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INTERACTIONS OF ACYL CARNITINES AND OTHER LYSINS WITH ERYTHROCYTES AND RECONSTITUTED ERYTHROCYTE LIPOPROTEINS

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SUMMARY

The effect of acyl carnitines, lysolecithin and palmitoyl choline on rat erythrocytes labelled with [^{3}H]cholesterol or [^{32}P]phosphoglycerides was studied. The amount of labelled lipid released from such cells was found to vary with the type of lysin used, its concentration and the acyl chain length in the case of acyl carnitines. The extent of hemolysis was not directly proportional to the amount of lipid released. Apoprotein derived from erythrocyte ghosts interacted with complex lipid mixtures to form insoluble lipoproteins at pH 4. Lysins depending on their charge and acyl chain lengths could prevent lipoprotein formation at pH 4.0 or could cause dissociation of such complexes at either pH 4.0 or pH 7.4. The mechanisms whereby lysins interact with intact membrane or reconstituted lipoproteins to release lipids are discussed.

INTRODUCTION

One approach for further understanding how lipids and proteins interact to form membranes has been to work with model systems comprising various types of proteins and lipid dispersions as reactive counterparts. For such studies, it has been possible to isolate apoprotein from erythrocyte[1–6] and other membranes[7], however the properties of the apoproteins differ depending on the procedure of isolation. Accordingly, one can deduce from various studies[8] that erythrocyte structural protein, water-insoluble at physiologic pH, reacts with lipids by hydrophobic interactions mainly. In the case of Maddy's apoprotein[5] which is water-soluble except in the isoelectric pH range of 3.7–4.8, interaction with lipid involves an ionic combination initially; the complex is then stabilized by hydrophobic interactions[9]. Model systems comprising proteins which are not denatured behave in a manner similar to Maddy's apoprotein with respect to their interaction with lipid[10,11]. Such studies point to the importance of both ionic and hydrophobic bonding for the formation and stability of lipoprotein complexes. The results from such studies however do not exclude the possibility that large portions of the lipoproteins artificially formed, are constituted

Abbreviation: CTA, cetyltrimethylammonium chloride.

by uninterrupted lipid bilayers, a situation which is also believed to exist in the membrane[12].

Previous studies established acyl carnitines as strongly lytic substances[13] the action of which could be inhibited by phospholipids, cholesterol and isolated membrane protein added to the bulk phase[14]. From these studies, it was apparent that lysins could react nonspecifically with a number membrane constituents, a finding which bears on the mechanism of lysis by these amphipathic substances. Since lytic activity was highly dependent on acyl chain length hydrophobic interactions had to be involved in some stage of the lytic process.

To further understand the mode of action of quaternary nitrogen amphipathic substances and other hemolysins, we have used model systems constituted by membrane apoprotein reacting with lipid dispersions. In this study we describe the effect of lysins on the formation and stability of lipoproteins *in vitro* and relate these results with the action of acyl carnitines and other lytic substances on erythrocytes prelabelled with [^{32}P]phosphoglycerides and [^{3}H]cholesterol.

EXPERIMENTAL

Materials

Acyl-DL-carnitines, palmitoyl choline and 1-palmitoyl-*sn*-glycero-3-phosphorylcholine were prepared as described earlier[13]. Cetyltrimethylammonium chloride (CTA) and sodium dodecyl sulphate were purchased from Fisher Scientific Co. and recrystallized several times from 80% ethanol by gradual addition of ether and cooling. Total lipids from rat erythrocytes were extracted by the method of Bligh and Dyer[15]. Labelled acyl carnitines were prepared by acylation of [*Me*-^{14}C]carnitine (Tracerlab, Mass. U.S.A.) diluted with carrier[13]. Labelled lipids were obtained by incubating rat liver preparations with [^{32}P]orthophosphate (New England Nuclear Corp.)[16]. ^{32}P-Labelled lecithin was separated from total hepatic lipids by preparative thin-layer chromatography and was degraded to the lysoanalogue by the action of phospholipase A2 from *Crotalus adamanteus* venom[17]. Total ^{32}P-labelled lipids from rat liver were admixed with erythrocyte total lipids in the proportion of 1:4 and the specific activity was calculated on the basis of counts per mg of total lipid.

