INTERACTION OF LYSOLECITHIN MICELLES AND LECITHIN VESICLES WITH APOLIPOPROTEIN GLN I FROM SERUM HIGH DENSITY LIPOPROTEINS*

Roy B. Verdery III and Alex V. Nichols

Donner Laboratory, Lawrence Berkeley Laboratory
University of California, Berkeley, CA. 94720

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SUMMARY

Lysolecithin and apoLP-Gln I, mixed without sonication and analysed ultracentrifugally, formed a complex of density 1.063-1.21 g/ml. Formation of this lysolecithin-apolipoprotein complex was accompanied by changes in apolipoprotein conformation and fluorescence. ApoLP-Gln I, when incubated with lecithin vesicles, primarily formed complexes of density < 1.063 g/ml and formed few complexes of density 1.063-1.21 g/ml. Preliminary studies on mixtures containing apoLP-Gln I, lysolecithin, and lecithin vesicles are also described and suggest that apoLP-Gln I in a 1.063-1.21 g/ml complex can bind up to 200 molecules of phospholipid per molecule of apolipoprotein.

The physical-chemical properties of several isolated human serum apolipoproteins have been investigated following their sonic relipidation with lecithin (1-4). Sonication of mixtures (1:1 by weight) of apolipoproteins and lecithin results in formation of lipid-apolipoprotein complexes, primarily of d 1.063-1.21 g/ml, and in considerable change in apolipoprotein conformation (1). Interaction of apolipoproteins with phospholipids in the absence of sonication has been less extensively examined (2,3). In the present communication we report our observations on the interaction, during incubation without sonication, of apolipoprotein Gln I (apoLP-Gln I)** primarily with lysolecithin as micelles and secondarily with lecithin as homogeneous single-bilayer vesicles.

MATERIALS AND METHODS

ApoLP-Gln I was isolated from normal human serum high density lipoproteins by methods previously described (5,6). The apolipoprotein used gave a single

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** Apolipoprotein Gln I, with carboxy-terminal glutamine, is the major protein found in human serum high density lipoproteins, d 1.063-1.21 g/ml (11)

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band on polyacrylamide gel electrophoresis (7). Concentration of apolipoprotein was determined by the method of Lowry et al. (8). Lecithin (egg, General Biochemicals, Chagrin Falls, OH.) vesicles labeled with (^{14}C)lecithin (algal, New England Nuclear, Boston, MA.) were prepared by the method of Huang (9). Solutions containing lecithin were radioassayed by liquid scintillation counting. Chromatographically pure lysolecithin (Supelco, Bellefonte, PA.) was used.

Fluorescence measurements were made at 25°C on an Aminco-Bowman spectrophotofluorometer equipped with a temperature-controlled cell holder. Circular dichroism (CD) measurements were made on a Cary 6005, and mean residue ellipticity was calculated assuming 116.6 for the mean residue weight (10).

All mixtures were incubated in 0.5 ml at room temperature (23°C) for 30 minutes before analysis. Incubations without lecithin were performed in 0.0005 M NH_4HCO_3 pH 8.2, and incubations with lecithin were performed in 0.02 M NaCl, 0.002 M Tris-HCl pH 8.0.

Sample mixtures were fractionated by sequential preparative ultracentrifugation (114,000 xg, 24 hr, 15°C) at densities of 1.063 (NaCl) and 1.21 (NaCl-NaBr) g/ml. The top 2 ml after centrifugation at d 1.063 g/ml, the top 1 ml after centrifugation at d 1.21 g/ml, and the remaining subnatant at d 1.21 g/ml were designated d < 1.063, d 1.063-1.21, and d > 1.21 fractions, respectively. Estimations of apolipoprotein quantities in ultracentrifugally separated fractions were made by fluorescence determinations standardized with samples of known protein and lipid content (12).

