Phospholipid Molecular Species Alterations in *Tetrahymena* Ciliary Membranes following Low-Temperature Acclimation†

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ABSTRACT: The molecular species compositions of phosphatidylcholine, phosphatidylethanolamine, and 2-(aminoethyl)-phosphonolipid, the three principal phosphoglycerides of *Tetrahymena pyriformis* ciliary membranes, have been determined by coupled gas chromatography–mass spectrometry of the *tert*-butyldimethylsilyl derivatives of their phospholipase C derived diglycerides. There were striking changes in the molecular species composition of phosphatidylcholine and phosphatidylethanolamine after the cells were chilled from 39 to 15 °C. The low temperature induced changes occurring in each phospholipid class were markedly different from those taking place in the other two. The molecular species patterns of the ciliary phosphoglycerides resembled in a qualitative way the patterns found in their microsomal counterparts. However, the relative proportions of many molecular species within each ciliary phospholipid class were very different from those of the corresponding microsomal component, and changes induced in a particular microsomal phospholipid class by chilling were often absent or even reversed in the equivalent ciliary phospholipid. Compositional changes in ciliary phospholipids during low-temperature acclimation were greater in nearly all respects than were concurrent changes in microsomal phospholipids. The molecular species data confirm that the cell possesses a variety of highly selective mechanisms allowing those organelles which accept phospholipids of microsomal origin to retain a surprising degree of independence in responding to environmental stress.

In numerous cell types, including the protozoan *Tetrahymena pyriformis* (Thompson & Nozawa, 1977), metabolic studies have identified the microsomal membrane as the principal assembly site for phospholipids. The modification of fatty acids by enzymatic desaturation is also carried out in microsomal membranes (Fukushima et al., 1977).

The processes whereby newly made microsomal phospholipids are transported to other functionally different membranes within the cell are not so well characterized. In some cell types, a gradual transformation of endoplasmic reticulum like membrane into structurally and functionally distinct organelles appears to take place through the process termed "membrane flow" (Morrê & Ovtracht, 1977). Cells also contain phospholipid transfer proteins, which can apparently catalyze a net flux of lipids from one membrane to another (Wirtz et al., 1980), as well as the more commonly observed one-for-one molecular exchange (Zilversmit & Hughes, 1976).

The in vivo specificity of these intracellular lipid transporting systems is poorly understood. However, it is well established that many membranes which derive their phospholipids from microsomes have compositional patterns which are markedly different from that of the donor microsomal membranes themselves. This is clearly the case with the *Tetrahymena* ciliary membrane, as illustrated in the preceding paper (Ramesha & Thompson, 1982). The ciliary membrane lipid composition does respond to compositional changes induced in the microsomal membrane but only after a lengthy delay. All available evidence points to a severely restricted movement of lipids between the organelles (Nozawa & Thompson, 1971; Ramesha & Thompson, 1982).

Because of the detailed information available with respect to the lipid composition and metabolism of *Tetrahymena* microsomal membranes (Dickens & Thompson, 1982), and now ciliary membranes (Ramesha & Thompson, 1982), the opportunity arose to compare, for the first time, the phospholipid molecular species compositions of ciliary membranes with those of microsomal membranes. Such a comparison, included in this report, provides information on the intracellular redistribution of individual phospholipid molecular species in low temperature stressed *Tetrahymena*.

Experimental Procedures

Materials. Phospholipase C from *Bacillus cereus* was either purchased from Boehringer Mannheim (Indianapolis, IN, grade II phospholipase C) or kindly provided by Dr. Kunihiko Saito, Kansai Medical University, Osaka, Japan (crude preparation from *B. cereus*). *tert*-Butyldimethylchlorosilane–imidazole reagent was purchased from Applied Science (State College, PA) in sealed 1-mL ampules and was opened just before use. All the solvents used were of analytical grade.

