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TRANSBILAYER DISTRIBUTION OF PHOSPHOLIPID FATTY ACYL CHAINS IN PHOTORECEPTOR MEMBRANE *

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Summary

The transverse distribution of the fatty acyl chains of the major phospholipids over the two faces of the photoreceptor membranes has been determined in bovine rod outer segment (stacked disk) preparations. For this purpose, the fatty acid composition of the phospholipids has been analyzed before and after treatment with trinitrobenzenesulfonic acid and phospholipase D. The latter agents are used under conditions in which they have been demonstrated to attack only the outer (cytoplasmic) face of the membrane.

After treatment with trinitrobenzenesulfonic acid or phospholipase D, the fatty acid composition of the unreacted phospholipids is the same as that before treatment, regardless of the extent of modification or hydrolysis attained. The fatty acid composition of phosphatidic acid, resulting from phospholipase D action, also remains unchanged during progressive hydrolysis.

These results indicate that the fatty acyl chains of the major phospholipids have the same composition on either side of the disk membrane. Together with our previously published evidence for the distribution of the major phospholipids in rod outer segment disk membranes, this means that both the phospholipids and their fatty acyl chains have a remarkably symmetrical distribution over the two membrane faces.

On the basis of literature data it is concluded that this approximate symmetry reflects the high mobility of the entire phospholipid pool of disk membranes, thus including appreciable transbilayer movements of the phospholipids.

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Abbreviation: Mops, 4-morpholinepropanesulfonic acid.

Introduction

We have recently been studying the transbilayer distribution of the phospholipids in the rod disk membranes by means of phospholipases [1] and trinitrobenzenesulfonic acid (TNBS) [2]. Reliable methods were developed, in which phospholipase D and TNBS in principle only attacked the outer leaflet of the bilayer of these membranes. A nearly symmetrical distribution of the three major phospholipids, phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine, was concluded.

The same methods have now been used to investigate the transbilayer distribution of the phospholipid fatty acyl chains. The results indicate a symmetrical distribution of the fatty acyl chains of all three major phospholipids.

Materials and Methods

Rod outer segment preparations. Stacked bovine rod outer segment disk membranes ('stacked disks') are prepared by sucrose-Ficoll 400 gradient centrifugation [3]. The preparation consists of stacked disks, partially surrounded by plasma membrane.

Rhodopsin content has been determined according to the method of van Breugel et al. [4].

Treatment with trinitrobenzenesulfonate. Treatment with 2,4,6-trinitrobenzene-1-sulfonic acid (TNBS) tetrahydrate (BHD Chemicals Ltd, Poole, U.K.) is performed in a buffer containing 40 mM Mops, 2 mM $CaCl_2$, 3 mM $MgCl_2$, 140 mM NaCl (pH 7.4). TNBS is added as a freshly prepared solution in the same buffer to a final concentration of 1 mM, representing a 5-fold excess with regard to the number of primary amino groups present in stacked disks (final rhodopsin concentration 3—4 μM). Incubation is carried out at 20°C in darkness under N_2. The reaction is stopped by lowering the pH through addition of excess ice-cold 0.2 M sodium acetate buffer (pH 5.5). After centrifugation (0°C, 100 000 × *g*, 30 min) lipid analysis is carried out as described below.

Treatment with phospholipase D. Stacked disks are resuspended in 20 mM Tris-maleate buffer (pH 6.0), containing 600 mM sucrose and 5% (w/w) Ficoll 400. Incubation with phospholipase D (Boehringer, Mannheim, F.R.G.) is carried out in an N_2 atmosphere at 30°C in darkness in the same buffer containing 40 mM $CaCl_2$ and enzyme solubilized in distilled water (final concentration about 0.7 U/ml; final rhodopsin concentration 30—40 μM). The reaction is stopped by adding excess ice-cold 0.16 M Tris-maleate buffer (pH 6.0) containing 10 mM EDTA. Lipid analysis is performed after centrifugation (0°C, 100 000 × *g*, 30 min).

