Study on the composition-structure relationship of lipophorins

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Abstract High density lipophorin ($HDL_p$is the main lipoprotein found in resting insect hemolymph. It has, in general, two molecules of apolipoproteins: apoLp-I (250 kDa) and apoLp-II (80 kDa) and a variable lipid content which ranges from 35% to 59% (w/w). Diacylglycerols (DG), phospholipids (PL), and hydrocarbons (HC) are the main lipid components, whereas cholesterol and triacylglycerols are minor components. DG content varies from 7 to 30%, PL from 11 to 24%, and HC from 0 to 15%. In order to determine the relationship between the lipid composition and the arrangement of lipid and protein components in the lipoprotein particle, a density-composition structural model was designed. The model was established by means of 12 sets of data on lipophorin density-composition relationships, and model validity was determined throughout lipoprotein space- and surface-filling conditions. Despite the differences among the lipid compositions of lipophorins, it is concluded that there are several unifying structural restrictions that govern the molecular organization of lipophorins. Quantitative treatment of the model indicates that lipophorin structure is consistent with the following. *1*) Spherical particles with a protein-rich outer layer of approximately 20-21 Å thickness, comprised of proteins, phospholipids, cholesterol, and small amounts of DG, and a lipid-rich core composed of HC, TG, and almost all the lipophorin DG. *2*) Apolipophorins have a lipid-embedded localization within the lipoprotein particle. They might represent one of the few examples of proteins containing β-shift structure, exerting strong hydrophobic interaction and having a lipid-embedded localization. *3*) On the lipophorin surface, apoproteins occupy about 31×10^3 Å^2 that represents from 62 to 82% of the total lipoprotein surface, depending on the size of lipophorin considered. *4*) The content of PL of each lipophorin is closely proportional to the lipoprotein size. *5*) Despite the importance of lipophorin as DG carrier, there are only small amounts of this component on the lipophorin surface which could reach a maximal surface lipid concentration of about 10 mol%. — **Soulages, J. L., and R. R. Brenner.** Study on the composition-structure relationship of lipophorins. *J. Lipid Res.* 1991. **32:** 407-415.

Supplementary key words lipophorin model • lipoprotein structure • insect lipoprotein • lipid transport • lipid-protein interaction

Lipophorin is the major lipoprotein in insect hemolymph. Lipophorins have been isolated from the hemolymph of several insect species and many of these studies have been summarized in recent reviews (1-3).

Lipophorins have a variable lipid content which ranges from 35% to 55% (w/w) depending on the insect species and developmental stage. Diacylglycerols (DG), phospholipids (PL), and hydrocarbons (HC) are the main lipid components, whereas cholesterol (Chol), free fatty acids (FFA), triacylglycerols (TG), and other hydrophobic compounds are minor. The content of DG varies from 7% to 30%, PL from 11% to 24%, and HC from 0% to 15%.

All lipophorins studied have two nonidentical apoproteins: apolipophorin I (apoLp-I) and apolipophorin II (apoLp-II) with molecular weights of about 250,000 and 80,000, respectively. In some adult insects that utilize large amounts of lipids as fuel for flight, an additional small apolipoprotein (apoLp-III) of about 17,000 is associated with the lipophorin. In these insect lipophorins the number of molecules of apoLp-III per lipoprotein particle is variable and depends on the metabolic state of the insect (4, 5). Despite the great importance of these lipophorins in the lipid metabolism of some insects, our study is focused on the structure-composition relationship of those lipophorins that do not contain apoLp-III.

Although the structure of lipophorin still remains unknown, several important basic studies have been carried out. Small angle X-ray scattering studies (6) and microscopic observations (7, 8) indicate that lipophorin has a spheric-like geometry. The localization of phospholipid molecules in the particle was studied by NMR spectroscopy (9). It was determined that all the PL molecules are located on the surface of the lipoprotein particle, in contact with water. Results obtained by calorimetric (10) and spectroscopic studies (6, 10) are consistent with the existence of a lipophorin lipid-core composed of hydrocarbons. Immunological studies and protease sensitivity

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Abbreviations: HDL_p, high density lipophorin; DG, diacylglycerol; PL, phospholipid; HC, hydrocarbon; Chol, cholesterol; FFA, free fatty acid; TG, triacylglycerol.