[^{3}H]Cholesterol was purchased from New England Nuclear and diluted to lower specific activity. Soluble apoprotein from bovine erythrocyte ghosts was prepared by the method of Maddy[5]. The protein precipitated between pH 3.7 and 4.8. It was stored in precipitated form at 0–5 °C in water adjusted to pH 4.0 with 0.1 M HCl.

Methods

Binding studies with Maddy's protein. The method was based on that of Zwaal and Van Deenen[9]. Maddy's protein precipitated at pH 4,0, was suspended in 0.05 M acetate buffer, pH 4.0, and adjusted to a concentration of 10 mg/ml. 0.5 ml of protein suspension was mixed with a known amount of lipid and/or lysin dispersion sonicated in ice. The volume was adjusted to 5 ml with acetate buffer. Incubations were carried out in a shaking ice–water bath for 15 min and the lipoprotein formed was collected by centrifugation at 3000 × *g* for 10 min and washed twice with distilled water (22 °C). Amounts of lipid or lysin bound to the protein were calculated from the counts ob-

tained in the precipitate. Over 95% of the protein was recovered in the lipoprotein precipitate.

The effect of lysins on the stability of the reconstituted lipoproteins was studied as follows: 5 mg of apoprotein were incubated at 37 °C for 30 min in either 0.05 M acetate buffer, pH 4.0, or 0.05 M Tris–acetate buffer, pH 7.0, containing 0.5 mg of sonicated [^{32}P]lecithin or 0.5 mg of sonicated 0.5 mg [^{3}H]cholesterol. The lipoprotein formed was collected by centrifugation, washed once with warm water (37 °C) and reincubated for 30 min with different amounts of lysins. After centrifugation and washing, the radioactivity remaining in the precipitate or in the supernatant *plus* washings was counted. To estimate the radioactivity of the lipoprotein complex, the twice washed pellet was suspended in 0.5 ml of water or less and an aliquot was transferred to a vial containing 0.6% (w/v) diphenyloxazole (PPO) in toluene–ethanol (2:1, v/v). Samples were counted with a Beckman LS133 spectrometer.

Studies on the release of lipids from erythrocyte membranes during lysis. 10 ml (50% hematocrit) of rat red blood cells prepared as described previously[1] were incubated at 37 °C for 3 h with total hepatic ^{32}P-labelled lipid (37 500 dpm/mg lipid phosphorus per ml or with 2 ml of [^{3}H]cholesterol (200000 dpm/ml) dispersed in saline as reported earlier[13]. The labelled erythrocytes were then washed several times with isotonic NaCl at pH 7.4 until the supernatant showed only background radioactivity. Finally the packed erythrocytes were resuspended in saline to give a 50% hematocrit. 3 ml of this suspension were then incubated with various weakly-lytic concentrations of lysins in a final volume of 5 ml saline for 10 min in a water bath with gentle shaking at 37 °C. Part of the suspended cells were centrifuged and the clear supernatant was used to estimate the amount of labelled lipid released. The remainder of the suspension was used to estimate percent hemolysis as described previously[13].

RESULTS

Results summarized in Table I show the release of labelled phospholipid from erythrocytes by weakly-lytic amounts of stearoyl-palmitoyl- and myristoyl carnitines, palmitoyl choline and (1-palmitoyl)-lysolecithin. For any particular lytic agent, there was some correspondence between the amount of lysin added, the amount of phospholipid released and the degree of hemolysis. The release of phospholipid and degree of hemolysis were also roughly proportional to the acyl chain length in the acyl carnitine series. However there was no precise proportionality between the amount of phospholipid released and the extent of hemolysis. For example, equivalent amounts of (1-palmitoyl)-lysolecithin and palmityol choline released similar quantities of phospholipid but caused different extents of hemolysis. Quite similar conclusions can be derived from results obtained with [^{3}H]cholesterol-labelled cells as shown in Table II. Here again there is only approximate proportionality between the amount of labelled cholesterol released, the amount of lysin added, the chain length in the acyl carnitine series and extent of hemolysis. Results in Tables I and II indicate that the lytic action of quaternary ammonium amphipaths is related not only to their ability of releasing lipids from the membrane but also to their direct reactivity with other membrane constituents, likely proteins.

