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The intramembranous domains of lipophilin in phosphatidylcholine vesicles are similar to those in the myelin membrane

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A membrane-permeable photolabel 3-(trifluoromethyl)-3-(*m*-[^{125}I]iodophenyl)diazirine (^{125}I-TID) has been used to label lipophilin in normal human myelin and after incorporation of purified lipophilin into phosphatidylcholine (PC) vesicles. The labelled protein was isolated and the specific activities for lipophilin from myelin and from PC vesicles was found to be $1.2 \cdot 10^{11}$ and $1.5 \cdot 10^{11}$ cpm/mol, respectively. The chromatographic profiles of tryptic peptides were similar in both cases and the specific activities of the C-terminal intramembranous fragments (residues 205–268) the same. We concluded that the organization of lipophilin in PC vesicles was similar to its organization in myelin and that the PC-vesicle system represents a good system in which to study the orientation and interaction of lipophilin with lipids.

Normal human myelin contains two major proteins and several minor proteins (see Ref. 1 for recent review of the proteins). One of these, lipophilin (a proteolipid type), comprises 50% of the total myelin protein. It has been isolated, purified [2] and incorporated into lipid vesicles [3]. The protein so incorporated into lipid vesicles has been used as a model membrane system for the orientation of this protein in the normal human myelin membrane. An extensive review of these model membrane studies has been published [4].

Although it appeared to be a good model system for the disposition of the protein in myelin, definite experimental evidence has only recently been obtained. With the elucidation of the primary structure of lipophilin by Stoffel et al. [5] it became possible to identify isolated peptide sequences from myelin with those known to be present in lipophilin. The development of a membrane permeable photolabel 3-(trifluoromethyl)-3-(*m*-[^{125}I]iodophenyl)diazirine (^{125}I-TID) by Brunner et al. [6] provided us with a radioactive label with which the purification of the intramembranous peptides could be followed easily. With the use of this photolabel we demonstrated that it represented an excellent method by which to label the intramembranous domains of lipophilin in normal human myelin [7]. In the present manuscript we demonstrate that the orientation of lipophilin in vesicles consisting of phosphatidylcholine (PC) and lipophilin is similar to the orientation of the protein in the human myelin membrane. We concluded that the vesicle system of PC and lipophilin is a good model system in which to study the interactions of this protein with lipids. The data obtained from such studies have direct application to understanding the disposition of lipophilin in myelin.

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Myelin was isolated from normal human white matter by the method of Lowden et al. [8]. Radioactively labelled myelin was prepared as described earlier [7]. For the isolation of tryptic peptides, a small amount of ^{125}I-TID labelled myelin ($1.44 \cdot 10^6$ cpm) was added to 30 mg of non-radioactive myelin.

The preparation and labelling of vesicles with [^{125}I]-TID. Lipophilin was incorporated into phosphatidylcholine (PC) vesicles containing 31% protein by the 2-chloroethanol method of Boggs et al. [3]. The procedure used to label PC vesicles containing lipophilin was identical to the one described for myelin [7].

The isolation of lipophilin and tryptic fragments. Lipophilin was isolated from both myelin and PC vesicles by the method of Gagnon et al. [2]. Tryptic fragments of myelin and PC vesicles were obtained by the procedure of Kahan and Moscarello [7]. Amino acid analysis was done on 50 pmoles of protein or peptide on a Water's Pico Tag system after hydrolysis in 5.7 M HCl in gas phase for 24 h. Peptides were sequenced in a Beckman 890M sequencer.

Phosphatidylcholine was obtained from Avanti Biochemicals, Birmingham, AL. Sephadex LH-20 and LH-60 were obtained from Pharmacia, Sweden. 3-(Trifluoromethyl)-3-(*m*-[^{125}I]iodophenyl)diazirine (^{125}I-TID) was purchased from New England Nuclear.

Time-course of labelling of myelin and phosphatidylcholine vesicles by ^{125}I-TID. In order to determine the time-course of labelling of myelin and phosphatidylcholine (PC) vesicles with ^{125}I-TID, the membrane suspensions were photolysed for periods of time varying from 0 to 2 min as described earlier [7]. After each time of photolysis 50 μl of labelled membrane suspensions were transferred to 1.95 ml of wash solution containing bovine serum albumin. The samples were centrifuged and washed three more times with buffer. Since a small amount of radioactivity was always bound to the vesicles in the absence of photolysis, this amount was subtracted from the cpm obtained at each of the experimental points. The data are shown in Fig. 1A. Although more rapid labelling was observed for the myelin samples at the early times, maximum labelling was obtained in both cases within 1–2 min. In repeat experiments these data were reproducible to within $\pm$2–4%.