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reactions indicate that apoLp-I is largely exposed to the external media, while apoLp-II, although being found next to apoLp-I, appears to be located in a deeper area (4, 7, 11–13). Spectroscopic studies using amphipathic and nonpolar fluorescent lipid analogs indicate a strong lipid-protein interaction that affects the order of the hydrocarbon chains of both polar and nonpolar lipophorin-lipid components (14). Moreover, after extensive proteolytic degradation of apoLp-I, neither peptide liberation from the lipophorin nor any change in lipid-protein interaction was detected (13), though an island lipid-embedded apolipoprotein localization is presumed.

Along with several distinctive features that exist between mammal lipoproteins and lipophorins, i.e., the nature of their lipid composition, there are two specially remarkable biochemical differences. Unlike mammal lipoproteins, lipophorins serve as a reusable shuttle for transport of lipids, at least DG and FFA (15–17). Second, in mammal lipoproteins change in either the lipid composition or in the relative lipid content always seems to be accompanied by a change in the apolipoprotein content and/or composition; on the other hand, lipophorins undergo remarkable changes in their lipid content and/or lipid composition without variations in the nature or content of their apolipoproteins.

The purpose of this study was to obtain further information about the relationship between the variation of the lipid composition and the molecular organization of lipophorin. Within this scope and using all the available density-composition data of lipophorins (twelve sets of data), we have developed a lipophorin structural model.

Of several aspects of lipophorin structural organization that remain to be elucidated, we have obtained information on *1*) the distribution of DG within the lipoprotein particle; *2*) the average localization of apolipoproteins; and *3*) the extension of the lipophorin surface area occupied by proteins.

Despite the marked lipid composition variations found among the insect lipophorins, we obtained important conclusions about the physicochemical restrictions that govern the modifications in the lipid composition of lipophorins.

RESULTS

General model assumptions

In the proposed model, it is assumed that lipophorins are spherical particles of radius, R_2, and density δ_2, that have a lipid core of radius R_1, composed of DG, HC, and TG, and an outer shell composed of PL, cholesterol, DG, and protein. For the mathematical treatment, the lipid core was subdivided into an apparent hydrophobic lipid core of radius R_0, composed of TG and HC (**Fig. 1**).

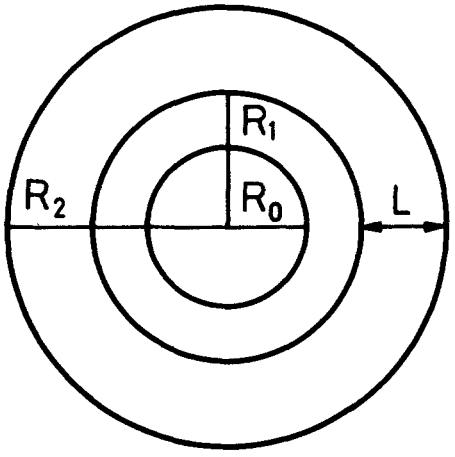

Fig. 1. Schematic representation of the arbitrary lipophorin subdivision used in the mathematical treatment.

From the compositional molecular weights (MW) of lipophorins, and assuming no structural water, the values of R_2 and R_0 were calculated according to equations 1 and 2, respectively.

$$R_2\,[\text{Å}] = \left[\frac{3 \times MW \times 10^{24}}{4 \times \pi \times \delta_2 \times N}\right]^{1/3} \qquad Eq.\ 1)$$

$$R_0\,[\text{Å}] = \left[\frac{3 \times MW \times X_o \times 10^{24}}{4 \times \pi \times \delta_0 \times N}\right]^{1/3} \qquad Eq.\ 2)$$

where N is Avogadro's number and δ_0 is the average density of the TG + HC apparent hydrophobic lipid core. δ_0 was calculated from the sum of the TG + HC weight fractions, X_0, and the density of individual components:

$$\delta_0^{-1} = \frac{\delta_{TG}^{-1} \times X_{TG} + \delta_{HC}^{-1} \times X_{HC}}{X_0} \qquad Eq.\ 3)$$

DG distribution model assumption

It has been well established that DG evokes major structural changes in natural (18) and artificial membranes (19, 20). The incorporation of DG into planar phospholipid membranes produces lamellar-hexagonal transitions by adding hydrocarbon volume to the phospholipids in such a way that lipids are forced to form high curvature monolayers, where the area of the polar groups is significantly reduced. Within lipid bilayers DG itself may occupy an area of 96 $Å^2$, increasing the area of the polar head group of PLs. These characteristics of DG molecules are the main factors of the strong

membrane-disturbing effect of DG. In this regard, it was determined (20) that the maximal area that PE and PC polar head groups can occupy in a planar bilayer structure (79 and 90 $Å^2$, respectively), is reached at very low DG levels of 5 and 20 mol%, respectively.