Fig. 1 illustrates the binding of ^{32}P-labelled lipid to Maddy's protein at pH 4.

TABLE I

RELEASE OF [^{32}P]PHOSPHOLIPID FROM RED BLOOD CELLS DURING HEMOLYSIS BY VARIOUS LYSINS

Lysins (*μmoles*)		*Bound labelled phospholipid* remaining* (*μg lipid P*)	*Labelled phospholipid* released* (*μg lipid P*)	*Hemolysis* (%)
No lysin		189.6	26.0	0.0
Stearoyl carnitine	$2 \cdot 10^{-1}$	157.6	41.6	3.5
	$10 \cdot 10^{-1}$	142.4	78.0	11.0
	$20 \cdot 10^{-1}$	81 6	141.3	16.0
Palmitoyl carnitine	$2 \cdot 10^{-1}$		38.0	2.0
	$10 \cdot 10^{-1}$		72.3	9.0
	$20 \cdot 10^{-1}$		128.2	13.0
Myristoyl carnitine	$2 \cdot 10^{-1}$		33.2	0.5
	$10 \cdot 10^{-1}$		66.0	1.0
	$20 \cdot 10^{-1}$		101.6	3.5
(1-Palmitoyl)-lysolecithin	$2 \cdot 10^{-1}$		36.0	2.5
	$10 \cdot 10^{-1}$		72.4	10.0
	$20 \cdot 10^{-1}$		122.0	13.0
Palmitoyl choline	$2 \cdot 10^{-1}$		35.6	0.0
	$10 \cdot 10^{-1}$		68.0	4.0
	$20 \cdot 10^{-1}$		120.8	7.0

* The amount of phospholipid bound or released was calculated from the radioactivity in each sample and the specific activity of the lipid used for labelling the cells.

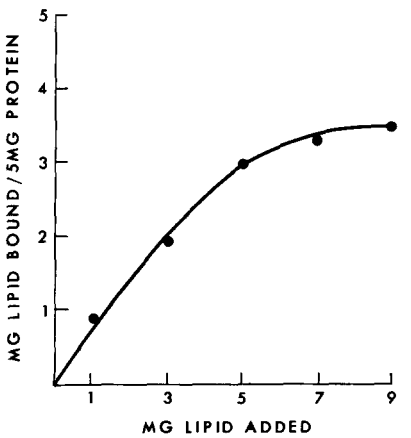

Fig. 1. Binding of total ^{32}P-labelled lipid with Maddy's erythrocyte membrane protein. 5 mg of Maddy's protein was incubated at 0 °C with different amounts of total lipid constituted with one part rat liver ^{32}P-labelled lipid and four parts unlabelled erythrocyte membrane lipid (spec. act. 33033 dpm/mg) in a total volume of 5 ml of 0.05 M acetate buffer, pH 4.0. After centrifugation the precipitate was washed twice with distilled water and counted.