RESULTS AND DISCUSSION

With increasing amounts of lysolecithin (Fig. 1), the fluorescence intensity of apoLP-Gln I increased and reached a maximum between 100:1 and 200:1 (molar ratio, lysolecithin:apolipoprotein). This change in fluorescence intensity is similar to that accompanying the interaction of lysolecithin with glu-

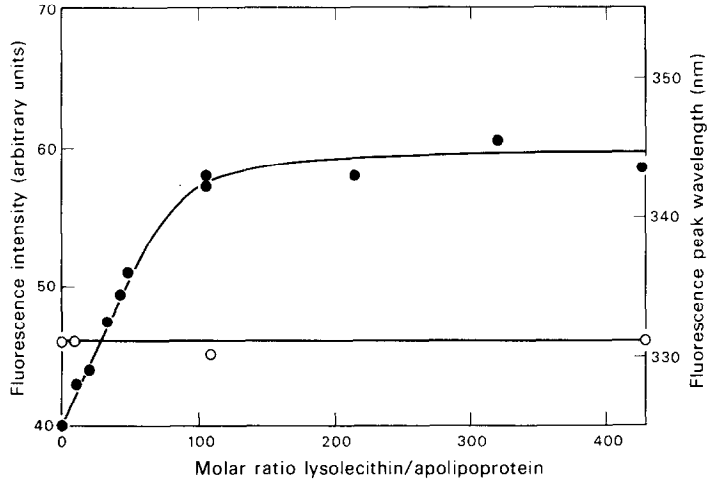

Fig. 1. Fluorescence intensity (●) and fluorescence peak wavelength (o) of apoLP-Gln I mixed with increasing amounts of lysolecithin. Fluorescence excitation was at 278 nm.

cagon (13) and suggests that some interaction between lysolecithin and apoLP-Gln I occurs in aqueous solution. At all amounts of added lysolecithin, the fluorescence peak wavelength (331 nm) remained constant and corresponded to the emission peak wavelength of tryptophan in an apolar environment (14).

Denaturation of apoLP-Gln I in aqueous solutions of guanidine HCl, as monitored by changes in fluorescence peak wavelength, occurred at a guanidine concentration of approximately 1 M (Fig. 2). In the presence of lysolecithin

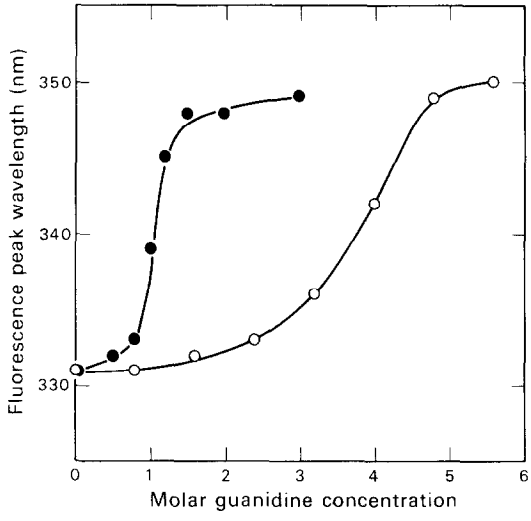

Fig. 2. Fluorescence peak wavelength of apoLP-Gln I (●) and a mixture of apoLP-Gln I and lysolecithin (o) at different guanidine HCl concentrations. Fluorescence excitation was at 278 nm.

(100:1 molar ratio, lysolecithin:apolipoprotein), the transition occurred at about 4 M guanidine, indicating that lysolecithin stabilized the apolipoprotein. Such stabilization is probably due to complex formation between lysolecithin and the apolipoprotein, since apolipoproteins are also protected against denaturation when combined with lipids in intact lipoproteins (15).

Changes in molar ellipticity caused by addition of lysolecithin to solutions containing apoLP-Gln I (Fig. 3) indicated an increase in helicity of the apolipoprotein. Such a change in the conformation of the apolipoprotein pro-