In view of these DG properties and taking into account the small size of lipophorin, which by itself spreads apart the polar head groups of the lipids at the lipophorin surface, it is reasonable to assume that only low amounts of surface DG would be compatible with lipophorin stability. For these reasons, it is assumed that the content of surface DG (N^S_{DG}) is low and directly related to the phospholipid content of each lipophorin (N_{PL}) (equation 4).

$$N^S_{DG} = \alpha \times N_{PL} \qquad Eq.\ 4)$$

The content of lipid core DG (N^{LC}_{DG}) is related to the available inner lipid space by lipophorin size, the thickness of the outer shell (L), and R_0 through equation 5

$$N^{LC}_{DG} = \frac{4 \times \pi}{3 \times V_{DG}} \times [(R_2 - L)^3 - R_o^3] \qquad Eq.\ 5)$$

where it is considered that there are no protein components within the lipid core space, and V_{DG} represents the molecular volume of diacylglycerols (1100 $Å^3$).

Since the total content of lipophorin DG (N^T_{DG}) must be the sum of the surface and lipid core DG, from the equations 4 and 5 N^T_{DG} is expressed as:

$$N^T_{DG} = \alpha \times N_{PL} + \beta \times [(R_2 - L)^3 - R_o^3] \qquad Eq.\ 6)$$

Equation 6 can be easily rearranged in a linear form which relates the total DG/PL ratio to the lipophorin size and the contents of TG, HC, and PL.

$$\frac{N^T_{DG}}{N_{PL}} = \alpha + \beta \times \frac{[(R_2 - L)^3 - R_o^3]}{N_{PL}} \qquad Eq.\ 7)$$

When the inclusion of the apoLp-II within the lipid core is considered equation 7 is modified as follows:

$$\frac{N^T_{DG}}{N_{PL}} = \alpha + \beta \times \frac{[(R_2-L)^3 - R_o^3 - \frac{3 \times V_{aa}}{4 \times \pi} \times N^i_{aa}]}{N_{PL}} \qquad Eq.\ 8)$$

Where V_{aa} is the molecular volume of amino acids and Ni_{aa} represents the number of amino acids included in the lipid core.

The linear structure of equations 7 and 8 allows an easy evaluation of model validity by linear regression analysis. Thus, the consistency of the model is restricted to a set of linear fitting parameters values: α, β, and the coeffcient of correlation, r. A value of β closely related to the actual molecular volume of DG (1,100 $Å^3$) must be expected, as well as a small value of α consistent with the lipophorin stability.

The Y_i values for each lipophorin were directly obtained from the compositional data in **Table 1** and they are shown in **Table 2**. However, for the determination of the X_i values, it is necessary to know the lipophorin outer shell thickness (L). A suitable value for L is the length of a PL molecule, but there are no experimental data of PL length in lipoproteins; the experimental values obtained in either natural or artificial membranes vary over a broad range. In order to calculate X_i, the L-value was modified between 16 and 25 Å, a range that includes the experimental values obtained in membranes (21, 22). Thus, there will be as many sets of X_i-values as L-values.

TABLE 1. Density and compositional data of lipophorins

Insect	Molecular Weight ($\times 10^{-3}$)			Lipids							
	ApoLp-I	ApoLp-II	HDL_p	DG	PL	HC	TG	Chol	Total	Density	Ref.
	kDa			*weight %*						*g/cm³*	
Manduca sexta											
Larvae	245	78	515	15.7	16.7	2.8	1.1	1.2	37.3	1.151	23
Prepupae-1	245	78	608	20.2	23.3	0.6	1.1	1.8	46.9	1.128	23
Prepupae-2	245	78	495	12.5	18.9	0.5	1.0	1.8	34.8	1.177	23
Pupae[a]	245	78	600	17.5	21.6	0.4	1.0	2.8	46.4	1.139	23
Larvae	285	81	600	14.1	12.6	5.0	2.3	2.0	37	1.155	24
Larvae	245	78	590	21.4	17.7	3.1	2.1	1.0	45.3	1.144	25
Larvae	245	78	713	30.3	20.8	2.3	0.7	0.6	54.7	1.112	25
Apis mellifera	250	80	559	13.8	14.4	2.2	4.2	6.5	41	1.13	26
Diatraea grandiosella	231	74	492	15.4	13.0		3.2	6.4	38	1.11	27
Musca domestica	253	85	514	6.0	20.0	6.0		2.2	34.2	1.145	28
Triatoma infestans[a]	255	78	628	19.5	14.9	5.7	1.9	2.1	47.0	1.10	29
Periplaneta americana	250	85	665	8.0	22.8	15.0	1.0	2.7	49.6	1.12	30, 31

The compositional molecular weights of lipophorins were calculated from the protein content of lipophorins and the apoLp molecular weights.
[a]These lipophorins have about 3% of FFA.

