Biochimica et Biophysica Acta 860 (1986) 503–509
Elsevier

BBA 73262

Incorporation of cytochrome b_5 into endoplasmic reticulum vesicles as protein-lysophospholipid micelles

Kirsten Christiansen and Jens Carlsen

Department of Biochemistry C, Panum Institute, University of Copenhagen, Copenhagen (Denmark)

(Received January 16th, 1986)
(Revised manuscript received May 27th, 1986)

Key words: Cytochrome b_5; Lysophosphatidylcholine; Endoplasmic reticulum

Cytochrome b_5 is incorporated into vesicles of the endoplasmic reticulum as protein-lysophosphatidylcholine micelles. Cytochrome b_5 becomes firmly bound to the membrane and at the same time lysophosphatidylcholine is acylated by acyltransferases of the endoplasmic reticulum and converted into the membrane component phosphatidylcholine. The possibility of an insertion of cytochrome b_5 into the endoplasmic reticulum in vivo by this mechanism is discussed.

Introduction

Cytochrome b_5, which together with NADH-cytochrome b_5 reductase and the desaturase forms a microsomal electron transport chain involved in fatty acid desaturation, is an amphiphilic protein with a hydrophilic domain containing the catalytical activity and a hydrophobic segment anchoring the protein to the membrane [1]. Reconstitution of cytochrome b_5 into lipid vesicles can result in two modes of insertion: a loosely and a tightly bound form. The loosely bound form is susceptible to attack by carboxypeptidase Y [2,3], and the tightly bound form is nonsusceptible to attack by carboxypeptidase Y [4,5]. This last mode of insertion of cytochrome b_5 is believed to be the physiological form, the form in which endogenous cytochrome b_5 is inserted into the endoplasmic reticulum [2,6]. Various chemical and physical studies have been focusing on the interaction of the hydrophobic segment of the protein with the lipid bilayer. Although different conclusions have been reached, it is most likely from photolabelling [4] and neutron scattering studies [7] that the hydrophobic segment and the highly charged C-terminal of cytochrome b_5, inserted in the tightly bound form, is located deep in the lipid bilayer with portions in both halves of the membrane and possibly spanning the membrane.

Cytochrome b_5 has been incorporated into microsomes by spontaneous insertion [2] and the amount of cytochrome b_5 in rat liver microsomes has been increased 20 times [8]. Different results have been obtained [2,6,9] but it is generally accepted that exogenous added cytochrome b_5 binds to microsomes in a form, using the criteria from the liposome experiments, is undistinguishable from the tight binding form. This is in contrast to the results obtained, if cytochrome b_5 is incorporated by spontaneous insertion into lipid vesicles composed of microsomal lipid [2,5]. Therefore it has been suggested, that proteins in the endoplasmic reticulum membrane [2,10] or one or more destabilizing factors [11] are required for insertion of cytochrome b_5 into microsomes in a tightly bound form.

Abbreviations: Cyt b_5, cytochrome b_5; lysoPC, lysophosphatidylcholine; PC, phosphatidylcholine.

Cytochrome b_5 is synthesized on free polysomes [12,13] and thus post-translationally inserted into the endoplasmic reticulum. How cytochrome b_5 is transported in the cytoplasm and how the highly charged C-terminal crosses the lipid bilayer of the endoplasmic reticulum membrane is still obscure. We have previously reported, that cytochrome b_5 can be incorporated into lipid vesicles as protein-lysophospholipid micelles [5]. Incorporated in this manner cytochrome b_5 become tightly bound to the vesicles independent of the lipid composition. In this report we present evidence that cytochrome b_5 can be incorporated into endoplasmic reticulum vesicles as protein-lysophospholipid micelles. Incorporated in this manner, cytochrome b_5 become firmly bound to the membrane. At the same time the lysophospholipid, which is incorporated together with cytochrome b_5, is converted to the membrane component phosphatidylcholine. The possibility that newly synthesized cytochrome b_5 or apocytochrome b_5 in vivo can be transported in the cytoplasm protected by the detergent lysophospholipid and inserted into the membrane as protein-lysophospholipid micelles is discussed.