Lipid analysis. The lipids in pellets of treated and untreated stacked disks are extracted twice with $CHCl_3/CH_3OH$ (2 : 1, v/v), containing 50 mg/l butylated hydroxytoluene, and washed with aqueous acidified 0.1 M KCl. All procedures are carried out under an N_2 atmosphere and all solutions are bubbled with N_2 before use.

Phospholipids are separated by two-dimensional thin-layer chromatography on plates coated with silica gel 60 HR (Merck, Darmstadt, F.R.G.) and 3% (w/w) magnesium silicate (Woelm, Eschwege, F.R.G.). The spots are quantita-

tively analyzed by phosphorus assay [1]. The accuracy of the determination is about 5% (relative standard error) for phosphatidylcholine and phosphatidylethanolamine and about 10% (relative standard error) for phosphatidylserine. When fatty acid analysis is performed in the next step, phospholipids are separated on plates precoated with silica gel 60 (Merck). In this case the elution solvents contain also 50 mg/l butylated hydroxytoluene. The lipids are visualized after spraying with 0.001% (w/v) aqueous 1-amino-2-hydroxy-4-naphthalenesulfonic acid solution. Precoated plates are cleaned before use by elution in $CHCl_3/CH_3OH/H_2O$ (65 : 25 : 4, v/v).

For fatty acid analysis of disk preparations, the total lipid extract is evaporated after addition of heptadecanoic acid (17 : 0; Supelco, Inc., Bellefonte, U.S.A.) as internal standard. Methylation of the fatty acids is carried out in CH_3OH with either 14% (w/v) BF_3 (5) or 10% (v/v) CH_3COCl. (Both reagents gave the same results). The samples are heated for 15 min at 100°C. The methyl esters are extracted by adding equal volumes of *n*-pentane and water. After separation by low-speed centrifugation the aqueous layer is re-extracted once with *n*-pentane. The combined pentane layers are dried over anhydrous Na_2SO_4 and evaporated. The residue is taken up in iso-octane and submitted to gas-liquid chromatography.

In the case of fatty acid analysis after thin-layer chromatography, the spots of the various lipid classes are scraped off and treated as above. Methylation of the fatty acids is carried out with CH_3COCl/CH_3OH, since the use of BF_3/CH_3OH makes silica gel stick to the glass wall.

The methyl esters are analyzed at 210°C in a Pye Unicam gaschromatograph model 204, equipped with an all-glass sample stream and flame ionization detector. Injection of the sample and detection are performed at 250°C. A 6 ft × 4 mm inner diameter column, containing 10% SP-2330 on 100/120 Supelcoport (Supelco, Inc.), is used to separate the methyl esters. Identification is by comparison with reference methyl ester mixtures PUFA No. 1, PUFA No. 2, GLC 50 and GLC 60 (Supelco, Inc.). Relative molar concentrations of each fatty acid in the sample are calculated with a Hewlett Packard integrator model HP 2280 A. The internal standard method is used and the integrated area of each peak is divided by the molecular weight of the corresponding methyl ester.

The overall recovery is always better than 95% as estimated by using heneicosanoic acid (21 : 0; Supelco, Inc.), added as internal standard to the suspensions prior to lipid extraction. The error in the fatty acid analysis appears to be 5% or less (standard deviation of the mean).

Results

Lipid composition of outer segments

The fatty acid composition of stacked disks and of the phospholipids present in these preparations is shown in Table I. The total amount of phospholipid is 62 ± 2 (n = 5) mol per mol rhodopsin. Appreciable amounts of diglycerides and free fatty acids (2 ± 1 and 4 ± 1 mol per mol rhodopsin, respectively) appear to be present consistently, whereas triglycerides are virtually absent.

TABLE I

FATTY ACID COMPOSITION OF ROD OUTER SEGMENTS AND THEIR MAJOR PHOSPHOLIPIDS

Fatty acids making up less than 0.3 mol% in any fraction are omitted. Values are averages with standard deviation for five preparations.