TABLE 2. Lipophorin composition and density-derived properties

Insect	R_2 (Å)	R_o (Å)	N_{DG}	N_{PL}	N_{Chol}	N_{DG}/N_{PL}
Manduca sexta						
Larvae	56.2	21.3	130	110	16	1.18
Prepupae-1	59.8	16.8	196	182	28	1.08
Prepupae-2	55.0	15.0	99	120	23	0.82
Pupae	59.3	15.6	173	171	44	1.01
Larvae	58.7	27.3	124	117	31	1.06
Larvae	58.9	24.3	208	139	15	1.5
Larvae	63.3	21.7	357	198	11	1.8
Apis mellifera (larvae)	58.1	25.3	128	107	94	1.2
Diatraea grandiosella	56.0	19.0	125	85	82	1.47
Musca domestica (larvae)	56.2	24.8	51	137	29	0.37
Triatoma infestans	60.9	28.3	202	129	34	1.56
Periplaneta americana	61.7	37.4	88	202	47	0.44

R_2 and R_o were calculated according to equations 1 and 2. N_{DG} and N_{Chol} were estimated assuming the following molecular weights: DG, 605; Chol, 386. When the molecules of PL per lipophorin particle were not given by the authors, they were calculated assuming a molecular weight of 750.

Table 2 also shows the values of R_2 and R_0 used for the calculation of the X_i-values for each lipophorin.

The least-square-fitting parameters obtained for several values of the outer shell thickness are shown in **Table 3**. The first line (N^i_{aa} = 0) shows those values for α, V_{DG} (β), and r obtained when no protein is included within the lipophorin lipid-core. Within the L-range of 19-23 Å there is a good agreement between the $V_{DG}(\beta)$ values and the actual molecular volume of DG (1,100 $Å^3$). The values of the slope represent strong evidence of model consistency, but it is also very important for model validity that small values of α are obtained in this range of L-values. Moreover, from the behavior of the goodness-of-fit measured through the r-values, we conclude that this model is consistent with the experimental data, and is best for an L-value near 20-21 Å, where r has its maximal value, $\alpha \simeq 0$, and the slope is inversely proportional to the actual volume of DG.

Up to this point, it has been considered that both apolipoproteins, apoLp-I and apoLp-II, were confined to the lipoprotein outer shell. Although, apoLp-I is probably not located deeper than as just considered, there is still great uncertainty about the localization of the apoLp-II (4, 7, 11, 13). Since it is not possible to rule out a deeper local-

TABLE 3. DG distribution: model-fitting parameters and their relationship with the thickness of the outer shell (L) and the apoLp-II localization

N^i_{aa}	Thickness of the Outer Shell (L)								
	16	18	19	20	21	22	23	24	25
					Å				
0									
α	−0.201	−0.152	−0.114	−0.062	0.001	0.007	0.164	0.257	0.360
V_{DG} (β)	1479	1283	1200	1133	1076	1031	997	972	962
r	0.710	0.729	0.738	0.741	0.745	0.743	0.738	0.732	0.719
180									
α	−0.250	−0.175	−0.118	−0.043	0.046	0.148	0.261	0.381	0.502
V_{DG} (β)	1296	1121	1052	996	953	921	900	891	888
r	0.748	0.768	0.776	0.777	0.777	0.771	0.762	0.749	0.728
360									
α	−0.283	−0.163	−0.081	0.024	0.147	0.278	0.421	0.560	0.699
V_{DG} (β)	1137	990	934	893	866	850	848	854	874
r	0.786	0.801	0.809	0.805	0.798	0.788	0.767	0.748	0.717
540									
α	−0.288	−0.107	0.034	0.153	0.311	0.468	0.630	0.778	0.915
V_{DG} (β)	1006	890	860	824	818	821	841	870	913
r	0.823	0.832	0.831	0.821	0.805	0.785	0.753	0.723	0.684
720									
α	−0.249	0.012	0.174	0.347	0.529	0.698	0.860	0.998	1.114
V_{DG} (β)	901	821	803	797	815	839	887	941	1.024
r	0.854	0.850	0.841	0.820	0.790	0.759	0.717	0.679	0.628

α and r are the Y-intercept and correlation coefficient, respectively, while V_{DG} (β) is the apparent molecular volume of DG obtained from the line-slope (β) throughout the equation: $V_{DG}(\beta) = \frac{4\pi}{3\beta}$.

ization of the apoLp-II, this possibility was checked by means of the regression analysis according to equation 8. For these calculations, it was considered that the apoLp-II has a molecular weight of about 80,000 and a total amino acid number of 720 (MW_{aa} = 110). From these data and considering a protein density of 1.37 g/cm^3 or 133 Å^3/amino acid, the values of X_i were calculated according to equation 8. Table 3 shows the results of the least squares fit of the data points obtained for different values of both unknown parameters: the degree of amino acid incorporation (N^i_{aa}) within the lipid core and the thickness of the lipophorin outer shell.

