ARTIFICIAL PROTEOLIPIDS:

CYTOCHROME c - DETERGENT COMPLEXES SOLUBLE IN ISOOCTANE

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SUMMARY

Some novel and artificial proteolipid systems which consist of cytochrome c and detergents of both negative (sodium dodecylsulfate) and positive (laurylpyridinium chloride) charge are described. At molar ratios of about 30 of each type of detergent to cytochrome c and at pH 8, 20-30% of the total cytochrome c can be extracted at room temperature into isooctane. Thus, simple synthetic detergents are able to effectively replace phospholipids, which are usually used in conjunction with alcohols or divalent cations for rendering proteins soluble in non-polar media.

It is known that various proteins become soluble in organic solvents by the addition of both lecithin and acidic phospholipids (1-6). In various cases, alcohols (2), divalent cations (7) or the use of low pH (6) may be required for effective extraction into the organic phase. It has been suggested that the acidic phospholipids are essential for making the protein complex soluble since they neutralize positive charges on the protein (2, 5) and enclose it with a non-polar envelope (8, 6).

The present communication reports novel proteolipid systems in which simple synthetic detergents effectively replace phospholipids, which are usually not well defined with regard to their composition. Alcohols, divalent cations, or low pH are not required in this case.

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EXPERIMENTAL

Materials were obtained from the following sources: cytochrome c (horse heart, type III) from Sigma; L-3-lecithin (pure, synthetic dipalmitoyl-L-3-lecithin) from Koch-Light; sodium dodecylsulfate (Texapon L100, recrystallized, ~99%) from Henkel; laurylpyridinium chloride (Dehyquart C, cryst., 94%, 6% water) from Henkel; isooctane, spectroscopically pure, from Fluka. Ethanol was analytical grade.

The aqueous phase (1-3 ml) was prepared from concentrated aqueous stock solutions except for lecithin, which was either dispersed in water or dissolved in ethanol. An equal volume of isooctane was added, and the two phases were shaken in a vortex mixer for about 10 minutes at room temperature. The phases separated either by low-speed centrifugation or by standing for about an hour. The amount of cytochrome c in the organic phase was determined by measurement of the absorbances at 25° C of the Soret-band maxima in both aqueous and organic phases, using a Cary Model 14 recording spectrophotometer. Assuming the same extinction coefficients in both phases (2), it appeared that the sum of the protein in both solvents agreed with the initial amount added.

RESULTS AND DISCUSSION

As is evident from the preliminary results presented in Table I, the anionic detergent SDS*, added to cytochrome c in the presence of lecithin and ethanol, renders the protein soluble in the organic solvent phase. At constant lecithin and ethanol concentrations, the amount of soluble cytochrome c depends critically on the molar ratio of added SDS to protein. A maximum amount of cytochrome c (about 70%) was extracted at a molar ra-

*Abbreviations: SDS, sodium dodecylsulfate; LPC, laurylpyridinium chloride.

TABLE I

EXTRACTION OF CYTOCHROME c INTO ISOOCTANE IN THE PRESENCE OF DETERGENTS AND PHOSPHOLIPIDS

Initial conditions, aqueous phase							
Cytochrome c concn. $\times 10^5$ (M)	SDS concn. $\times 10^5$ (M)	LPC concn. $\times 10^5$ (M)	pH	Molar ratio SDS/cytochrome c	Molar ratio LPC/cytochrome c; or lecithin/cytochrome c	Fraction of protein soluble in organic phase	Remarks
5	25	--		5	20	0.38	1.0×10^{-3} M lecithin and 30% ethanol (v/v) added initially
5	40	--		8	20	0.60	
5	45	--	8 to 9	9	20	0.73	
5	50	--		10	20	0.39	
5	100	--		20	20	0.06	
5	25	--		5	--	0.42	
5	50	--	8 to 9	10	--	0.62	colored emulsions formed
5	100	--		20	--	0.31	
5	700	--		140	--	0.0	
8	80	80		10	10	0.04	
8	120	120		15	15	0.19	
8	160	160		20	20	0.17	
8	200	200	7.4 to	25	25	0.18	
8	240	240	7.7	30	30	0.18	
8	320	320		40	40	0.18	
8	120	80		15	10	0.0	
8	120	160		15	20	0.0	
8	240	240	5.7	30	30	0.12	
8	240	240	6.7	30	30	0.18	
8	240	240	7.7	30	30	0.26	
8	240	240	8.0	30	30	0.22	
8	240	240	8.4	30	30	0.23	
8	240	240	9.6	30	30	0.08	

tio of SDS to protein of 9:1. By comparison with previous work on systems involving acidic phospholipids and lecithin in which similar optimal ratios were found (2, 4) it appears that the anionic detergent may effectively replace the acidic phospholipids. In the absence of both lecithin and ethanol, colored emulsions containing the cytochrome c are formed, again with maximum yield at a molar ratio of about 9:1 between SDS and the protein (see Table I).

As mentioned above, effective neutralization of the charges on the protein by the detergent would occur with simultaneous formation of a non-polar environment around the protein by the hydrocarbon chains. Further addition of detergent, with binding at the low-affinity sites (9) would increase the number of excess negative charges, which may decrease the solubility of the complex in the organic medium.

The role of the zwitterionic lecithin in rendering the protein complexes soluble is less easily comprehended (4). Assuming the presence of both positive and negative amphiphiles to be essential for formation of isooctane-soluble protein complexes, it was attempted to use mixtures of SDS and the cationic detergent LPC. According to the results shown in Table I, LPC is indeed able to form isooctane-soluble cytochrome c complexes in conjunction with SDS, and, therefore, may replace the lecithin. The aliphatic cationic detergent cetyltrimethylammonium bromide may also be used instead of LPC. In these systems, ethanol is not required. Maximum extraction occurs at pH 8 of the aqueous phase (LPC : SDS = 1:1). In the absence of either SDS or LPC, or at molar ratios between them differing significantly from 1:1, practically no extraction of the cytochrome c into isooctane could be observed.

These systems described, involving relatively simple and chemically defined detergents, are considered to serve as new and convenient models for the study of proteins and their complexes in non-polar media, as may apply to membrane proteins. A more detailed investigation of these systems is in progress and will be reported elsewhere.

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