	Outer segments	Phosphatidyl-choline	Phosphatidyl-ethanolamine	Phosphatidyl-serine
Phospholipid composition		(mol% of total phospholipids)		
		36.0 ± 1.5	44.1 ± 1.6	15.2 ± 0.9
Fatty acids		(mol% of total fatty acids)		
16 : 0	19.9 ± 0.3	30.6 ± 2.2	12.6 ± 0.3	4.1 ± 0.4
18 . 0	22.1 ± 0.6	19.4 ± 1.0	25.0 ± 0.4	21.0 ± 1.1
18 : 1 ω 9	3.3 ± 0.1	4.5 ± 0.5	4.2 ± 0.1	1.5 ± 0.4
18 : 2 ω 6	<0.1	0.9 ± 0.1	0.9 ± 0.1	<0.1
20 : 4 ω 6	4.8 ± 0.1	2.7 ± 0.1	2.4 ± 0.1	4.3 ± 0.5
22 : 4 ω 6	1.6 ± 0.1	0.4 ± 0.1	0.8 ± 0.1	3.0 ± 0.2
22 : 5 ω 6	2.3 ± 0.1	0.9 ± 0.1	1.5 ± 0.1	1.6 ± 0.3
22 : 5 ω 3	1.9 ± 0.2	1.4 ± 0.1	1.4 ± 0.1	3.3 ± 0.2
22 . 6 ω 3	43.0 ± 0.4	35.9 ± 2.2	50.2 ± 0.8	48.1 ± 0.8
24 : 4	} 1.2 ± 0.1	<0.1	<0.1	3.9 ± 0.4
24 : 5		<0.1	<0.1	9.3 ± 0.7

Treatment with TNBS

Treatment of stacked disks with TNBS leads to trinitrophenylation of phosphatidylethanolamine and phosphatidylserine (40 and 38% modification, respectively, after 60 min). Phosphatidylcholine does not react with TNBS. Hence, the fatty acid composition of this phospholipid can serve as a control to indicate the reliability of the method, notably of the retention of acyl chain unsaturation during the experiments. This seems to be satisfactory. After separation of the phospholipids by thin-layer chromatography, the fatty acid composition of the unmodified phospholipids is determined.

Fig. 1 summarizes the results after 10, 20, 30 and 60 min of treatment with TNBS. The unmodified phospholipids appear to have the same fatty acid composition as before incubation with TNBS, regardless of the degree of modification attained. For practical reasons only the four most abundant fatty acids are

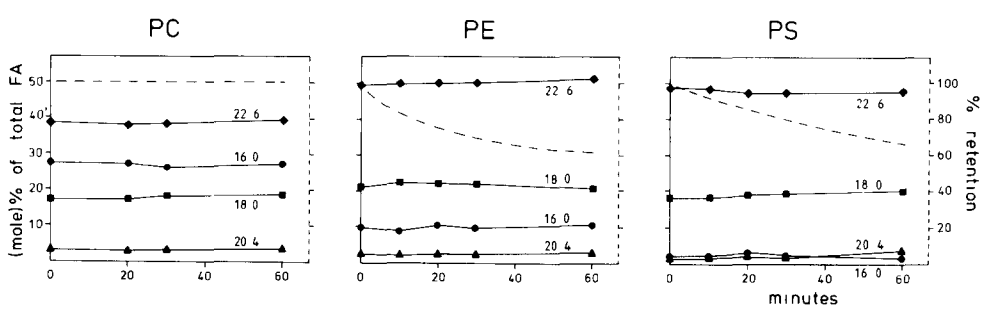

Fig. 1. Fatty acid composition of unmodified phospholipids of bovine stacked disk preparations before and after treatment with TNBS. Closed symbols (solid lines) represent content of indicated fatty acids in phosphatidylcholine (PC), (residual) phosphatidylethanolamine (PE) and phosphatidylserine (PS). Broken lines represent the percent of native phospholipid left.

presented, viz., palmitic acid (16 : 0), stearic acid (18 : 0), arachidonic acid (20 : 4) and docosahexaenoic acid (22 : 6). Clearly, the fatty acid composition of the phospholipids accessible to TNBS is the same as that of the non-accessible phospholipids. Consequently, the products of the reaction, i.e., trinitrophenyl derivatives of phosphatidylethanolamine and phosphatidylserine, must retain the same fatty acid composition as the mother compounds during the course of reaction with TNBS. Analysis of these derivatives indicates that this is indeed the case, but exact calculation is hampered by the presence of the (more saturated) diglycerides and free fatty acids near the spots of trinitrophenyl phosphatidylethanolamine and trinitrophenyl phosphatidylserine (close to the solvent front).