Unlike the results previously obtained for $N^i_{aa} = 0$, the incorporation of protein within the lipoprotein lipid-core enables us to obtain a set of coherent model-fitting parameters. Although each degree of amino acid incorporation has an L-value that gives a line-slope closely related to the actual molecular volume of DG, i.e., N^i_{aa} = 180, L = 18 or N^i_{aa} = 360 L = 16, these $V_{DG}(\beta)$ values are accompanied by negative α-values. Obviously, negative α-values have no physical meanings. Moreover, for a given N^i_{aa} value different from zero, there is no coincidence between the line-slope related to the actual V_{DG} and the maximal value of the correlation coefficient as observed at $N^i_{aa} = 0$.

From the regression analysis of the experimental data to the DG distribution model assumption, it is concluded that apolipoproteins are confined to the outer shell, and that lipophorins have only a very low content of surface DG.

However, the conditions imposed by equations 7 and 8 do not imply any restriction about the filling of the outer shell space. Therefore, this last restriction must also be considered.

Outer shell space-filling condition

The second model condition accounts for the space filling of the outer shell of lipophorins.

In agreement with the DG distribution and the amount and molecular volumes of the surface components, the following equation can be expressed:

$$\frac{4}{3}\pi \times [R_2^3 - (R_2 - L)^3] = N_{Chol} \times V_{Chol} + N_{PL} \times (V_{PL} + \alpha \times V_{DG}) + V_{APO} \qquad Eq.\ 9)$$

The left term of the equation represents the total outer shell volume and the right term includes the condition of $N^S_{DG} = \alpha \times N_{PL}$, and excludes the presence of hydrocarbons and triacylglycerols from the outer shell space. In this equation V_{APO} is the volume occupied by the apolipoproteins and V_{PL}, V_{Chol}, and V_{DG} represent the molecular volumes of the lipid components.

Equation 9 can be rearranged allowing linear correlation analysis. Thus:

$$\frac{4}{3}\pi \times [R_2^3 - R_1^3] - N_{Chol} \times V_{Chol} = N_{PL} \times (V_{PL} + \alpha \times V_{DG}) + V_{APO} \qquad Eq.\ 10)$$

Due to the low content of cholesterol, this rearrangement has little effect on the space-filling condition, but it allows us to study the model validity by means of the Y-intercept and the slope of the plot, which give the best linear fit when $X_i = N_{PL}$ and Y_i is the left term of the equation 10.

Once again, the uncertainty of the L-value requires that for fitting of the data points to equation 10, several L-values must be considered.

The best fit of the data points was reached for an L-value of about 20 Å, which is near to that obtained when the DG distribution condition was checked (**Fig. 2**).

From the actual molecular volumes of DG and PL and the line-slope values, it is possible to obtain new α-values. For an L-value of 20 Å, the number of surface DG per PL molecule has a value of 0.130. This α-value is slightly higher than that previously obtained, but it is also low enough to be compatible with the lipophorin stability.

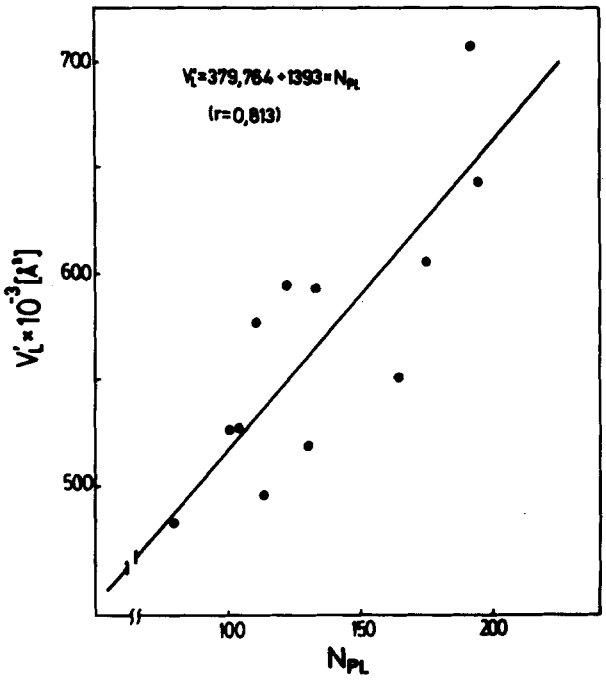

Fig. 2. Data points of $V_L = \frac{4}{3}\pi [R_2^3 - (R_2 - 20)^3] - N_{Chol} \times 602$, were plotted against N_{PL} according to equation 10. The line is the least-squares fit of the data that render the best linear correlation coefficient value (L = 20).

