Biochimica et Biophysica Acta, 455, (1976) 796–805

BBA 77531

EFFECT OF THE HYDROPHILE-LIPOPHILE BALANCE OF NON-IONIC DETERGENTS (TRITON X-SERIES) ON THE SOLUBILIZATION OF BIOLOGICAL MEMBRANES AND THEIR INTEGRAL *b*-TYPE CYTOCHROMES

ERIK SLINDE and TORGEIR FLATMARK

Department of Biochemistry, University of Bergen, Årstadveien 19, N-5000 Bergen (Norway)

(Received May 13th, 1976)

SUMMARY

The solubilization of four integral membrane proteins (i.e. cytochrome *b*-561 of the chromaffin granule membrane, cytochrome b_5 of the endoplasmic reticulum and the mitochondrial *b*-type cytochrome(s) as well as cytochrome *c* oxidase) has been studied at 0 °C using the non-ionic detergents of the Triton X-series having the common hydrophobic 4(1,1,3,3-tetramethylbutyl)phenoxy (*t*-octyl-phenoxy) group and a variable average number ($\bar{n}$) of polar ethylene oxide units added. Following a pre-extraction of peripheral membrane and matrix proteins with low and high salt concentration and a weak non-ionic detergent (Tween 20, average hydrophile-lipophile balance ($\overline{\text{HLB}}$) = 16.7), the amount of heme proteins solubilized by subsequent Triton X-solutions was measured. With the detergents tested the degree of solubilization decreased in the sequence cytochrome *b*-561 > cytochrome b_5 > mitochondrial cytochrome(s) *b* and parallelled the effect of the detergents on light scattering and the phospholipid to protein ratio of the three membranes. For all the *b*-cytochromes, the solubilizing power of the detergent increased with decreasing average length of the polar ethylene oxide chain and the hydrophile-lipophile balance as long as clouding did not occur (e.g. Triton X-114, $\bar{n}$ = 7.5 and $\overline{\text{HLB}}$ = 12.4). Thus, the greatest difference in the degree of solubilization of the three cytochromes was observed with Triton X-405 ($\bar{n}$ = 40 and $\overline{\text{HLB}}$ = 17.9). All the cytochromes were most efficiently solubilized (i.e. approx. 90 %) by Triton X-100 ($\bar{n}$ = 9.5 and $\overline{\text{HLB}}$ = 13.5).

INTRODUCTION

The use of soluble amphiphiles (detergents) appears to provide the most generally useful solubilization method presently available for integral membrane proteins. In the last few years polyoxyethylated phenols like Triton X-100 has been

Abbreviations: *t*-octyl-phenoxy, 4(1,1,3,3-tetramethylbutyl)phenoxy; $\overline{\text{HLB}}$, average hydrophile-lipophile balance.

TABLE I

CHEMICAL AND PHYSICAL PROPERTIES OF THE NON-IONIC DETERGENTS OF THE TRITON X-SERIES[a]

Detergent	Average number of ethylene oxide moieties[b]	Cloud point (°C)[c]	Average hydrophile - lipophile balance	Average molecular weight	Critical micellar concentration (%, w/w)
Triton X-15	1	insol.	3.6	250	—
Triton X-35	3	insol.	7.8	338	—
Triton X-114	7–8	22	12.4	536	0.009
Triton X-100	9–10	65	13.5	628	0.01
Triton X-102	12–13	88	14.6	756	—
Triton X-165	16	100	15.8	910	—
Triton X-305	30	100	17.3	1526	—
Triton X-405	40	100	17.9	2066	—

[a] From Rohm and Haas Company Independence Mall West, Philadelphia, Pa. 19105
[b] CH_3-$C(CH_3)_2$-CH_2-$C(CH_3)_2$-C_6H_4-$O(CH_2$-$CH_2O)_nH$.
[c] 1 % solution.

widely used (for review, see ref. 1) e.g. in the study of membrane bound cytochromes. Their use has, however, been largely empirical, and in our attempts to isolate the mitochondrial *b*-type cytochromes we have found few guidelines on the mechanism of action of these detergents [1].