Materials and Methods

Chemicals

Lysophosphatidylcholine (egg) was obtained from Serdary Research Lab., London, Canada. [1-^{14}C]Palmitoyllysophosphatidylcholine was obtained from Amersham International, Amersham, U.K. Crystalline bovine serum albumin, trypsin (type III-S) and apronitin was from Sigma, and Aquasol from New England Nuclear, Boston, MA, U.S.A. DH-990 (2-(3,5-di-*t*-butyl-1-hydroxyphenyl)thio)hexanoic acid) was a generous gift of Merrell Dow Pharmaceuticals Inc. (Indianapolis, IN, U.S.A.). Carboxypeptidase Y tested for lack of endopeptidase activity [14] was a generous gift from Carlbiotech, Copenhagen, Denmark.

Cytochrome b_5 and [^{3}H]cytochrome b_5

Amphiphilic cytochrome b_5 was isolated from pig liver essentially as described by Strittmatter et al. [15], and was obtained in sodium deoxycholate after Sephadex G-75 chromatography. Cytochrome b_5 was trace labeled by reductive alkylation as described by Jentoft and Dearborn [16]. To cytochrome b_5, 0.06 μmol, in 50 mM phosphate buffer (pH 8.1) containing 1% sodium deoxycholate, was added 2.5 μmol recrystallized $NaCNBH_3$ and 1 mCi [^{3}H]$NaCNBH_3$ (specific activity: 12 mCi/μmol). After mixing 0.02 μmol HCHO, prepared by hydrolysis of paraformaldehyde, was added and the reaction allowed to proceed for 2 h at room temperature. Labile adducts and excess of reagents was removed by extensive dialyses against 20 mM Tris-acetate (pH 8.1) containing 0.2 mM EDTA and 1% sodium deoxycholate. The specific activity of the final product was about $25 \cdot 10^7$ cpm/μmol.

Detergent-free cytochrome b_5

Detergent-free cytochrome b_5 was obtained by removal of sodium deoxycholate by dialysis at room temperature for 48 h.

Cytochrome b_5-lysophospholipid micelles

Cytochrome b_5-lysophosphatidylcholine micelles was obtained by exchange of the sodium deoxycholate with [^{14}C]lysophosphatidylcholine as described earlier [5]. The final molar ratio of lysophosphatidylcholine to cytochrome b_5 after dialysis was if nothing else was stated 300.

Preparation of subcellular fractions

The subcellular fractions were isolated from pig liver, rough and smooth endoplasmic reticulum by the method described by Dallner [17] and mitochondria by the method of Fleischer and Kervina [18]. The purity of the fractions were determined by measuring the activities of cytochrome oxidase (EC 1.9.3.1) [19] and the rotenone insensitive NADH-cytochrome *c* reductase (EC 1.6.2.3) [20]. The amount of protein was determined by the method of Lowry et al. [21] with bovine serum albumin as standard. The subcellular fractions were stored at -80°C. Trypsin treatment of the vesicles were performed with 90 μg of trypsin per mg of vesicle protein at 30°C for 2 h. The vesicles were collected by centrifugation at 100 000 $\times$ *g* for 1 h, and suspended in 20 mM Tris-HCl (pH 7.4) containing 0.15 M KCl and apronitin in a 3-times molar excess of the added trypsin. NADH-cytochrome b_5 reductase activity was measured as described by Mihara and Sato [22].