The results also show that no phospholipid with a particular fatty acid composition has been preferentially attacked.

Treatment with phospholipase D

Treatment of stacked disks with phospholipase D leads to the hydrolysis of 50% phosphatidylcholine and 42% phosphatidylethanolamine after 60 min, and gives rise to 37% phosphatidic acid after this time. A final level of 40% hydrolysis is reached after 180 min. Since phospholipase D hardly hydrolyzes phosphatidylserine under these conditions, the fatty acid composition of this lipid can serve as a control in the same way as that of phosphatidylcholine in the TNBS approach.

Fig. 2 presents the results obtained with phospholipase D in the same way as those for the treatment with TNBS in Fig. 1. Again the unmodified phospholipids have the same fatty acid composition as before the incubation, regardless

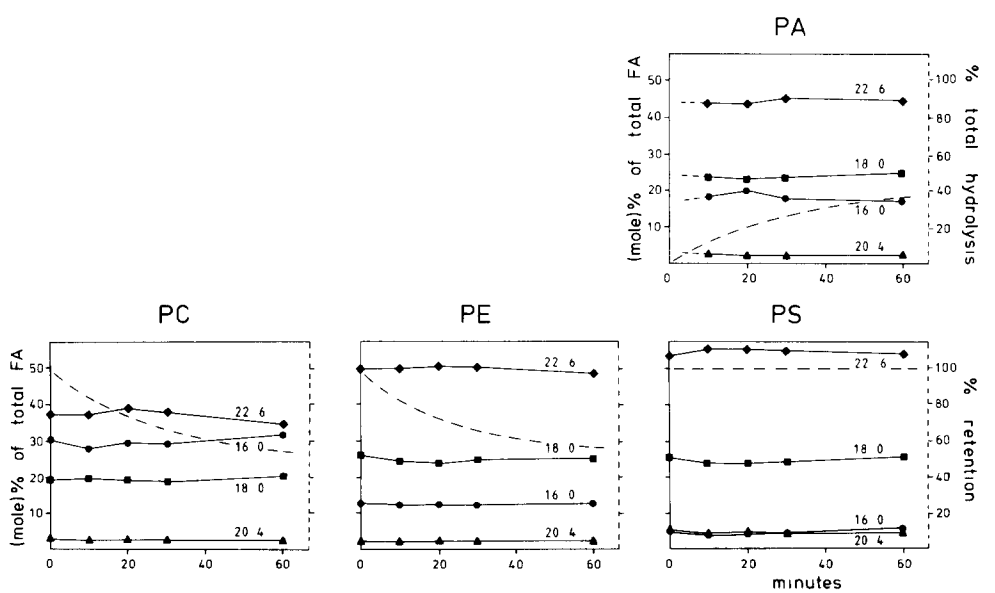

Fig. 2. Fatty acid composition of phospholipids of bovine stacked disk preparations before and after phospholipase D treatment. Presentation as in Fig. 1. PA: phosphatidic acid, the hydrolysis product. Broken line in top right figure (PA) represents percent of total phospholipid hydrolysis.

of the degree of hydrolysis reached. The fatty acid composition of the product, phosphatidic acid, also remains constant during hydrolysis. This composition clearly reflects the derivatives of the phosphatidic acid from roughly equimolar amounts of phosphatidylcholine and phosphatidylethanolamine.

There is no indication for preference of the enzyme for phospholipids with a particular fatty acid composition.

