dogfish were caught off the coast of Maine and maintained in flow-through circulating sea-water tanks at ambient water temperatures during July and August, 1991. Blood (10 ml) was obtained by caudal puncture and centrifuged in the presence of the protease inhibitor PMSF. The plasma was centrifuged in salt solutions sufficient to obtain VLDL (density less than 1.007 g/ml), LDL (density 1.007 - 1.065 g/ml) and HDL (density 1.065-1.21 g/ml) in an ultracentrifuge as described by Mills et al (ibid, 1977). Fractions were verified by electron microscopy. Aliquots of the isolated fractions were electrophoresed (6.0% SDS-PAGE) and transferred to nitrocellulose filters by semi-dry blotting techniques in methanol-Tris:SDS buffer. The blots were probed with a 1:1000 dilution of anti-chicken apoprotein B antibody (courtesy of Dr. D. Williams). Two sharp bands appeared on the blots, the first migrating with an approximate molecular weight of 350 kD, the second of about 50 kD. The high molecular weight protein (350 kD) corresponds well to what has been shown previously for avians (360 kD; Gehrke, L., Bast, R.E., and Iland, J. (1981) JBC 256:2522). However, the identification of the lower molecular weight component (50 kD) is not known at the present time. It is possible that this protein could be a homolog to apo B48, a truncated hepatic/intestinal version of apolipoprotein B100, or a breakdown product. It is interesting that this protein does not appear in the HDL ultracentrifugal fraction.

The results verify the work of Mills et al (ibid, 1977) and further identify the major protein component of elasmobranch VLDL and LDL as apoprotein B. It appears that common mechanisms for transport and presumably cellular uptake of lipids exist from mammals to elasmobranchs; further, the apoprotein B molecule appears to be quite conserved on the basis of the cross-reactivity of the elasmobranch protein with an antibody to chicken apoprotein B. These studies will facilitate future work on the regulation of this protein by hormones and its role in the uptake of lipids into specific cells, particularly the oocyte.

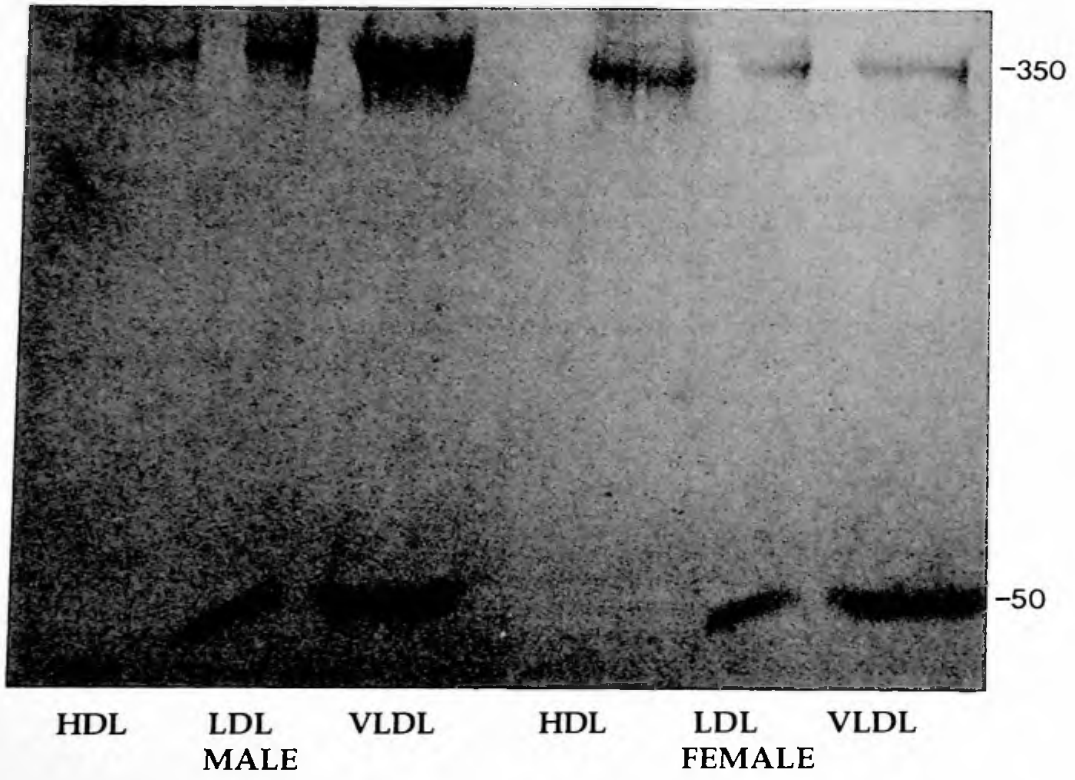

Figure 1 - Western blot of male and female dogfish (Squalus acanthias) isolated lipoproteins. Male and female dogfish plasma was ultracentrifuged to obtain several lipoprotein density classes. The lipoproteins were separated on 6.0 % SDS-PAGE, transferred to nitrocellulose membranes and probed with rabbit anti-chicken apolipoprotein B100 antibody. Two proteins, approximately 350 kD and 50 kD respectively, were isolated. VLDL = very-low density lipoprotein; LDL = low density lipoprotein; HDL = high density lipoprotein.

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