Incubation

Detergent-free cytochrome b_5 or cytochrome b_5-lysophosphatidylcholine micelles was incubated with subcellular fractions. Unless stated to the contrary, each incubation mixture contained in 0.6 ml: 50 mM Hepes adjusted to pH 7.4, 3.5 mM ATP, 0.17 mM CoA, 10 mM cysteine, 0.1 mM EDTA, 3 mM $MgCl_2$, 150 mM KCl and 1.3 mM oleic acid bound to 0.2 mM defatted bovine serum albumin. The [^{3}H]cytochrome b_5 and [^{14}C]lysophosphatidylcholine concentration were if nothing else was stated 3.3 μM and 1 mM, respectivly. The amount of vesicle protein was indicated. The incubation was performed at 37°C usually for 10 min or as stated. The reaction was stopped by placing the tubes in ice, and the separation of membrane fragments from unincorporated [^{3}H] cytochrome b_5 and [^{14}C]lysophosphatidylcholine was performed by centrifugation at $100\,000 \times g$ in a Beckman Airfuge or ultracentrifuge (model L8-70). The supernatant was removed and in order to ensure incorporation and not just adhesion of the micelles to the membrane vesicles, the pellet was washed once by resuspending in 0.6 ml of 50 mM Hepes, pH 7.4 containing 0.5 mM KCl and the centrifugation repeated. Wash of the pellet released 10% of the radioactivity associated with the membrane into a supernatant. A second wash did not release further radioactivity into a supernatant. The amount of radioactivity in the supernatant, wash and in the pellet after solubilization in 0.5% Triton X-100 was determined by measuring the radioactivity in Aquasol in a liquid scintillation counter Tri-Carb 4530 (Packard Instruments C., Inc., U.S.A.). The amount of protein in the pellet was determined on the Triton X-100 solubilizate, measured in the presence of sodium dodecylsulphate [23]. Incorporation of cytochrome b_5 into membrane fractions were confirmed by polyacrylamide gel electrophoresis with SDS (13% polyacrylamide) carried out according to Laemmli [24]. The proteins were stained with Coomassie brilliant blue.

Lipid extraction and analysis

Lipid extraction of the supernatant and the pellet after wash was performed by adding chloroform/methanol (2 : 1, v/v) according to Fox and Zilversmit [25]. The lipid was dissolved in a known volume of chloroform/methanol/water (25 : 15 : 2, v/v). Analysis of the conversion of lysophosphatidylcholine into phosphatidylcholine was performed by thin-layer chromatography in the solvent system chloroform/methanol/water (65 : 25 : 4, v/v). The spots were visualized by iodine vapor. After evaporation of the iodine, the spots were scraped from the plate into scintillation vials and the radioactivity counted in 4 ml of Aquasol.

Results

In a previous study it has been shown, that cytochrome b_5-lysophospholipid micelles can be formed by detergent exchange [5]. If such preformed cytochrome b_5-lysophospholipid micelles are incubated with vesicles of the endoplasmic reticulum, both cytochrome b_5 and lysophospholipid become incorporated into the membrane. In Table I is shown the results obtained if micelles containing increasing amount of cytochrome b_5 is incubated with a constant amount of smooth endoplasmic reticulum. Independent of the ratio of cytochrome b_5 to lysophospholipid in the performed micelles, the same ratio of ^{14}C to ^{3}H is found in the membrane vesicles after incubation, indicating that it is cytochrome b_5 in form of protein-lysophosphatidylcholine micelles, which is incorporated into the membrane. As also seen in Table I about 30% of the incorporated lysophosphatidylcholine is acylated by endogenous acyltransferases after 10 min of incubation as the ^{14}C label is found associated with phosphatidylcholine. The same ratio of ^{14}C to ^{3}H in the preformed micelles as in the membrane vesicles is also obtained after incubation of a constant amount of micelles with different amounts of vesicle protein (Table II). However, the amount of total label incorporated into the vesicles did not increase proportionally with protein concentration. One of the reasons for this may be, that the fusion is very rapid and even at 'zero-time', there is a considerable amount of label associated with the membrane. Furthermore, not all micelles fuse with the membrane, and incomplete fusion at pH 7.4 is a well-known phenomena [26].

Part of the incorporated lysophosphatidylcholine become acylated and part degraded to free

TABLE I

INSERTION OF CYTOCHROME b_5-LYSOPHOSPHATIDYLCHOLINE MICELLES INTO SMOOTH ENDOPLASMIC RETICULUM VESICLES

The incubation mixture contained 600 nmol [^{14}C]lysophosphatidylcholine and varying amounts of [^{3}H]cytochrome b_5 (0.5, 1, 2 or 3 nmol), 2.0 mg of vesicle protein in a volume of 0.6 ml. The incubation time was 10 min at 37°C. The values represent the average of double determinations.