The Y-intercept is a measure of the protein localization. Considering the analysis of the DG distribution condition, apoLps are confined to the lipophorin outer shell; then the value of the Y-intercept should be consistent with such a previous result. Taking into account that the molecular volume of an amino acid is 133 $Å^3$ and the total amino acid number of both apoLps is about 3,000 (corresponding to a protein density of 1.37 g/cm^3 and an amino acid molecular weight of 110 kDa), from the Y-intercept obtained for an L-value of 20 Å (Fig. 2), it was calculated that approximately 95% of the amino acids of the apoLps are confined to the lipophorin outer shell and the remaining 5% (150 amino acid residues) would be located within the lipophorin core.

When one compares the values of L, α, and the protein localization obtained when both the DG distribution model condition and the outer shell space-filling condition render the best fit for the data, there is good agreement between the two model conditions, confirming the consistency of the premises on which the structural model is based.

Lipophorin surface space-filling condition

Assuming that the surface area occupied by apolipoproteins at the lipoprotein surface is constant and independent of the lipid composition, a simple mathematical relation may be derived. Thus, equation 11 expresses the relation between the lipophorin size and the content of surface lipid of any given lipophorin.

$$4 \times \pi \times R_{\frac{1}{2}}^2 - N_{Chol} \times A_{Chol} = N_{PL} \times (A_{PL} + \alpha \times A_{DG}) + A_{APO} \qquad \textit{Eq. 11)}$$

A_{Chol}, A_{PL}, and A_{DG} are the molecular areas of the lipid components and the A_{APO} is a constant that represents the area occupied by apoLps at the lipophorin surface.

On the basis of a linear regression analysis of $A_L = 4 \times \pi \times R^2_2 - N_{Chol} \times A_{Chol}$ against N_{PL}, it is possible to estimate the area occupied by apoLps at the lipophorin surface (Y-intercept) as well as a new independent α-value from the slope of the line. However, there are no experimental data about the surface areas occupied by cholesterol and diacylglycerol molecules at the level of membrane or lipoprotein surface, required to obtain first the Y_i values, and second a new α-value. Therefore, it was considered that only the hydroxyl group of either cholesterol or diacylglycerol molecules is located at the lipophorin surface, and, according to the measurements of CPK space-filling model, a value of 20 $Å^2$ was adopted for the cross-sectional areas of hydroxyls (32).

Fig. 3 shows the linear plot of A_L against N_{PL}. The goodness of the fit ($r = 0.810$) supports these model assumptions. From the Y-intercept, a value of 31,159 $Å^2$ was obtained for the surface area occupied by apolipoproteins. It is interesting to note that apoLps cover a large

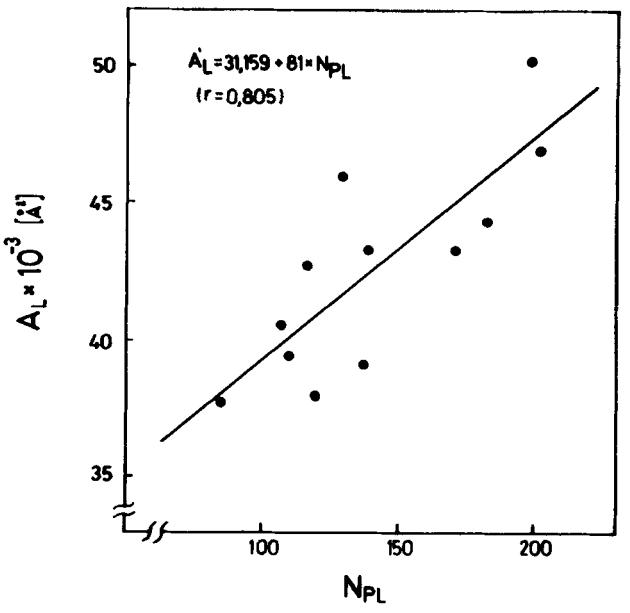

Fig. 3. Data points of $A_L = 4\pi R_{\frac{1}{2}}^2 - N_{Chol} \times 20$ were plotted against N_{PL} according to equation 11. The line is the least-squares fit of the data points. The Y-intercept represents the surface area occupied by proteins (31,159 $Å^2$).

proportion of the total lipophorin surface and that, depending on the size of the lipophorin considered, may represent from 62 to 82% of the lipophorin surface.