Since a large number of oligomers is available in the Triton X-series having a common hydrophobic *t*-octyl-phenoxy group and different average number of ethylene oxide units added (Table I), it is possible to study the role of the hydrophilic moiety on the efficiency of solubilization of integral membrane proteins. In the present study such experiments have been conducted on three different metabolically active biological membranes of mammalian cells which differ in composition and function. The proteins selected were cytochrome *b*-561 of the chromaffin granule membrane recently isolated in highly purified form [2] and shown to be the only heme protein of this membrane [3]. The other proteins were microsomal cytochrome b_5 which has been shown to be an amphiphilic protein [4] and the *b*-cytochromes of the mitochondrial inner membrane which are very hydrophobic proteins [5]. The main advantage of using these heme proteins as a reference is that they all have been isolated and partly characterized and their assay is relatively simple and accurate due to their prosthetic group protoheme IX. In addition, the characteristic α-absorption bands in their reduced form offer a convenient control of their yield as well as possible modification as a result of the solubilization process [6].

MATERIALS AND METHODS

Isolation of subcellular particles. Bovine kidney cortex mitochondria were isolated by a large scale procedure using the sedimentation conditions previously described [7]; the angle GSA rotor and the swinging bucket HB-4 rotor of the Sorwall RC2-B refrigerated centrifuge were used. The chromaffin granules were isolated from fresh bovine adrenal glands [8] and the pellet P_c was used. Bovine liver

microsomes were isolated as described [9]. The pellets of the different organelles were frozen immediately after isolation and stored in liquid nitrogen until used.

Pre-extraction of the isolated subcellular particles. Since all the *b*-type cytochromes studied are known to be hydrophobic integral membrane proteins [2, 4, 5], sequential extraction has been employed to remove water soluble proteins and peripheral membrane proteins. The pellets of subcellular particles were suspended in 10 mM phosphate buffer pH 6.9 (adjusted with KOH) and resuspended in a Thomas C homogenizer. The volume was adjusted to 42 ml and sedimented in the Sorvall SS-34 rotor (R_{max} = 10.6 cm, R_{min} = 5.7 cm, 15 000 rev./min, 30 min, S_{min} = 1400 S). (S_{min}, the lightest particle to be sedimented completely). The supernatant was decanted off, and the sediment was treated as above, but now with 1 M KCl, 10 mM phosphate buffer, pH 6.9, and re-sedimented. The final pre-extraction was carried out as above using the weak non-ionic detergent Tween 20 (2 % (w/v)), 10 mM phosphate, pH 6.9) with a $\overline{HLB}$ of 16.7 [10].

Extraction with polydisperse non-ionic detergents of the Triton X-series. The pre-extracted membrane pellets were subjected to a 2 % (w/v) detergent solution of the Triton X-series containing 20 % (w/v) glycerol, 1 mM EDTA, 10 mM phosphate at pH 6.9. Since glycerol has been successfully used to protect e.g. cytochrome P-450 from denaturation [11, 12], this compound was introduced to protect the *b*-type cytochromes studied. EDTA was introduced to prevent lipid peroxidation [13]. The membrane preparations were homogenized in a Thomas A homogenizer and sedimented in Corex tubes in the Sorvall SS-34 rotor (R_{max} = 10.2 cm, R_{min} = 7.0 cm, 15 000 rev./min, 30 min, S_{min} = 850 S). The supernatant was decanted off, and the pellets resuspended by homogenization in the buffer containing Triton X-100. All experiments were carried out at 0–4 °C.

Spectrophotometry. Difference spectra were determined in a Shimadzu MPS 5000 recording spectrophotometer calibrated against the α-band of cytochrome *c* (550 nm). Baseline corrections were performed when necessary. The *b*-type cytochromes were assayed at 25 °C by the difference in absorbance at the α-peak and the trough at higher wavelengths after reduction with solid dithionite. The wavelength couple used was 561–575 nm for cytochrome *b*-561, 557–575 nm for cytochrome b_5, 561.5–575 nm for the mitochondrial *b*-type cytochromes and 605–630 nm for cytochrome *c* oxidase. Due to clouding of the non-ionic detergent Triton X-114 at this temperature (Table I), samples containing this detergent were diluted 1 : 1 with a 5 % (w/v) Triton X-405 solution prior to spectrophotometric analysis.