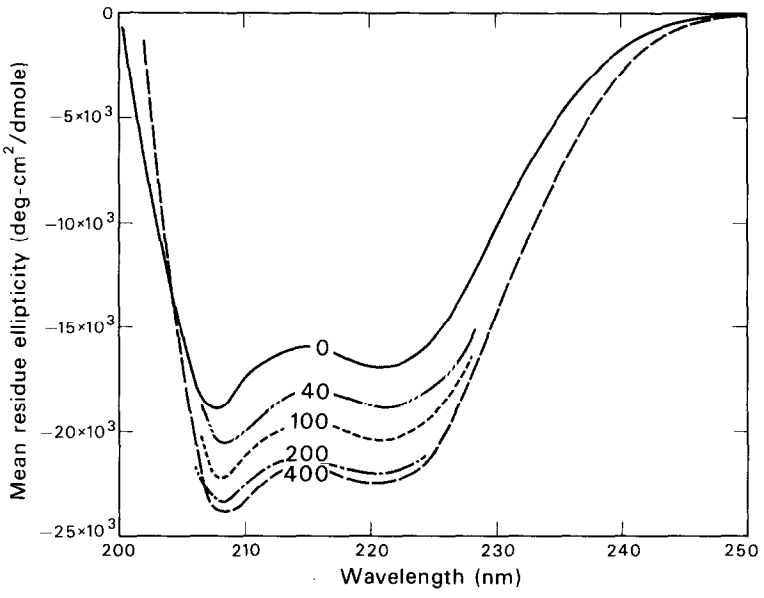

Fig. 3. Circular dichroism spectra of apoLP-Gln I with different amounts of added lysolecithin. Numbers refer to the molar ratio lysolecithin:apolipoprotein.

vides further evidence of interaction and binding of lysolecithin with apoLP-Gln I. ApoLP-Gln I showed as much change in molar ellipticity upon incubation with lysolecithin as has been reported to result from its cosonication with lecithin (1).

Complex formation during incubation of apoLP-Gln I with lysolecithin was further evaluated by ultracentrifugal fractionation of mixtures of apoLP-Gln I and lysolecithin (Table I). In these experiments, the amount of apoLP-Gln I was constant (0.14 mg) and was incubated with increasing amounts of lysolecithin. Flotation of apolipoprotein into the d 1.063-1.21 fraction clearly indicates complex formation between the apolipoprotein and lysolecithin in a mixture of molar ratio of 170:1 (lysolecithin:apolipoprotein); CD and fluorescence data (Figs. 1 and 3) suggest complexes were also formed at the 34:1 ratio, but such complexes must have been of density > 1.21 g/ml.

We conducted similar ultracentrifugal fractionation studies on mixtures of apoLP-Gln I and homogeneous single bilayer lecithin vesicles (Table II).

TABLE I

Distribution of apoLP-Gln I after ultracentrifugal fractionation of lysolecithin-apoLP-Gln I mixtures.

Fraction	ApoLP-Gln I distribution		
	no lysolecithin	34:1*	170:1
d < 1.063	0**	0	0
d 1.063-1.21	0	0	48
d > 1.21	100	100	52

* Molar ratio (lysolecithin:apolipoprotein) of mixture.
** % of recovered apolipoprotein.

TABLE II

Distribution of apoLP-Gln I and (^{14}C)lecithin after ultracentrifugal fractionation of lecithin vesicle-apoLP-Gln I mixtures.

Fraction	Component	Distribution of (^{14}C)lecithin and apoLP-Gln I			
		10:1*	30:1	100:1	1000:1
d < 1.063	apoLP-Gln I	0**	8	38	100
	(^{14}C)lecithin	84	92	96	98
d 1.063-1.21	apoLP-Gln I	0	2	4	0
	(^{14}C)lecithin	6	5	3	1
d > 1.21	apoLP-Gln I	100	90	58	0
	(^{14}C)lecithin	10	3	1	1

* Molar ratio (lecithin:apolipoprotein) of mixture.
** % of recovered component.

Apolipoprotein was maintained constant (0.092 mg) and incubated with increasing amounts of lecithin. Under these conditions, apoLP-Gln I formed complexes with lecithin of d < 1.063 g/ml and showed little tendency to form complexes of d 1.063-1.21 g/ml. These observations are of considerable interest since other forms of phospholipid-apolipoprotein interaction, which include cosonication of apoLP-Gln I and lecithin, and incubation of apoLP-Gln I with lyso-

lecithin, result in formation of complexes primarily of d 1.063-1.21 g/ml and few complexes of d < 1.063 g/ml (1). Our preliminary CD results indicate an increase in helical content of the apolipoprotein during complex formation with the lecithin vesicles.