In order to calculate another α-value from the line-slope, it was also necessary to adopt a value for the area of the polar head group of phospholipids. Unlike the cases of DG and cholesterol, several experimental values of PL polar head groups have been reported. Obviously, the head group areas depend on their nature and on the membrane composition. For pure egg PC, a value of 75.6 $Å^2$ was determined (20), while the incorporation of increasing amounts of DGs, up to 20 mol%, or cholesterol, up to 50 mol%, increased the polar head group area up to 96 $Å^2$ (20). In a similar way only 5 mol% of DG increase the egg PE polar head group up to its maximal (bilayer compatible) value of 79 $Å^2$ (20).

Since PC and PE are the main PL components of lipophorins, the above data are useful to estimate limit values for the areas of lipophorin PL polar head groups. The comparison of the line-slope value (81 $Å^2$) with any of those areas of PL polar head groups measured in the presence or absence of either DG or cholesterol indicates that, once again, only small amounts of DG would be located on the lipophorin surface. Thus, adopting the least value of egg PC polar head group are (pure PC) of 75.6 $Å^2$, and considering one value of A_{DG} = 20 $Å^2$, a maximal value of (0.27) was obtained. On the other hand, negative α-values were obtained when PL areas larger than 81 $Å^2$ were used. Then, since the presence of both DG and cholesterol on the lipophorin surface cannot be eliminated, an α-value lower than 0.27 is inferred.

DISCUSSION

Lipid organization and transport

Throughout this study we have demonstrated that, although there is an apparently random variation in the lipid composition, there are some structural factors that control such composition variations. Our results clearly show two of these factors. *1*) After the quantitative analysis of the outer shell space-filling and the lipoprotein surface-filling conditions (Figs. 2 and 3), it was found that the phospholipid content of lipophorins increases proportionally to the lipoprotein size increase. *2*) The results obtained after subjecting the density composition data to the three different model conditions strongly support the hypothesis that the superficial DG content of lipophorins is markedly low. Consequently, the total DG content of lipophorins is approximately proportional to their size and it decreases together with an increase in the HC and TG contents of lipoproteins. By analyzing the lipophorin properties used in the present study (Tables 1 and 2), it was deduced that these lipoproteins undergo important changes in size and in the lipid content of the hydrophobic core. Our results indicate that the unique condition required for an increase in the lipid content of the lipoprotein core is the incorporation of PL molecules, in order to cover the lipoprotein surface requirements. However, the incorporation of a third apolipoprotein (apoLp-III) that occurs only when lipophorins are loaded with large amounts of DGs, may indicate that under circumstances when the content of DG is high, apoLp-I and apoLp-II are not able to maintain particle stability. Even though the size of lipophorin may vary considerably with an in crease on the PL content, there is probably a limit for the increase in lipid content of those lipophorins that only have apoLp-I and apoLp-II as protein components.

Although it is known that most of the net transfer of DG from the lipophorin to the tissues does not require that cells incorporate or degrade apolipoproteins (16), the mechanism of such lipid transfer is not as yet clear.

The spontaneous removal of a particular molecule from the surface of membranes or lipoproteins depends not only on its surface concentration, but also on the molecular packing of the surface lipids (33, 34). Thus from the high degree of molecular packing of lipophorin surface lipids (14) and the low surface concentration of DG as shown by the present results, only a very slow spontaneous transfer of DG from lipophorin to tissues or to other lipoproteins might be expected. This could also be the reason for the presence of a lipid transfer protein that promotes the transfer of DG between lipophorins, at least in the hemolymph of *Manduca sexta* (35, 36) and *Musca domestica* (28). The interaction of the lipid transfer protein with the lipophorin surface would affect the packing of lipophorin surface lipids, favoring the hydration and removal of DG. It is probable that insects have, in general, a lipid transfer protein-like factor that could facilitate the transfer of DG from lipophorin to tissues. Since a rapid transbilayer movement of DG was observed in natural (18, 37) and artificial membranes (38), one might not expect that the rate of exchange between the inner core and the surface of lipophorin constitutes a rate-limiting process for the removal of DG from the lipophorin surface.