Micelle composition			Incorporated label			Product formed
Cyt b_5 (nmol)	LysoPC (nmol)	LysoPC/ Cyt b_5	^{3}H (nmol)	^{14}C (nmol)	^{14}C/^{3}H	PC (nmol)
0	600	–	–	240	–	128
0.5	600	1200	0.26	340	1308	118
1	600	600	0.54	351	649	109
2	600	300	1.07	344	321	105
3	600	200	1.64	341	207	96
3	–	–	0.45	–	–	–

fatty acids. As shown in Table II the metabolism of the lysophospholipid increases with protein concentration. The amount of ^{14}C label, which is not incorporated, but remains in the supernatant, has been analyzed on thin-layer chromatography. The results show that most of the ^{14}C label is lysophospholipid. In the experiment in Table II using 3.3 mg of vesicle protein, the label in the supernatant is recovered as lysophosphatidylcholine (59%), phosphatidylcholine (13%) and free fatty acid (28%).

The incorporation of the protein-detergent micelles is not dependent on the acylation of lysophosphatidylcholine to phosphatidylcholine. When DH-990, a hypolipidemic drug, which is an inhibitor of the lecithin acyltransferase, acyl-coenzyme A: 1-acyl-*sn*-3-phosphocholine [27], is added to the incubation mixture, the amount of lysophospholipid and cytochrome b_5 incorporated is not different from the control without DH-990, even if the acylation is inhibited more than 90% (Table III).

Cytochrome b_5 incorporated into the endoplasmic reticulum as protein-lysophospholipid micelles become firmly bound to the membrane. It cannot be removed by treatment of the membrane with 0.5 M KCl. The cytochrome b_5 is nonsusceptible to attack by carboxypeptidase Y when incubated at 30°C at pH 6.5 for 2 h in the molar ratios of cytochrome b_5 to carboxypeptidase Y of 20 : 1 and 5 : 1 as used by others [2,9] (Table IV). Even at a ratio of 2 : 1, the degradation of the

TABLE II

EFFECT OF INCREASING PROTEIN CONCENTRATION ON INCORPORATION AND METABOLISM OF CYTOCHROME b_5-LYSOPHOSPHATIDYLCHOLINE MICELLES IN SMOOTH ENDOPLASMIC RETICULUM VESICLES

The incubation mixture contained 600 nmol [^{14}C]lysophosphatidylcholine, 2 nmol [^{3}H]cytochrome b_5 and increasing amounts of vesicle protein in a volume of 0.6 ml. The incubation time was 10 min at 37°C. The values represent the average of double determinations. FFA, free fatty acids.

Vesicle protein (mg)	Incorporated label			Lipid metabolites		
	^{3}H (nmol)	^{14}C (nmol)	^{14}C/^{3}H	LysoPC (nmol)	PC (nmol)	FFA (nmol)
1.0	0.90	308	342	202	93	13
2.1	1.13	364	322	189	136	39
3.3	1.14	416	364	142	172	102
4.4	1.22	429	351	124	163	142

TABLE III

EFFECT OF DH-990 ON INCORPORATION OF CYTOCHROME b_5-LYSOPHOSPHATIDYLCHOLINE MICELLES INTO SMOOTH AND ROUGH ENDOPLASMIC RETICULUM VESICLES

The incubation mixture contained 600 nmol [^{14}C]lysophosphatidylcholine, 3 nmol [^{3}H]cytochrome b_5, 2,3 mg of vesicle protein and 1.2 nmol of DH-990 in a volume of 0.6 ml. The values represent the average of double determinations. ER, endoplasmic reticulum.