TABLE II

RELEASE OF [^{3}H]CHOLESTEROL FROM RED BLOOD CELL DURING HEMOLYSIS BY VARIOUS LYSINS

Lysins (*μmoles*)		*Bound labelled cholesterol* remaining* (*μg*)	*Labelled cholesterol* released* (*μg*)	*Hemolysis* (%)
No lysin		238.9	7.8	0.0
Stearoyl carnitine	$2 \cdot 10^{-1}$	234.0	19.5	6.5
	$10 \cdot 10^{-1}$	171.3	63.0	18.0
	$20 \cdot 10^{-1}$	150.5	104.2	21.0
Palmitoyl carnitine	$2 \cdot 10^{-1}$		13.8	5.0
	$10 \cdot 10^{-1}$		54.3	10.0
	$20 \cdot 10^{-1}$		80.2	15.0
Myristoyl carnitine	$2 \cdot 10^{-1}$		11.2	3.0
	$10 \cdot 10^{-1}$		52.8	3.5
	$20 \cdot 10^{-1}$		73.7	4.5
(1-Palmitoyl)-lysolecithin	$2 \cdot 10^{-1}$		18.0	3.5
	$10 \cdot 10^{-1}$		59.6	11.0
	$20 \cdot 10^{-1}$		82.9	16.0
Palmitoyl choline	$2 \cdot 10^{-1}$		12.1	2.0
	$10 \cdot 10^{-1}$		53.2	6.0
	$20 \cdot 10^{-1}$		74.3	10.0

* The amount of cholesterol bound or released was calculated from the radioactivity in each sample and the specific activity of the cholesterol used for labelling the cells.

The amount of lipid bound to the protein was estimated from the counts obtained in the lipoprotein fraction and from the specific activity of the lipid calculated on the basis of the total lipid mixture. It could be argued however that there was selective binding of phospholipids and that counts obtained did not represent total lipids bound. If lanes I and V of Fig. 2 are compared, it is seen that lipid binding to Maddy's protein is nonselective and reflects the composition of the original lipid mixture. Zwaal and Van Deenen observed a similar phenomenon with their apoprotein preparation[9]. Binding to protein increased until the amount of lipid added to the incubation mixture was about 5 mg. At this point, the lipid–protein ratio in the precipitate was approximately 0.6 and was not markedly increased by further addition of lipid. This value is in good agreement with the original lipid–protein ratio found in erythrocyte ghosts ranging from 0.58–0.80 and is similar to that found by Zwaal and Van Deenen[9] working with neuraminidase-treated apoprotein.

Table III illustrates the binding of palmitoyl DL-[^{14}C]carnitine to apoprotein and to reconstituted lipoprotein. The binding was not markedly affected by the ionic strength of the reactive mixture, nor was it greatly increased in reconstituted lipoprotein. Other data (not shown) indicated that hepatic ^{32}P-labelled lysolecithin was also bound to apo- and lipoprotein to similar extents but in this case ionic strength increased the binding. It seems from these results that interactions of lysins with