The effect of detergents on the equilibrium light scatter of the membrane suspensions was measured in a dual beam instrument (Shimadzu MPS-50L recording spectrophotometer). Using 520 nm light [14], the intensity of the direct beam (0°) was measured, i.e. $A' = (I_0/I_p+I_d)$ where I_0, I_p and I_d represent the incident light, the parallel transmitted and the diffuse transmitted light, respectively. The membrane suspension was added to the basic incubation medium to a final concentration of 0.7–1.4 mg protein/ml and the equilibrium light intensity thus measured represents the value of 0 % solubilization. 100 % solubilization was arbitrarily defined as the light intensity obtained when 6 M urea containing 2 % (w/v) deoxycholate pH 8.5 served as the suspension medium; the changes varied from 85 to 95 % of the initial apparent absorbance. The extent of the optical change brought about by the non-

ionic detergents was expressed in percent of this value. The temperature was measured in the cuvette by a thermocouple, and the experiments were performed at 2 °C.

Hydrophobicity/fractional charge ratio. The hydrophobicity/fractional charge ratio [15] was calculated from the amino acid composition of the *b*-type cytochromes as described [16].

Protein. Due to interference of the detergents with the ordinary Lowry method a modified version of this method was used [17].

Chemicals. The Triton X-series, a product of Rohm and Haas Company was obtained through the Sigma Chemical Co.. Tween 20 SD was from Atlas Chemical (Germany). The detergents were used as supplied by the manufacturers.

RESULTS

The effects of the non-ionic detergents on the solubilization of specific membrane proteins as well as on the phase transition of the phospholipid bilayer reported in the present study concerned systems in equilibrium and the kinetic aspects have not been considered. Although the time required to reach equilibrium was not exactly determined for each organelle, solubilization in dilute aqueous solutions appears to be a quite rapid process as measured by light scattering [18, 19]. The monomeric species of the detergents are the active solubilizing forms [20] and a constant free concentration of the detergents was maintained by selecting the total concentrations far above their critical micellar concentration (Table I) and the critical threshold concentration determined for the damage of liposomes of egg lecithin [21]. The detergent lipid ratio (by weight) was > 10 in all cases i.e. far above that needed for phase transition in pure phospholipid liposomes and biological membranes [1].

Pre-extraction of the isolated subcellular particles

All the subcellular particles were pre-extracted, first by hypoosmotic shock followed by high salt concentration and finally by Tween 20 as described in Methods section. Pre-extraction with Tween 20 has previously been successfully used in studies on cytochrome *b*-561 from chromaffin granule membranes [2] and was performed in order to make the membrane preparations comparable on a relative basis. Part of the Tween 20 may, however, bind to the membranes and slightly increase the concentration of species with the effective hydrophile-lipophile balance in the final solubilization (for review, see ref. 1).

Experimental evidence has recently been presented that rat liver mitochondria contain a number of *b*-type cytochromes some of which appear to be extractable by KCl [22]. The α-band of these cytochromes has been found to be centered at 556 nm and 558 nm [22]. On the other hand, the integral *b*-type cytochromes of the inner membrane (respiratory chain), with their α-bands centered at higher wavelengths (for review, see ref. 23), have been found not to be extractable under such mild conditions [5]. Using cytochrome *c* oxidase as a marker enzyme of the mitochondrial inner membrane we could not detect (spectroscopically) any solubilization of this membrane by the pre-extraction procedure.

The chromaffin granules contain some (approx. 40 %) cytochrome *b*-561 that is released by the three pre-extractions.

In the case of the microsomal membrane preparations 37 % of the cytochrome

TABLE II

Solubilization of cytochrome *b*-561 of chromaffin granule membrane, microsomal cytochrome b_5, mitochondrial *b*-cytochromes and cytochrome *c* oxidase. 2.5 g (wet wt.) of chromaffin granules, 2 g (wet wt.) of microsomes and 20 g (wet wt.) of mitochondria were pre-extracted as described in Material and Methods. The pre-extracted membranes were then exposed to non-ionic detergents (2 %, w/v) of the Triton X-series. The final protein concentrations were 0.3 mg/ml (chromaffin granules), 1.2 mg/ml (microsomes) and 6.5 mg/ml (mitochondria).