Acceptor vesicle	Inhibitor (DH-990)	Incorporated label			Product formed PC (nmol)
		^{3}H (nmol)	^{14}C (nmol)	^{14}C/^{3}H	
Smooth ER	−	1.02	382	375	101
Smooth ER	+	1.02	390	382	14
Rough ER	−	0.96	370	385	251
Rough ER	+	1.07	446	417	33

protein is low. At cytochrome b_5 to carboxypeptidase Y molar ratios of 1:2 and 1:5 the degradation is only 15% in comparison to a control value of 7%. As also seen in Table IV, cytochrome b_5 inserted by fusion is less susceptible to attack by carboxypeptidase Y than cytochrome b_5 incorporated into microsomes by spontaneous insertion. The greater stability of cytochrome b_5, using our method of incorporation, is also seen in trypsin-treated vesicles, vesicles in which 20% of the protein and 80% of the NADH-cytochrome b_5 reductase activity is removed.

Cytochrome b_5 can be incorporated into both smooth and rough endoplasmic reticulum as protein-lysophospholipid micelles (Table III). Mitochondria will not incorporate cytochrome b_5 as lysophospholipid micelles. The mitochondrial membrane disrupts under condition where the endoplasmic reticulum membrane is stable to lysophospholipid. After incubation of 3 mg of vesicle protein with 600 nmol of lysophosphatidylcholine in a volume of 0.6 ml (detergent concentration, 0.05%), 80% of the endoplasmic reticulum (rough and smooth) is recovered as vesicle protein while only 30% of the mitochondrial protein can be recovered in a mitochondrial membrane pellet. In contrast, cytochrome b_5 when added without de-

TABLE IV

AMOUNT OF CYTOCHROME b_5 RELEASED FROM SMOOTH ENDOPLASMIC RETICULUM VESICLES AFTER CARBOXYPEPTIDASE Y TREATMENT

To vesicles (5 mg of protein), containing 5 nmol of [^{3}H]cytochrome b_5 inserted either by (A) fusion as lysophospholipid micelles or (B) spontaneous insertion, was added either buffer (control) or increasing amounts of carboxypeptidase Y (CPY). The samples were incubated at 30°C for 2 h after pH adjustment to 6.5 with acetic acid. The membranes were pelleted and the amount of radioactivity in the supernatant determined. ER, endoplasmic reticulum.

Cyt b_5/CPY (molar ratio)	Cytochrome b_5 released (%)			
	A		B	
	ER vesicles	ER vesicles (trypsinized)	ER vesicles	ER vesicles (trypsinized)
Control	7	6	8	8
20:1	9	7	12	15
4:1	12	9	17	19
2:1	11	9	21	21
1:2	15	18	25	26

TABLE V

INCORPORATION OF CYTOCHROME b_5 INTO SUBCELLULAR FRACTIONS OF PIG LIVER

[^{3}H]Cytochrome b_5 ((A) 2.6 nmol; (B) 10 nmol) was incubated with 1.9 mg of protein of either of the subcellular fractions for 10 min at 37°C. ER, endoplasmic reticulum.

Subcellular fraction	Cytochrome b_5 incorporated (nmol/mg of protein)	
	A	B
Smooth ER	0.44	1.56
Rough ER	0.47	1.60
Mitochondria	0.44	1.51

tergent, can be incorporated into both endoplasmic reticulum (both smooth and rough) and into mitochondria (Table V). The incorporation of cytochrome b_5 is in this case the same for all three subcellular fractions per mg of protein, even if cytochrome b_5, if at all present, is only a minor component of the mitochondria.

Discussion

The previously described method for reconstitution of proteins into liposomes [26] and used for incorporation of cytochrome b_5 into lipid vesicles in a tightly bound form [5], has in this study been used to incorporate cytochrome b_5 into its natural membrane, the endoplasmic reticulum. The method, which is based on incubation of preformed cytochrome b_5-lysophospholipid micelles with membrane vesicles, results in insertion of cytochrome b_5 in the membrane. The lysophospholipid, which is incorporated together with the protein, is acylated by the enzymes of the endoplasmic reticulum into phospholipid, and part is degraded to free fatty acid. The incorporation of cytochrome b_5 as protein-lysophospholipid micelles is not dependent on a conversion of the lysophospholipid to phospholipid, shown in experiments where the lecithin acyltransferase activity is inhibited.