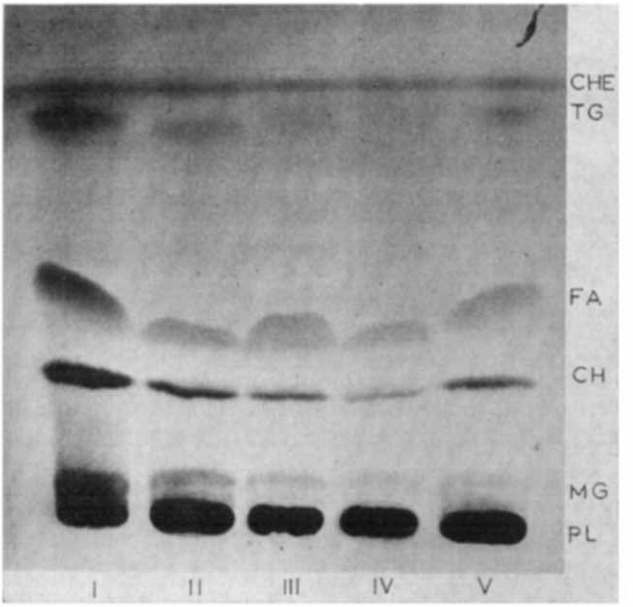

Fig. 2. Analysis of lipids bound to apoprotein and released from reconstituted lipoprotein by lysins. 10 mg of Maddy's protein were incubated with 10 mg of total lipid (liver/stroma lipid 1:4, w/w) at 0 °C for 15 min. Sample I contains the lipids bound to lipoprotein at pH 4 and were extracted from the complex by the Bligh and Dyer procedure[15]. Sample V contains the lipids prior to binding. Samples II, III and IV contain the lipids released from lipoprotein by addition of $2 \cdot 10^{-6}$ moles of palmitoyl carnitine, palmitoyl choline and (1-palmitoyl)-lysolecithin, respectively, to a bulk phase buffered at pH 7.0 with 0.05 M Tris–acetate. Samples were chromatographed on silica gel G with light petroleum (b.p. 60–90 °C)–ether–acetic acid (70:30:1, v/v/v). Components were visualized with I_2 vapour. CHE, cholesterol ester; TG, triglyceride; FA, fatty acid; CH, cholesterol; MG, monoglyceride; PL, phospholipid.

TABLE III

BINDING OF PALMITOYL-DL-[^{14}C]CARNITINE TO APOPROTEIN AND RECONSTITUTED LIPOPROTEIN AND THE EFFECT OF 1 M NaCl

The incubation mixture contained palmitoyl [^{14}C]carnitine (spec. act. 38709 dpm/mg) and 5 mg Maddy's protein or lipoprotein complex formed from 5 mg of protein and 5 mg lipid (Fig. 2) in 5 ml of 0.05 M acetate buffer, pH 4.0. In one set of experiments 1 M NaCl was also added to the incubation mixture.

Palmitoyl carnitine added (moles)	*Radioactivity bound (dpm)*	
Apoprotein	(−NaCl)	(+NaCl)
$5 \cdot 10^{-7}$	3957	3246
$10 \cdot 10^{-7}$	7668	6440
Lipoprotein		
$5 \cdot 10^{-7}$	4866	3871
$10 \cdot 10^{-7}$	9298	7122

apoprotein involve mainly hydrophobic bonding and are not appreciably dependent on charge. Depending on the lysin used however, slightly different mechanisms of interaction may become apparent reflecting the different reactivities of the heterogeneous protein fraction used. It is also evident from our results that the mode of binding of wedge-shape amphipaths is different from complex lipid mixtures which adopt larger aggregate forms in aqueous media and involve initially, ionic interactions with protein[9].

The association of apoproteins with lipid or lysin at pH 4 was further studied by sucrose density gradient ultracentrifugation as prescribed by Zwaal and Van Deenen[9]. Results not shown indicated that reconstituted lipoprotein separated into three fractions varying in lipid/protein ratios, however, none had the ratio of 0.6 usually obtained for bulk reconstituted lipoprotein or found for erythrocyte membrane. Addition of low concentrations of ^{14}C-labelled palmityol carnitine or ^{32}P-labelled lysolecithin to apoprotein or to reconstituted protein did not significantly alter the sedimentation profiles. These experiments did indicate however that lysins could bind with apoprotein directly and with all reconstituted lipoproteins formed.

Results in Fig. 3 show that with an increase in lysin concentration there is at pH 4, a corresponding decrease in lipoprotein formation. The effect is much more marked in the case of cationic detergents, such as CTA. Palmitoyl carnitine is also positively changed at this pH as could be deduced from the titration curves of acetyl and octanoyl carnitines which indicated pK values of 3.5 and 3.7, respectively. Lysolecithin has no charge at this pH whereas sodium dodecyl sulphate is negatively charged.

Results in Fig. 4 indicate that with increase of lysin concentration in the incubation mixture, dissociation of ^{32}P-labelled lipid reconstituted lipoproteins increases proportionally. Again marked effects are seen only with cationic detergents such as CTA, palmitoyl choline and palmitoyl carnitine. Thus a net positive charge appeared to be an important criterion for detergent action at pH 4.