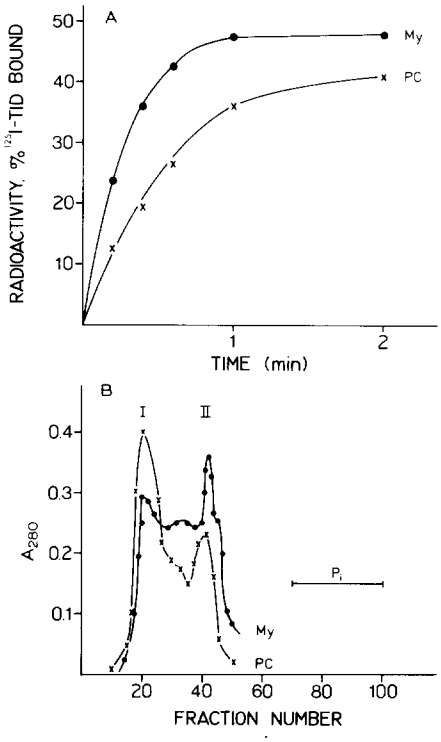

Fig. 1. (A) Time-course of binding of ^{125}I-TID to phosphatidylcholine vesicles containing 31% protein (0.38 mg lipophilin/0.8 mg PC). A total of 30 mg of vesicles in 0.3 ml was incubated with $4.5 \cdot 10^6$ cpm ^{125}I-TID and irradiated for various times. At each time shown, 50 μl of the vesicle suspension was removed, washed with 3×500 μl of vesicle buffer and counted. The amount of radioactivity bound to the vesicles without photolysis (time zero) was subtracted from the amount bound after photolysis for various times. The amount of radioactivity is expressed as a percentage of the total radioactivity original present in each sample. (B) Sephadex LH-60 gel filtration of ^{125}I-TID labelled peptides, generated by tryptic digestion of 30 mg of myelin (My, ●) and phosphatidylcholine vesicles (PC, ×). The digest was reduced and alkylated, extracted with acidified chloroform/methanol, concentrated and chromatographed on a Sephadex LH-60 (2.5×75 cm) column. The column was eluted with a flow rate of 5.6 ml/h. Fractions of 2.8 ml were collected and counted for ^{125}I and absorbances at 280 nm were recorded. The fractions under peaks I and II were pooled and concentrated by rotary evaporation for further purification [7] P_i indicates the phosphorus-containing fractions. The radioactive profile which was superimposable on the protein profile is not shown.

When lipophilin was isolated from both vesicle suspensions by the method of Gagnon et al. [2], the specific activities were $1.27 \cdot 10^{11}$ cpm/mol

TABLE I

SPECIFIC ACTIVITIES OF LIPOPHILIN AND THE C-TERMINAL FRAGMENT

Lipophilin was isolated from myelin and from phosphatidylcholine vesicles by the method of Gagnon et al. [2]. An aliquot was used to measure radioactivity and a second aliquot was used for amino acid analysis. The C-terminal tryptic fragment was isolated from the Sephadex LH-60 column as described by Kahan and Moscarello [7]. The specific activity was obtained as described for the intact protein.

	Specific activity (cpm/mol)	
	Myelin	PC vesicles
Lipophilin	$1.27 \cdot 10^{11}$	$1.5 \cdot 10^{11}$
C-terminal fragment (205–268)	$3.1 \cdot 10^{10}$	$2.8 \cdot 10^{10}$

and $1.5 \cdot 10^{11}$ cpm/mol for lipophilin from myelin and PC vesicles, respectively (Table I), demonstrating that the extent of labelling of lipophilin in both vesicle systems was similar.

The isolation of tryptic peptide fragments from myelin and PC vesicles. Myelin and PC vesicles labelled with ^{125}I-TID were digested with trypsin and the resulting digest was reduced and alkylated prior to fractionation on a Sephadex LH-60 column as described earlier by Kahan and Moscarello [7]. The chromatograms obtained from tryptic fragments from both vesicle systems were similar and are shown in Fig. 1B. Both digests were resolved into two major peaks I and II although some material eluting in fractions 32–37 in the myelin samples was less pronounced in the vesicle samples and probably arose from other proteins in myelin. The fractions under peaks I and II were pooled and two peptides representing and N-terminal fragment (from peak I) containing residues 1–49 and a C-terminal peptide (from peak II) containing residues 205–268 were isolated as described earlier [7]. The identity of the peptides under peaks I and II was confirmed by N-terminal amino acid sequence analyses (Table II).

The specific activities of the C-terminal peptides isolated from lipophilin in myelin and PC vesicles are shown in Table I. The specific activities are almost identical demonstrating that the extent of intramembranous localization of this peptide was the same in both cases.

TABLE II

N-TERMINAL AMINO ACID SEQUENCES OF THE FRAGMENTS UNDER PEAKS I AND II FROM SEPHADEX LH-60 COLUMN

Peak	Myelin	PC vesicles
I	Gly-Leu-Leu-Glu-Cys-	Gly-Leu-Leu-Glu-
II	Met-Tyr-Gly-Val	Met-Tyr-Gly-Val

The data in the present investigation demonstrated that the orientation of lipophilin in PC vesicles represents a good model system in which to study the orientation of lipophilin in myelin. This is based on the following evidence. After labelling with ^{125}I-TID, the specific activity of lipophilin isolated from PC vesicles was the same as that of lipophilin isolated from myelin (Table I). The chromatographic profile (Fig. 1B) of the tryptic digest of lipophilin in PC vesicles was similar to that of lipophilin in myelin. Two major peaks were obtained in each case, one contained the N-terminal peptide (residues 1–49) while the other contained the C-terminal peptide (residues 205–268). The C-terminal peptide from each digest was purified and the specific activity of the peptide from PC vesicles was found to be the same as that from myelin vesicles. Although we have not isolated the other peptides, the fact that the specific activities of the protein from both vesicle systems were the same and the specific activities of the C-terminal peptides were the same indicates that the intramembranous organization of this protein is similar in myelin and PC vesicles insofar as this can be determined by this method. Since the PC-vesicle system represents a good model system for studying protein–lipid interactions, the data can be useful to understanding the orientation of lipophilin in the human myelin membrane.

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