Discussion

Lipid composition of outer segments

Our data for the phospholipid composition of rod outer segments are in agreement with those in previous publications [6—8]. The degree of unsaturation of the fatty acids is higher than given in the first reports (see Ref. 8), but closely resembles that in more recent publications [7,9,10]. This probably reflects the higher purity of the present rod outer segment preparations as compared to about 10 years ago. The presence of appreciable amounts of free fatty acids and diglycerides, and their fatty acid composition, will be dealt with in a separate report.

Transbilayer distribution of fatty acyl chains

In two previous studies [1,2] we have presented considerable evidence for an approximately symmetrical distribution of phospholipids (phospholipid head groups) over the two faces of disk membranes. Under carefully defined conditions, both phospholipase D and the amino group reagent TNBS appeared to behave as probes, which is principle only act on the outer leaflet of the disk membranes. Therefore, these approaches can be used as well for investigating the transbilayer distribution of the fatty acyl chains.

The fatty acid composition of the phospholipid classes and their modification products during the course of action of either TNBS or phospholipase D is constant within experimental error. This leaves little doubt that the fatty acyl chains of each of the major phospholipid classes in the disk membrane are symmetrically distributed over the two faces of this membrane, at least on the time scale of our experiments. Together with the approximately symmetrical distribution of the phospholipid head groups [1,2], this demonstrates that the lipid part of the disk membrane displays no transverse asymmetry, in contrast to its major protein rhodopsin.

To our knowledge, this is the first time that a symmetrical transverse fatty acyl chain distribution is found in a biological membrane. In all previous reports a higher unsaturation of the inner leaflet has been observed [11—16]. It may be significant that, with one exception (mouse brain synaptosomes [14]), these data refer to plasma membranes in which an asymmetrical phospholipid distribution has been found as well. Hence, the number of observations, at least for intracellular membranes, is too small to permit generalization at this time with respect to transverse fatty acyl chain distribution.

Our present results show that no phospholipid with a particular fatty acid composition is preferentially attacked by or protected against TNBS or phospholipase D, which suggests lateral randomness of the fatty acyl chains in the outer face of the membrane. Recent cross-linking studies of Crain et al. [17]

also 'indicate a random array of lipids with respect to their fatty acid content in the rod disk membrane'.

Transbilayer movement of phospholipids

The approximately symmetrical distribution of the phospholipids over the two faces of the disk membrane raises the question as to whether this represents a static situation or a dynamic equilibrium.

From the autoradiographic work of Young and coworkers, it is well known that in many species the visual pigment rhodopsin, soon after its synthesis in the inner segment, becomes incorporated in the infoldings of the plasma membrane at the base of the rod outer segments (for a review see Ref. 18). Later they form a package of labeled disks, wnich gradually moves to the apical end of the outer segment. The specific radioactivity of this rhodopsin remains constant until the labeled disks are scavenged by the pigment epithelium, which in rat takes about 10 days [19] and in frog about 40 days [20]. Clearly, the rhodopsin molecule remains to the end in the disk in which it is originally incorporated. In addition, it remains asymmetrically inserted in the membrane with the carbohydrate-bearing N-terminus at the inside of the disk [21]. Its mobility in the outer segment is apparently limited to rotation around an axis perpendicular to the disk membrane [22,23] and to lateral diffusion in this membrane [24,25].

The synthesis and fate of the phospholipids of rod outer segments, studied by autoradiographic and biochemical approaches, show a quite different pattern. Most evidence derives from experiments with frog, but in rabbit [26,27] the same general pattern is observed. De novo synthesis of phospholipids takes place in the microsomes of the inner segment and the phospholipids are exported to the outer segment as demonstrated with a wide variety of radioactive precursors: glycerol [28—32], fatty acids [33], choline [26,30,34], ethanolamine [27,31] and serine [27,29,31]. All evidence [28,33,35—38] suggests that these phospholipids are used in the formation of new disks at the base of outer segments, together with the newly synthesized rhodopsin. However, autoradiography does not reveal the banding phenomenon observed with rhodopsin. On the contrary, the radioactivity always diffuses quickly throughout the entire outer segment and any localized radioactivity at the base of the outer segment due to phospholipids is always short-lived [28,30,33]. These autoradiographic data are nicely confirmed by recent biochemical experiments of Anderson and coworkers [29—32]. They find that a pulse label of [2-^{3}H]glycerol is rapidly incorporated into the phospholipids of frog outer segments and, subsequently, the specific radioactivity of all major phospholipids declines exponentially with a halftime of about 20 days.