Detergent*	Cytochrome *b*-561		Cytochrome b_5		Mitochondrial *b* cytochromes		Cytochrome *c* oxidase	
	Solubilized (%)	Recovery (%)	Solubilized (%)	Recovery (%)	Solubilized (%)	Recovery (%)	Solubilized (%)	Recovery (%)
Triton X-405	70	102	25	100	3	84	0	88
Triton X-305	73	102	32	98	5	96	0	104
Triton X-165	80	98	69	104	14	102	1	105
Triton X-102	81	102	79	102	29	100	2	103
Triton X-100	88	95	93	100	88	103	88	101
Triton X-114	91	96	90	99	83	105	28	78

* For details see Table I.

b_5 was solubilized by Tween 20, whereas osmotic shock and high salt concentration has no solubilizing effect on this protein.

Solubilization of cytochromes by polyoxyethylated t-octyl-phenols

The membrane fractions obtained by pretreatment with hypoosmotic shock, high salt concentration and Tween 20, were exposed to 2 % (w/v) solutions of the various Triton X-species. The solubilization of the three different *b*-type cytochromes is shown in Table II and Fig. 1 summarizes the effect of the average hydrophile-lipophile balance value of the detergents on the degree of solubilization. It is seen that the cytochrome *b*-561 of the chromaffin granule membranes is solubilized rather efficiently with any of the detergents used (Table II and Fig. 1), whereas the microsomal cytochrome b_5 is brought into solution less efficiently the higher the average hydrophile-lipophile balance. On the other hand, only a minor fraction of the mitochondrial *b*-cytochromes is solubilized by detergents of low average hydrophile-lipophile balance (Table II and Fig. 1). For comparison, it is seen that only Triton X-100 and X-114 are able to solubilize cytochrome *c* oxidase at a measurable extent. From Table II and Fig. 1 it is also seen that Triton X-100 is the most effective detergent for the solubilization of all the membrane proteins studied. Similar observations have been reported for the solubilization by non-ionic detergents of the core component of a virus [24], integral proteins of a bacterial membrane [25], and of hormonally sensitive adenylate cyclase [26].

Phase transition of the phospholipid bilayer

Solubilization of biological membranes by detergents involves a disruption of the phospholipid bilayer and the formation of mixed phospholipid-detergent micelles

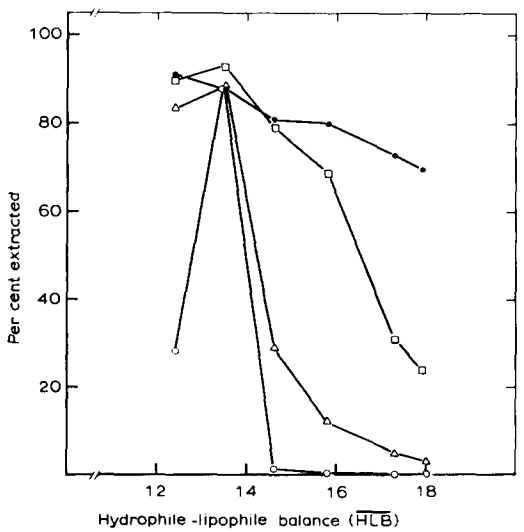

Fig. 1. Solubilization of cytochrome *b*-561 of chromaffin granule membranes, (●); microsomal cytochrome b_5, (□); mitochondrial *b*-cytochromes, (△) and cytochrome *c* oxidase, (○) by non-ionic detergents (Triton X-series) with different $\overline{\text{HLB}}$ values. For experimental details see Table I.

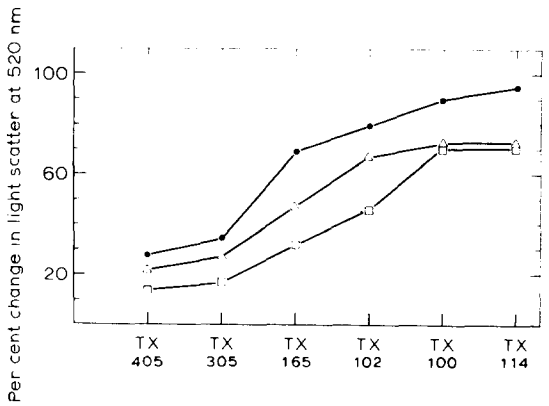

Fig. 2. Solubilization of chromaffin granule membranes (●) (0.82 mg protein/ml); microsomes (△) (0.71 mg protein/ml) and mitochondrial membranes (□) (1.4 mg protein/ml); as measured by a change in the equilibrium light scatter; 2 °C.