Results in Fig. 5 show that as the chain length of acyl carnitines is decreased from C18 to C10, there is a corresponding decrease in the ability of the detergent to dissociate ^{32}P-labelled lipoproteins. Detergency must therefore involve hydrophobic interaction of these lysins with lipoprotein. Qualitative results illustrated in Fig. 2, lanes II to IV show that the release of lipids by these detergents is not specific for ^{32}P-labelled components but includes cholesterol and other neutral lipids. Results summarized in this figure indicate detergent effects at pH 7.4 however essentially the same release pattern was obtained at pH 4 when palmitoyl carnitine was the agent tried.

Results summarized in Fig. 6 indicate that for equal concentrations of detergents, only positively charged surfactant substances are highly effective in releasing lipid from lipoprotein at pH 4. At physiological pH, although zwitterionic amphipaths are effective, the detergency of negatively charged sodium dodecyl sulphate is very much increased whereas that of positively charged CTA is markedly decreased. These effects must correlate with variation in charge of the protein as a function of pH.

DISCUSSION

The binding of lipids to Maddy's protein pretreated with neuraminidase, positively charged at pH 4 was studied in detail by Zwaal and Van Deenen[9]. Their results

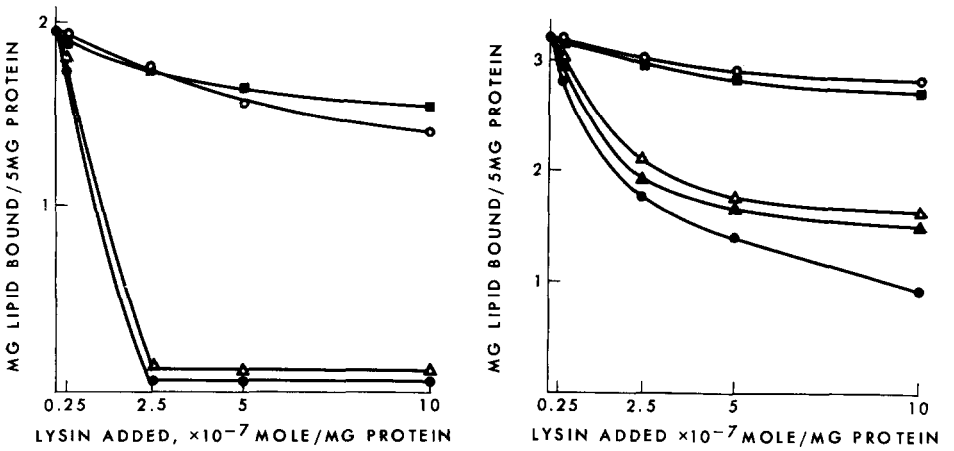

Fig. 3. Effect of preincubation of ^{32}P-labelled lipid with palmitoyl carnitine, and anionic and cationic lysins before addition of membrane protein. 3 mg ^{32}P-labelled lipid (132272 cpm) were preincubated 10 min at 37 °C with different amounts of palmitoyl carnitine, (1-palmitoyl)-lysol ecithin, sodium dodecyl sulfate or CTA in 4.5 ml of 0.05 M acetate buffer, pH 4.0. After addition of 5 mg membrane protein, the samples were incubated again for 15 min at 37 °C. The precipitates formed were then washed twice and counted. ●—●; palmitoyl carnitine, △—△; CTA, ■—■; sodium dodecyl sulfate, ○—○; (1-palmitoyl)-lysolecithin.

Fig. 4. The effect of lysins on lipoprotein dissociation at pH 4.0. 5 mg Maddy's protein were incubated with 5 mg ^{32}P-labelled lipid (75116 dpm) at 0 °C for 15 min in 5 ml, 0.05 acetate buffer, pH 4. After washing by addition of 10 ml cold distilled water, the precipitated lipoprotein complex was reincubated with different amounts of lysins dissolved in 5 ml of buffer for 15 min at 37 °C. After centrifugation, the precipitates obtained were washed twice with warm water and counted. ●—●; palmitoyl carnitine, ▲—▲; CTA, △—△; palmitoyl choline, ■—■; sodium dodecyl sulfate, ○—○; (1-palmitoyl)-lysolecithin.

indicated that lipoprotein formation involved initially an ionic interaction between the protein and negatively charged lipid micelles. The complex then becomes resistant to high ionic strength by hydrophobic bonding and is stable to 8 M urea. Analogous findings were made by Sweet and Zull[10] who studied lipoprotein formation using liposomes and albumin as a model system. Here again, complex formation involved bonding of a dual character. They suggested that an initial ionic interaction was followed by a penetration of apolar segments of protein into the bilayered structure of the liposomes.