These observations demonstrate that, in contrast to rhodopsin, the major phospholipids of the outer segments exhibit a high interdiscal mobility, at least in frog and rabbit. This conclusion may probably be extrapolated to vertebrate species other than frog and rabbit, since the phospholipid and fatty acid composition of vertebrate photoreceptor membranes is rather similar [8]. The pool of exchanging phospholipids must include the inner leaflet of the disk bilayer, since otherwise more persistent banding of the radioactive label derived from a phospholipid precursor should have been detected. The fact that

exchange of fatty acyl chains also seems to take place in outer segments [33] only adds an additional dimension to the dynamic behaviour of these phospholipids. In other words, there is most likely a transbilayer movement of rod outer segment phospholipids and the halftime of this phenomenon can be expected to be of the order of hours rather than days. Although direct evidence for this transbilayer movement is still not available, it is reasonable to assume that the approximately symmetrical transbilayer distribution of the phospholipids and their fatty acyl chains reflects the high mobility of the entire phospholipid pool of bovine disk membranes in vivo.

If this assumption is correct, the question arises why in our experiments only maximally 40% of the total phospholipids (viz., phosphatidylethanolamine and phosphatidylcholine) can be hydrolyzed by phospholipase D, and maximally 35% (viz., phosphatidylethanolamine and phosphatidylserine) can be modified by TNBS (see Ref. 2). In the case of a continuous transbilayer movement of phospholipids, ultimately the entire pool of (susceptible) phospholipids would become accessible to the reagents, even when these do not penetrate the membrane at all. One possibility is that the products, phosphatidic acid and trinitrophenyl phospholipids, are not subject to transbilayer movement. The simplest explanation, however, is that the transbilayer mobility of all phospholipids is greatly reduced after isolation and/or under the conditions of incubation (30°C, 40 mM Ca^{2+} in the case of phospholipase D treatment, 20°C in the case of TNBS treatment). Whereas most experimental evidence [1,2] is in agreement with this explanation, the strongest argument against transbilayer movement of phospholipids under our experimental conditions is provided by the combination experiments of Figs. 4 and 7 of Ref. 2. These show that pretreatment of the disk membranes with phospholipase D or TNBS alters neither the size nor the composition of the accessible phospholipid pool. This seems to eliminate the possibility that we would arrive artifactually at a symmetrical phospholipid distribution during the reagent incubations. However, this does not exclude that an asymmetric distribution of phospholipids would be maintained in vivo by a mechanism dependent on active metabolism.

Concluding remarks

Many questions regarding the phospholipids of rod outer segments remain to be answered. Which mechanisms are involved in their high mobility, both in interdiscal transfer and in intradiscal transbilayer movements? What is the physiological relevance of a membrane system with a 'static' major protein and very 'dynamic' phospholipids? Is there a relation with the high content of docosahexaenoic acyl chains, not only a highly unsaturated (22 : 6), but an essential fatty acid as well? The answers to these and other questions on the phospholipid dynamics of disk membranes must clearly await further investigations.