[1]. In the present study the transition from bilayer to the mixed micellar form was followed by measurement of the change in 0° light scattering using the wavelength 520 nm [14] where the pigments of the three membranes do not significantly absorb the incident light.

Aliquots of the membrane preparations (0.7–1.4 mg protein/ml) were exposed to a 2% (w/v) solution of the various species of the Triton X-series at 2 °C for at least 10 min. Based on the change in 0° light scattering thus induced, it is seen from Fig. 2 that for all the membranes studied the extent of phase transition, i.e. solubilization, depends on the chain length of the ethylene oxide moiety of the detergent, being highest with detergents having a low average hydrophile-lipophile balance.

DISCUSSION

It is now generally accepted that the phospholipids of biological membranes are arranged as a bilayer which is largely fluid under physiological conditions (for review, see ref. 27). The bilayer is interrupted by globular protein molecules (integral proteins) which are amphiphilic, with their hydrophobic ends embedded in the bilayer, and their hydrophilic ends protruding into the aqueous phase. The solubilization of such integral membrane proteins by detergents may therefore involve two primary processes i.e. (1) disintegration of the phospholipid bilayer and (2) binding of the detergent to proteins, disposed in the membrane either as monomeric molecular species or as specific and functional subunit aggregates, to form soluble protein-detergent complexes some of which may contain lipid.

The fluidity of the membrane lipids influences the effect of detergents on the phospholipid bilayer [1, 21]. In the present comparative study the temperature was selected well below the phase transition temperature for the membrane phospholipids [28] in order to eliminate the effect of a variation in the physical state of the bilayer lipids of the membranes as well as to protect the heme proteins from denaturation [6]. Thus, in order to account for the difference in the degree of solubilization of the four integral membrane proteins observed in the present study other possibilities

should be considered, notably the difference in the phospholipid to protein ratio and the phospholipid composition of the three membranes as well as the physico-chemical properties of the proteins and their mode of association with the membrane. Furthermore, the criteria of protein solubilization should also be considered.

Solubilization of membrane proteins is generally defined in terms of experimental convenience. When centrifugation is applied as the discriminating analytical step, solubilization usually means that the material under study does not sediment at a centrifugal effect of $10^7 \times g \cdot \text{min}$ [29]. This criterion is highly arbitrary since the sedimentation coefficient of (lipo)-protein-detergent complexes and detergent-free proteins depends on the density and viscosity of the medium, the R_{max} and R_{min} of the liquid column as well as the size and density of the complexes/proteins. If for instance the compartment dimensions are that of the commonly used Beckman Ti 50 angle-rotor ($R_{min} = 3.8$ cm, $R_{max} = 8.1$ cm) the lightest complex that sediments completely in the actual medium at $10^7 \times g \cdot \text{min}$ corresponds to an *S*-value of 76 S and a molecular weight of approx. $3.5 \cdot 10^6$. In the present study, however, the lightest complex which sediments completely was selected to have an *S*-value of 850 S in the actual medium (Table II and Fig. 1), which means that the supernatant may contain lipoprotein-detergent complexes of molecular weight up to at least $4 \cdot 10^7$. This high value was selected in order to recover in the supernatant as quantitatively as possible even large protein-detergent complexes which may easily be formed between hydrophobic lipoproteins and non-ionic detergents.

The light scattering experiments described in this paper provide additional evidence for the conclusion, based on model studies on liposomes [21], that the overall phospholipid composition contributes to the difference in the extent of solubilization. From Fig. 2 it is seen that the transition of the phospholipids from bilayer to the mixed micellar form is more extensive with all the detergents, the higher the phospholipid content (i.e. mitochondria < microsomes < chromaffin vesicles). Thus, the overall lipid to protein ratio, expressed as μmol phospholipid/mg protein has been determined as 0.29–0.34 (mitochondrial inner membrane [30]), 0.31 and 0.41 (smooth and rough endoplasmic reticulum [30]) and 2.2 (chromaffin granule membrane [31]). It should also be noted that the chromaffin granule membrane in addition has an unusually high content (15 %, by weight) of lysolecithin (for review, see ref. 32) which may contribute to the sensitivity of these membranes to solubilization even by non-ionic detergents of high average hydrophile-lipophile balance. On the other hand, physico-chemical properties of the four integral membrane proteins and their mode of binding to the respective membranes may also contribute to the observed difference in their resistance to solubilization by non-ionic detergents.