The success of our composition–structure correlationship derives from the apparent equilibrium that exists among the contents of the main lipid components of lipophorins. The maintenance of such equilibrium imposes several restrictions on the lipid transport mechanism. Thus, the net transfer from the lipophorin of a unique type of lipid molecule would not be possible. For example, in order to maintain the sphere-like geometry of lipophorin, the net transfer of DG must be accompanied by the transfer of PL. In this case, it is also interesting to note that the lipid transfer protein that has recently been described also has the capacity for binding and transferring PL (35).

Apolipophorin localization

Based on the results obtained from the quantitative analysis of the DG distribution and the outer shell space-filling conditions, it is concluded that lipophorin structure is consistent with the existence of an outer shell of about 20–21 Å thickness where phospholipids, cholesterol, apoproteins, and a small fraction of diacylglycerols are located. The estimated value for the outer shell thickness represents an average measure between the lengths of the outer lipid components and the apoprotein depth in the lipoprotein particle. Since the best fit of experimental data to the model conditions was reached for an L-value very similar to those values of PL lengths measured in membranes (21, 22), it may be inferred that protein penetration into the lipoprotein particle is rather deep; it would reach values of about 20 Å.

This conclusion supports the hypothesis that not only is there an ionic interaction of proteins with phospholipid polar groups, but also a hydrophobic interaction with lipid hydrocarbon chains or regions.

A high degree of β-structure in native lipophorin apoproteins (11, 39) and in apoLp-I reconstituted by lipids (39) has been reported. It has long been thought that membrane integral proteins could interact with the membrane hydrophobic regions by means of α-helical structure. However, there are reports about the existence of two integral membrane proteins where β-strands are found (40, 41).

Taking into account the above information, it may be suggested that either apoLp-I or both lipophorin apoproteins represent another expample of proteins with β-structure that interact hydrophobically with lipids.

A deep protein localization, as shown in our results, is consistent with previous experimental studies. First, both apoproteins are extremely insoluble in aqueous medium and they may only be solubilized with detergents (7, 39) or under highly denaturing conditions (39). Second, studies carried out in our laboratory have indicated that apoproteins exert a strong ordering effect lipophorin lipids, affecting the hydrocarbon chain mobility of outer shell components as well as the internal ones of lipophorin (14). This strong lipid-protein interaction, which has been studied by fluorescent polarization of fluorescent lipid analogues both amphipathic and nonpolar, is not modified even after extensive proteolytic degradation of apoLp-I (13). Simultaneously, using energy transfer for Trp-containing peptides or native PAGE for peptides in general, no peptide liberation could be detected either during or after an extensive proteolysis.

The general organization of protein and lipid components of lipophorin was also studied by small-angle X-ray scattering (6). The analysis of the data obtained from two out of three lipophorins led the authors (6) to a model that is consistent with lipid-embedded proteins. Although the structure derived from the X-ray scattering model does not show a strict correlation between the lipid composition and space-filling of particles, it presents strong experimental evidence regarding the deep localization of apoproteins.

Concerning lipophorin structure, the surface molecular arrangements must be well determined in order to understand its behavior as a lipid carrier.

Although, we are aware that the topology of lipids and proteins deserves further examination, the present study provides information related to the lipophorin surface extension, which is covered by lipids and proteins. Accordingly, proteins occupy a large and constant area on the lipophorin surface that represents between 62 and 82% of the total lipoprotein surface, depending on the lipophorin size.

In this regard, our results are markedly different from those obtained when the lipoprotein lipid space-filling model (LLSFM), developed to study the structure of human lipoproteins (42, 43), was applied to insect lipophorins (1). The application of the LLSFM to lipophorins renders lipoprotein surfaces fully covered by proteins. Obviously, this is not consistent with the physiological lipid transfer role of lipoproteins. This difference is probably derived from the large molecular area attributed to amino acids by the LLSFM. It must be noted that while apolipophorins have an apparent lipid-embedded localization and a β-sheet secondary structure, according to the LLSFM, the lipid-protein interaction is restricted to the polar head groups of phospholipids and the molecular area of amino acids is consistent with α-helical structure.

Finally, in our study, the possibility of a protein incorporation deeper than that limited by the outer shell thickness was considered. According to the outer shell space-filling analysis, a small inclusion of protein (about 150 amino acid residues) might occur within the lipid core. However, no protein would be located in the lipid core according to the DG distribution statistical analysis. Therefore, as a result of these differences and because of the large statistical error found in those studies, neither of these two possiblities should be discarded.

Manuscript received 22 May 1990 and in revised form 30 August 1990.

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