Cytochrome b_5 incorporated by our method become firmly bound to the membrane and is resistent to attack by carboxypeptidase Y even if high ratios of carboxypeptidase Y to cytochrome b_5 are used. Using the same high amounts of essentially endopeptidase free carboxypeptidase Y [14], cytochrome b_5 inserted by spontaneous insertion, is released to some extend. This agrees with data published earlier [9] although a different conclusion was reached. It has been stated, that cytochrome b_5 inserts spontaneously into the tightly bound form in microsomes [2] while it inserts as the loosely bound form in liposomes made of mcirosomal lipid. This discrepancy is difficult to explain, but the possibility exists, that it reflects a lack of test system rather than a difference in mode of insertion. The use of carboxypeptidase Y is probably not always a valid test for distinguishing between the different modes of insertion of cytochrome b_5. The test is useful when cytochrome b_5 is inserted into lipid vesicles, but steric hindrance and the availability of other substrates in natural membranes could slow down the action of carboxypeptidase Y dramatically, and thus give false results. It is noteworthy, that cytochrome b_5 incorporated as protein-lysophospholipid micelles into lipid vesicles is resistent to carboxypeptidase Y independent of the lipid composition [5].

Cytochrome b_5 can by our method be incorporated both into smooth and rough endoplasmic reticulum, but not into mitochondria, which lyse under the incubation conditions. The mitochondrial membrane apparently is more sensitive to lysophospholipid than the endoplasmic reticulum. The phenomena cannot be explained solely as a result of lack of acyltransferase activity in the mitochondrial membrane [28] as the endoplasmic reticulum under conditions where the acyltransferase activity is inhibited (Table IV) show no sign of lysis.

Cytochrome b_5 itself apparetly lack specificity for the endoplasmic reticulum membrane. Thus, we have shown that cytochrome b_5 will be incorporated in vitro into endoplasmic reticulum and mitochondria to the same degree. Cytochrome b_5 is also found in various subcellular fractions (mitochondria, endoplasmic reticulum and Golgi) soon after labelling in vivo [29]. The fact that cytochrome b_5 in vivo is found in highest concentration in the endoplasmic reticulum, may reflect (as stated by Okada et al. [30]) an affinity for other more specifically inserted membrane components. These membrane components could be of protein as well as lipid nature. If of lipid nature, lysophospholipids could be such components. Lysophospholipids are both water soluble and membrane soluble components present in small amounts in all biological materials [31]. They are formed as intermediates in de novo lipid biosynthesis at the endoplasmic reticulum and they also occur as degradation products, when fatty acids of the phospholipids are renewed or changed into specific species by the combined action of phospholipases and acyltransferases, reactions which also takes place at the endoplasmic reticulum. Thus, lysophospholipids are formed close to the membrane in which newly synthesized cytochrome b_5 inserts in vivo.

Cytochrome b_5 could be protected from aggre-

gation if it is transported in the cytoplasm as protein-lysophospholipid micelles. The problem of how both hydrophobic and charged amino acid residues can insert and even cross a lipid bilayer could be circumvented, if cytochrome b_5 is inserted into the endoplasmatic reticulum as lysophospholipid micelles. Whether this mechanism operates in vivo, remains to be established. That soluble components might be involved in insertion of proteins synthesized on free ribosomes, has been reported for the mitochondrial protein, ornithine carbamyltransferase [32,33]. The posttranslational import of the peroxisomal protein, catalase is also stimulated by a soluble factor [34]. In neither the mitochondrial nor the peroxisomal protein import, is the nature of the soluble factor known.

Acknowledgement

The expert technical assistance of Ms. Birthe Nystrøm and Ms. Birgit Harder is gratefully acknowledged.