Although all the three *b*-type cytochromes do contain a higher percentage of hydrophobic amino acid residues [2, 4, 5] than one would statistically expect for soluble proteins [33], there seems to be no correlation between the hydrophobicity/fractional charge ratio [15] and the degree of solubilization. The hydrophobicity/fractional charge ratio has been calculated from the previously determined amino acid composition of the *b*-type cytochromes [2, 4, 5] and found to be $3.79 \cdot 10^3$ for cytochrome *b*-561, $2.13 \cdot 10^3$ for cytochrome b_5, and $5.19 \cdot 10^3$ as an average value for the mitochondrial *b*-type cytochromes. The differential solubility of the three cytochromes may therefore rather reflect a difference in the association with the membrane notably, how far the proteins penetrate the membrane and/or to what extent

they interact with other polypeptides in the membrane. The experimental evidence so far indicates that cytochrome *b*-561 [19, 34] and cytochrome b_5 [35] are both easily accessible to reaction with hydrophilic as well as macromolecular electron donors and acceptors. On the other hand, the mitochondrial *b*-type cytochromes appear not to be accessible to hydrophilic redox active components from either side of the membrane [23]. Thus, the differential solubility of the three *b*-type cytochromes may partly be explained by a variable degree of penetration of the phospholipid bilayer by the proteins. This conclusion is also supported by the fact that cytochrome *c* oxidase, known to span the mitochondrial inner membrane (for review, see ref. 36], is hardly solubilized by detergents of $\overline{HLB} > 14$ even under conditions where a high degree of phase transition of the inner membrane phospholipid bilayer has taken place (Fig. 2).

In the present study no distinction has been made between the possibility of differential extraction of the two *b*-type cytochromes of the mitochondrial inner membrane [23]. However, the Triton X-100 extract contains both cytochromes in approximately equal amounts and can be separated by velocity sedimentation at a very high centrifugal effect as compared to that used in the present study (unpublished results).

From Table II it is seen that a difference in solubility of the mitochondrial *b*-type cytochromes and cytochrome *c* oxidase exist, notably in Triton X-114 which efficiently solubilizes the mitochondrial inner membrane (Fig. 2) and approx. 90 % of the *b*-type cytochromes whereas only approx. 25 % of the cytochrome *c* oxidase is non-sedimentable at the selected centrifugal effect. Since Triton X-114 has a rather low average hydrophile-lipophile balance (Table I and Fig. 1) and is a very efficient membrane solubilizer (Fig. 2), the cytochrome *c* oxidase, which sediments at the low centrifugal effect applied, must represent rather large aggregates of this protein. This difference in solubility between the *b*-type cytochromes and cytochrome *c* oxidase may be explained by the tendency of the latter cytochrome to form membranes in the presence of this detergent [37], and therefore represents a useful approach in the isolation of these mitochondrial cytochromes.

A major problem in the use of non-ionic detergents for the solubilization of membranes is their polydispersity, which is a result of the synthetic polymerization process [38]. It is, however, probable that an even higher selectivity may be obtained in the extraction process by the use of monodisperse non-ionic detergents, which should be possible to prepare not only on a small scale basis [39, 40].

ACKNOWLEDGEMENTS

This study was supported in part by grant No. C.11.14-3 from the Norwegian Research Council for Science and the Humanities. We are indepted to Professor M. Farstad, Laboratory of Clinical Biochemistry for the use of his spectrophotometer, and to Mrs. T. Marøy and Mr. C. Punvani for technical assistance.