Because of the significant differences in ultracentrifugal properties of the lysolecithin-apoLP-Gln I and lecithin-apoLP-Gln I complexes, we made a preliminary study of complexes formed during incubation of mixtures of apoLP-Gln I, lysolecithin, and lecithin. In this study a lysolecithin:apoLP-Gln I mixture, preincubated at a molar ratio of 170:1 (0.14 mg apolipoprotein), was further incubated with increasing concentrations of lecithin vesicles (Table III). The

TABLE III

Distribution of apoLP-Gln I and (^{14}C)lecithin after ultracentrifugal fractionation of lecithin vesicle-lysolecithin-apoLP-Gln I mixtures.

Fraction	Component	Distribution of (^{14}C)lecithin and apoLP-Gln I				
		17:170:1*	57:170:1	170:170:1	510:170:1	1000:170:1
d < 1.063	apoLP-Gln I	2**	3	9	52	78
	(^{14}C)lecithin	1	12	66	88	95
d 1.063-1.21	apoLP-Gln I	63	68	63	43	20
	(^{14}C)lecithin	65	68	24	10	4
d > 1.21	apoLP-Gln I	35	29	28	5	2
	(^{14}C)lecithin	34	20	10	2	1

* Molar ratio (lecithin:lysolecithin:apolipoprotein) of mixture.
** % of recovered component.

molar ratio of 170:1 was selected because substantial complex formation between the two components was previously observed at this value. When a relatively small amount of lecithin was present, lecithin:lysolecithin:apoLP-Gln I molar ratio 57:170:1, almost all of the lecithin was found in association with the apolipoprotein and both were distributed between the d 1.063-1.21 and d > 1.21

fractions. This was in contrast to the observations described above where, in the absence of lysolecithin, all of the lecithin floated at d < 1.063 g/ml and the apolipoprotein was either in the d < 1.063 or d > 1.21 fractions. Separate ultracentrifugal fractionation of a lecithin-lysolecithin mixture (containing no apoLP-Gln I) of molar ratio 57:170 (lecithin:lysolecithin) resulted in approximately 74% of the labeled lecithin floating into the d < 1.063 fraction. Hence, the incorporation of lecithin into the d 1.063-1.21 fraction in the 57:170:1 mixture suggests that lysolecithin "primes" the apolipoprotein, permitting interaction and complex formation with lecithin.

At a molar ratio of 170:170:1 (lecithin:lysolecithin:apoLP-Gln I), most of the recovered apoLP-Gln I was in the d 1.063-1.21 fraction while most of the lecithin was in the d < 1.063 fraction. It would thus appear that incorporation of lecithin into the d 1.063-1.21 fraction can proceed only up to some saturating amount, with excess lecithin "spilling over" into the d < 1,063 fraction. If it is assumed that the molar ratio of lecithin:lysolecithin in the d 1.063-1.21 fraction is the same as in the whole mixture, then a molar ratio of total phospholipid:apoLP-Gln I of about 200:1 can be calculated for the d 1.063-1.21 fractions isolated from each of the mixtures containing lecithin, lysolecithin and apoLP-Gln I. This suggests a definite limit in the binding capacity of apoLP-Gln I for the phospholipids investigated. Further work is in progress to determine the phospholipid binding capacity of apoLP-Gln I.

It should also be noted that even at the highest level of lecithin in the incubation mixture (molar ratio 1000:170:1), 20% of the apoLP-Gln I was still in the d 1.063-1.21 fraction. In the absence of lysolecithin, the ultracentrifugal fractionation of a comparable incubation mixture consisting of lecithin and apoLP-Gln I at a molar ratio of 1000:1 (lecithin:apoLP-Gln I) showed only negligible amounts (< 4%) of apoLP-Gln I in the d 1.063-1.21 fraction.

These results show that preincubation of apoLP-Gln I with lysolecithin facilitates incorporation of lecithin into complexes of d 1.063-1.21 g/ml. In the absence of lysolecithin, formation of lecithin-containing complexes of

d 1.063-1.21 g/ml is negligible and, under the conditions used, other facilitating procedures such as sonication are required for their production (16).

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