References

1 Strittmatter, P. and Dailey, H.A. (1982) in Membranes and Transport (Martonosi, A.N., ed.), Vol. 1, pp. 71–82, Plenum Press, New York
2 Enoch, H.G., Fleming, P.J. and Strittmatter, P. (1979) J. Biol. Chem. 254, 6483–6488
3 Tajima, S. and Sato, R. (1980) J. Biochem. 87, 123–134
4 Takagaki, Y., Radhakrishnan, R., Wirtz, K.W.A. and Khorana, H.G. (1983) J. Biol. Chem. 258, 9136–9142
5 Christiansen, K. and Carlsen, J. (1985) Biochim. Biophys. Acta 815, 215–222
6 Poensgen, J. and Ullrich, V. (1980) Biochim. Biophys. Acta 596, 248–263
7 Gogol, E.P. and Engelman, D.M. (1984) Biophys. J. Biophys. Soc. 46, 491–495
8 Strittmatter, P., Rogers, M.J. and Spatz, L. (1972) J. Biol. Chem. 247, 7188–7194
9 Bendzko, P., Prehn, S., Pfeil, W. and Rapoport, T.A. (1982) Eur. J. Biochem. 123, 121–126
10 Kreibich, G., Sabatini, D.D. and Adesnik, M. (1983) in Methods in Enzymology (Fleischer, S. and Packer, L., eds.), Vol. 96, pp. 530–542, Academic Press, New York
11 Greenhut, S.F. and Roseman, M.A. (1985) Biochemistry 24, 1252–1260
12 Rachubinski, R.A., Verma, D.P.S. and Bergeron, J.J.M. (1980) J. Cell Biol. 84, 705–716
13 Okada, Y., Frey, A.B., Guenther, T.M., Oesch, F., Sabatini, D.D. and Kreibich, G. (1982) Eur. J. Biochem. 122, 393–402
14 Carlsen, J. and Christiansen, K. (1985) Anal. Biochem. 148, 542–545
15 Strittmatter, P., Fleming, P., Connors, M. and Corcoran, D. (1978) in Methods in Enzymology (Fleischer, S. and Packer, L., eds.), Vol. 52, pp. 97–101, Academic Press, New York
16 Jentoft, N. and Dearborn, D.G. (1983) in Methods in Enzymology (Fleischer, S. and Packer, L., eds.), Vol. 91, pp. 570–579, Academic Press, New York
17 Dallner, G. (1978) in Methods in Enzymology (Fleischer, S. and Packer, L., eds.), Vol. 52, pp. 71–83, Academic Press, New York
18 Fleischer, S. and Kervina, M. (1974) in Methods in Enzymology (Fleischer, S. and Packer, L., eds.), Vol. 31, pp. 6–41, Academic Press, New York
19 Appelmans, F., Wattiaux, R. and DeDuve, C. (1955) Biochem. J. 59, 438–445
20 Sottocasa, G.L., Kuylenstierna, B., Ernster, L. and Bergstrand, A. (1967) J. Cell Biol. 32, 415–438
21 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
22 Mihara, K. and Sato, R. (1978) in Methods in Enzymology (Fleischer, S. and Packer, L., eds.), Vol. 52, pp. 102–108, Academic Press, New York
23 Wang, C.-S. and Smith, R.L. (1975) Anal. Biochem. 63, 414–417
24 Laemmli, U.K. (1970) Nature 227, 680–685
25 Fox, P.L. and Zilversmit, D.B. (1982) Biochim. Biophys. Acta 712, 605–615
26 Christiansen, K. and Carlsen ,J. (1983) Biochim. Biophys. Acta 735, 225–233
27 Parthasarathy, S., Kritchevsky, D. and Baumann, W.J. (1982) Proc. Natl. Acad. Sci. USA 79, 6890–6893
28 Daum, G. (1985) Biochim. Biophys. Acta 822, 1–42
29 Borgese, N. and Gaetani, S. (1980) FEBS Lett. 112, 216–220
30 Okada, Y. and Omura, T. (1978) J. Biochem. (Tokyo) 83, 1039–1048
31 Ansell, G.B., Dawson, R.M.C. and Hawthorne, J.N. (1973) Form and Function of Phospholipids, 2nd edn., pp. 459–467, Elsevier, Amsterdam
32 Argan, C., Lusty, C.J. and Shore, G.C. (1983) J. Biol. Chem. 258, 6667–6670
33 Miura, S., Mori, M. and Tatibana, M. (1983) J. Biol. Chem. 258, 6671–6674
34 Fujika, Y. and Lazarow, P.B. (1985) J. Biol. Chem. 260, 5603–5609