REFERENCES

1 Helenius A. and Simons, K. (1975) Biochim. Biophys. Acta 415, 29–79
2 Silsand, T. and Flatmark, T. (1974) Biochim. Biophys. Acta 359, 257–266
3 Terland, O., Silsand, T. and Flatmark, T. (1974) Biochim. Biophys. Acta 359, 253–256

4 Spatz, L. and Stittmatter, P. (1971) Proc. Natl. Acad. Sci. U.S. 68, 1042–1046
5 Yu, C., Yu, L. and King, T. E. (1975) Biochim. Biophys. Res. Commun. 66, 1194–1200
6 Garewal, H. S. and Wasserman, A. R. (1974) Biochemistry 13, 4063–4071
7 Slinde, E., Morild, E. and Flatmark, T. (1975) Anal. Biochem. 66, 151–158
8 Helle, K. B., Flatmark, T., Serck-Hanssen, G. and Lønning, S. (1971) Biochim. Biophys. Acta 226, 1–8
9 Kamath, S. A. and Rubin, E. (1972) Biochim. Biophys. Res. Commun. 49, 52–59
10 Becher, P. (1967) in Nonionic Surfactants (Schick, M. J., ed.), pp. 604–626, Marcell Dekker Inc., New York
11 Imai, Y. and Sato, R. (1974) J. Biochim. 75, 689–697
12 Imai, Y. and Sato, R. (1974) Biochem. Biophys. Res. Commun. 60, 8–14
13 Ernster, L. and Nordenbrand, K. (1967) Methods Enzymol. 10, 574–580
14 Hunter, F. E. and Smith, D. E. (1967) Methods Enzymol. 10, 689–696
15 Bigelow, C. C. (1967) J. Theoret. Biol. 16, 187–211
16 Welsher, H. D. (1969) Int. J. Prot. Res. 1, 253–265
17 Wang, C. S. and Smith, R. L. (1975) Anal. Biochem. 63, 414–417
18 Roodyn, D. B. (1962) Biochem. J. 85, 177–189
19 Flatmark, T., Terland, O. and Helle, K. B. (1971) Biochim. Biophys. Acta 226, 9–19
20 Makino, S., Reynolds, J. A. and Tanford, C. (1973) J. Biol. Chem. 248, 4926–4932
21 Inoue, K. and Kitagawa, T. (1976) Biochim. Biophys. Acta 426, 1–16
22 Cheah, K. S. (1975) in International Symposium on Electron-Transfer Chains and Oxidative Phosphorylation Sept. 15–18, Fasano-Italy, Univ. of Bari Meeting
23 Wikstrøm, M. K. F. (1973) Biochim. Biophys. Acta 301, 155–193
24 Stormberg, K. (1972) J. Virol. 9, 684–697
25 Umbreit, J. N. and Strominger J. L. (1973) Proc. Natl. Acad. Sci. U.S. 70, 2997–3001
26 Storm, D. R., Field, S. O. and Ryan, J. (1976) J. Supramol. Struct. 4, 221–231
27 Singer, S. J. (1974) Ann. Rev. Biochem. 43, 805–833
28 Chapman, D. and Wallach, D. F. H. (1968) in Biological Membranes, Physical Fact and Function (Chapman, D., ed.), pp. 125–202, Academic Press, London
29 Coleman, R. (1974) Biochem. Soc. Trans. 2, 813–816
30 Coleman, A., Nachbaur, J. and Vingnais, P. M. (1971) Biochim. Biophys. Acta 249, 462–492
31 Winkler, H., Hørtnagel, H. and Smith, A. D. (1970) Biochem. J. 118, 303–310
32 Winkler, H. and Smith, D. (1975) in Handbook of Physiol., Section 7, Endocrinology, Vol. VI Adrenal Gland, pp. 321–339
33 Capaldi, R. A. and Vanderkooi, G. (1972) Proc. Natl. Acad. Sci. U.S. 69, 930–932
34 Terland, O and Flatmark, T. (1975) Abstr. Comm. Meet. Fed. Eur. Biochem Soc. 10, No. 1098
35 Ernster, L. (1958) Acta Chem. Scand. 12, 600–602
36 Harmon, H. J., Hall, J. D. and Crane, F. L. (1974) Biochim. Biophys. Acta 344, 119–155
37 Chuang, T. F., Awasthi, Y. C. and Crane, F. L. (1973) Bioenergetics 5, 27–72
38 Shachat, N. and Greenwald, H. L. (1967) in Nonionic Surfactants (Schick, M. J., ed.), pp. 8–43, Marcell Dekker, Inc., New York
39 Simons, K., Helenius, A. and Garoff, H. (1973) J. Mol. Biol. 80, 119–133
40 Freeman Allen, C. and Rice, L. I. (1975) J. Chromatogr. 110, 151–155