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References

1 Drenthe, E.H.S., Bonting, S.L. and Daemen, F.J.M. (1980) Biochim. Biophys. Acta 603, 117—129
2 Drenthe, E.H.S., Klompmakers, A.A., Bonting, S.L. and Daemen, F.J.M. (1980) Biochim. Biophys. Acta 603, 130—141
3 Schnetkamp, P.P.M., Klompmakers, A.A. and Daemen, F.J.M. (1979) Biochim. Biophys. Acta 552, 379—389
4 Van Breugel, P.J.G.M., Geurts, P.H.M., Daemen, F.J.M. and Bonting, S.L. (1978) Biochim. Biophys. Acta 509, 136—147
5 Morrison, W.R. and Smith, L.M. (1964) J. Lipid Res. 5, 600—608
6 Anderson, R.E., Maude, M.B. and Zimmerman, W. (1975) Vision Res. 15, 1087—1090
7 Miljanich, G.P., Sklar, L.A., White, D.L. and Dratz, E.A. (1979) Biochim. Biophys. Acta 552, 294—306
8 Daemen, F.J.M. (1973) Biochim. Biophys. Acta 300, 255—288
9 Hendriks, T., Klompmakers, A.A., Daemen, F.J.M. and Bonting, S.L. (1976) Biochim. Biophys. Acta 433, 271—281
10 Stone, W.J., Farnsworth, C.C. and Dratz, E.A. (1979) Exp. Eye Res. 28, 387—397
11 Perret, B., Chap, H.J. and Douste-Blazy, L. (1979) Biochim. Biophys. Acta 556, 434—446
12 Emmelot, P. and van Hoeven, R.P. (1975) Chem. Phys. Lipids 14, 236—246
13 Renooij, W. and van Golde, L.M.G. (1977) Biochim. Biophys. Acta 470, 465—474
14 Fontaine, R.N., Harris, R.A. and Schroeder, F. (1980) J. Neurochem. 34, 269—277
15 Fong, B.S. and Brown, J.C. (1978) Biochim. Biophys. Acta 510, 230—241
16 Sandra, A. and Pagano, R.E. (1978) Biochemistry 17, 332—338
17 Crain, R.C., Marinetti, G.V. and O'Brien, D.F. (1978) Biochemistry 17, 4186—4192
18 Young, R.W. (1976) Invest. Ophthalmol. 15, 700—725
19 Young, R.W. (1967) J. Cell Biol. 33, 61—72
20 Hall, M.O., Bok, D. and Bacharach, A.D.E. (1969) J. Mol. Biol. 45, 397—406
21 Adams, A.J., Tanaka, M. and Shichi, H. (1978) Exp. Eye Res. 27, 595—605
22 Brown, P.K. (1972) Nat. New Biol. 236, 35—38
23 Cone, R.A. (1972) Nat. New Biol. 236, 39—43
24 Poo, M. and Cone, R.A. (1974) Nature 247, 438—441
25 Liebman, P.A. and Entine, G. (1974) Science 185, 457—459
26 Masland, R.H. and Mills, J.W. (1979) J. Cell Biol. 83, 159—178
27 Mizuno, A. (1976) J. Biochem. 80, 45—52
28 Bibb, C. and Young, R.W. (1974) J. Cell Biol. 62, 378—389
29 Anderson, R.E., Kelleher, P.A., Maude, M.B. and Maida, T.M. (1980) Neurochem. Intern. 1, 29—42
30 Anderson, R.E., Maude, M.B., Kelleher, P.A., Maida, T.M. and Basinger, S.F. (1980) Biochim. Biophys. Acta 620, 212—226
31 Anderson, R.E., Kelleher, P.A. and Maude, M.B. (1980) Biochim. Biophys. Acta 620, 227—235
32 Anderson, R.E., Maude, M.B. and Kelleher, P.A. (1980) Biochim. Biophys. Acta 620, 236—246
33 Bibb, C. and Young, R.W. (1974) J. Cell Biol. 61, 327—343
34 Basinger, S. and Hoffman, R. (1976) Exp. Eye Res. 23, 117—126
35 Kinney, M.S. and Fisher, S.K. (1978) Proc. R. Soc. Lond., Ser. B 201, 149—167
36 Papermaster, D.S., Converse, C.A. and Siu, J. (1975) Biochemistry 14, 1343—1352
37 Besharse, J.C. and Pfenninger, K.H. (1978) J. Cell Biol. 79, 97a
38 Papermaster, D.S., Schneider, B.G. and Besharse, J.C. (1979) J. Cell Biol. 83, 275a