In the present study, apoprotein was not treated with neuraminidase and consequently at pH 4, which is within the isoelectric pH range, the protein would bear no net positive charge. It is clear however from the work of Zwaal and Van Deenen[9] and our own results that sialic acid groups do not play an important role in the binding since the presence of intact sialoprotein does not affect the proportion of lipid in reconstituted lipoprotein. Consequently, at pH 4 the initial ionic interaction of lipids could involve positively charged areas of protein unaffected by the presence of sialoprotein. This conclusion is supported by the fact that addition of positively charged but not of uncharged or negatively charged amphipaths to lipids greatly diminished their interaction with protein.

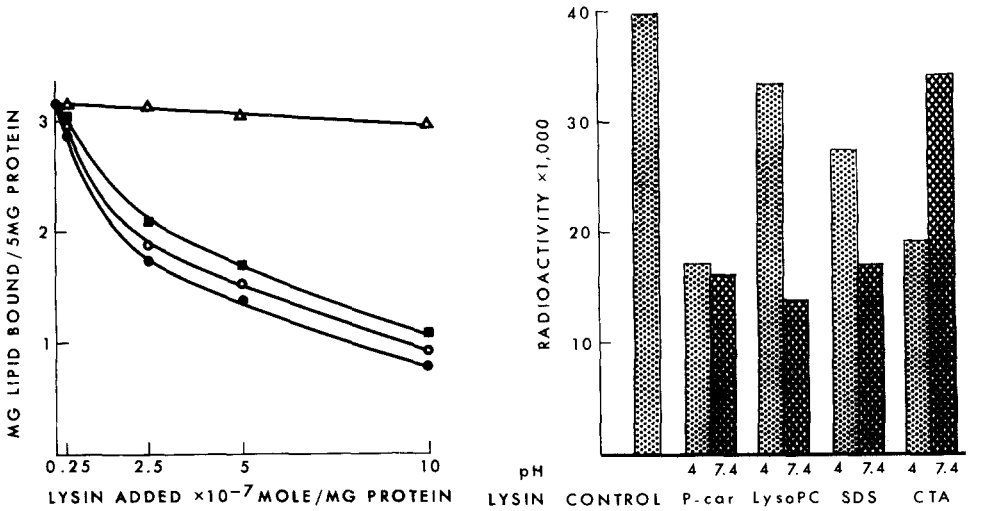

Fig. 5.The effect of acyl chain length of carnitine esters on lipoprotein dissociation. Conditions for this experiment are as stated for Fig. 5. ●—●; stearoyl carnitine, ○—○; palmitoyl carnitine, ■—■; myristoyl carnitine, △—△; decanoyl carnitine.

Fig. 6. A comparison of detergency of lysins on lipoprotein at pH 4.0 and pH 7.4. Lipoprotein prepared from 5 mg Maddy's protein and 5 mg of total lipid containing 75628 dpm was incubated with $1 \cdot 10^{-6}$ moles of lysin in 5 ml of 0.05 M acetate buffer pH 4.0 or in 5 ml 0.05 M Tris–acetate buffer, pH 7.4 at 37 °C for 30 min. After centrifugation the precipitate was washed twice and counted. P-car, palmitoyl carnitine; LysoPC, (1-palmitoyl)-lysolecithin; SDS, sodium dodecyl sulphate; CTA, cetyltrimethylammonium chloride.

It can be estimated from Fig. 4 that 1.25 μmoles of palmitoyl carnitine in the bulk phase can displace approximately 1.5 mg of lipid (equivalent to approximately 3 μmoles of lipid of average mol.wt 500) from reconstituted lipoprotein, yet on a molar basis apoprotein under similar conditions will bind no more palmitoyl carnitine than 20% of this amount of lipid released (*cf.* Table III). If the basic structure of the lipoprotein formed does indeed involve bilayers of lipid interrupted by apolar segments of protein as suggested in the case of Sweet and Zull[10], the release of lipids by smaller amounts of cationic detergents at pH 4 can be explained by a micellar dispersion of the bilayers. The positively charged micelles formed would be unable to reassociate with protein segments of like charge. Also their lyotropic mesomorphic character may be less compatible with lipoprotein formation in any case.

When lipoprotein is formed at pH 4 and the insoluble complex is transferred to a medium of pH 7.4, there results no dissociation of lipid and no increase in solubility of the lipoprotein. Yet this change in pH ought to neutralize positive charges due to imidazole groups in the protein which have been suggested as binding sites for lipid at pH 4 (ref. 9). Also the protein fraction as a whole would become more negatively charged. This further implies that once formed, the lipoprotein is stabilized by hydrophobic interactions between lipids and apolar segments of the protein. Addition of uncharged lysins would result in the formation of micelles with a net negative surface because at pH 7.4 the acidic character of phosphatidylserine and the inositides would be intensified. Since the protein bears net negative charge, recombination of

these micelles would not easily occur. The increased detergency at pH 7.4 of anionic lysins such as sodium lauryl sulfate could be explained on a similar basis. On the other hand, addition of a cationic detergent at physiological pH would result in the formation of positively charged micelles which are freer to recombine with the oppositely charged protein.

Besides possessing positively charged regions which favour binding to oppositely charged lipid micelles at pH 4, the apoprotein not treated with neuraminidase must contain other sites which can bind ionic amphipaths such as palmitoyl carnitine or lysolecithin. These sites need not be sialic acid groups, since the binding of palmitoyl carnitine is not markedly decreased in the presence of 1 M NaCl. This implies that hydrophobic sites in the protein fraction are accessible and directly implicated in this case. The accessibility of such sites to palmitoyl carnitine and not to complex lipid mixtures could be easily explained on the basis of the lyotropic mesomorphic properties of wedge-shaped amphipaths, being quite different from those of lipids adopting large aggregate forms in water. The slight decrease in binding of palmitoyl carnitine at high ionic strength (Table III) could be an indication that apoprotein or reconstituted lipoprotein binds these lysins in monomer form. It is well known that increase in ionic strength decreases the critical micelle concentration of ionic detergents. It follows from this that interaction of lysins with lipoprotein is hydrophobic in character and very likely involves monomeric penetration into the lipoprotein complex.

As regards the mechanism of lysis by acyl carnitines and lysolecithin, we have presented evidence indicating that their interaction with the membrane is hydrophobic[14]. There results from their penetration into labelled erythrocytes, a release of [^{3}H]cholesterol and [^{32}P]lecithin which is however not proportional to the extent of hemolysis. When constituents such as apoprotein or reconstituted lipoproteins are isolated and incubated with lysins, complexes are formed which are mainly hydrophobic in character. Assumably lysin penetration into the membrane is followed by similar interactions which cause release of lipid constituents. During lysis the ratio of lipid displaced to lysin added is usually high and consequently a simple exchange reaction is not involved[18]. Rather, these results are compatible with the idea that there are extensive regions in the membrane which contain lipid bilayers and dispersion of these aggregates as micelles by relatively fewer wedge-shaped molecules could account for massive loss of lipids accompanying detergent action. However the lytic action of amphipathic quaternary ammonium derivatives cannot be explained entirely on the basis of their ability to disperse lipid bilayers. These substances react hydrophobically with isolated membrane apoproteins and during lysis it is likely that they do so with proteins of intact membranes as well.

ACKNOWLEDGEMENT

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