Culture Conditions. Tetrahymena pyriformis, strain NT-1, was cultured in the medium previously described by Fukushima et al. (1976). The cells were grown isothermally at 39 or at 15 °C and were harvested in their mid-logarithmic phase of growth [(2.2–2.5) × 10^5 cells/mL].

Isolation of Cilia and Analysis of Ciliary Lipids. Cilia were isolated after deciliating the cells by the Ca^{2+}-shock procedure described elsewhere (Ramesha & Thompson, 1982). Lipid extraction from cilia and separation into major phospholipid classes were done as described in the preceding paper (Ramesha & Thompson, 1982).

Molecular Species Determination. For analysis of the phospholipid molecular species, the isolated ciliary phospholipids were digested with phospholipase C, and the resulting diglycerides were converted to *tert*-butyldimethylsilyl (*t*-BDMS) ethers as described by Dickens & Thompson (1982).

The carbon number composition of *t*-BDMS diglycerides was analyzed with a Varian Model 3700 gas chromatograph with a 3% OV-1 packed column (diameter 0.25 in., length 6 ft) at 300 °C.

The molecular species composition of the *t*-BDMS derivatives was analyzed with a Finnigan Model 4000 gas chromatograph–mass spectrometer (GC–MS) equipped with a de-

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Table I: Distribution of Carbon Numbers in Ciliary Membrane Phospholipids[a]

retention time (min)	C_n	2-(aminoethyl)-phosphonolipid 39 °C	2-(aminoethyl)-phosphonolipid 15 °C	phosphatidylethanolamine 39 °C	phosphatidylethanolamine 15 °C	phosphatidylcholine 39 °C	phosphatidylcholine 15 °C
9.8	26			2.52	4.03	1.22	0.75
11.9	27			2.51	0.34	6.91	0.73
14.7	28			7.09	6.23	3.61	15.78
16.8	29			5.26	0.91	6.62	0.79
21.6	30	0.46	0.54	13.35	17.91	10.57	16.69
24.6	31	tr[b]	tr[b]	13.70	4.11		
						20.25[c]	3.11[c]
24.6	e32[d]	5.44	3.72				
32.2	32	5.61	3.86	27.10	34.25	33.52	17.13
36.7	33	*e*	*e*	15.39	10.90		
36.7	e34	84.79	90.32			13.49	34.63
48.0	34	3.68	7.07	13.08	21.33	3.80	10.40

[a] Values are expressed as mole percent and are the average of two analyses obtained from pooled samples by using a FID detector. [b] Trace amounts of C_{31} are masked under the C_{e32} peak. [c] This peak contains significant proportions of both C_{31} and C_{e32}. [d] Small e signifies the presence of an alkyl ether side chain at the *sn*-1 position. [e] Small amounts of C_{33} are masked under the much larger C_{e34} peak.

dicated Data General computer for data accumulation and manipulation. For GC–MS, samples were separated on a packed SP 2100 column at 300 °C and admitted to the mass spectrometer operating at a ionization voltage of 70 eV. Selected ion retrieval was used to identify and quantify the major phospholipid molecular species as described in Dickens & Thompson (1982).

Results and Discussion

Temperature-Induced Changes in Carbon Number Distribution. The three major ciliary phosphoglycerides, 2-(aminoethyl)phosphonolipid (AEPL), phosphatidylethanolamine (PE), and phosphatidylcholine (PC), were purified by preparative thin-layer chromatography. Each phospholipid was then digested with phospholipase C, and the resulting mixtures of diglycerides were converted into *tert*-butyldimethylsilyl (*t*-BDMS) ethers. The *t*-BDMS diglycerides were then separated by high-temperature gas chromatography into a series of peaks based on their carbon numbers (C_n = combined number of carbon atoms in the two attached fatty acids). The distribution of diglyceride moieties for the three phosphoglycerides, based on these carbon numbers, is shown in Table I.

AEPL exhibited the simplest pattern of the three phospholipid classes, having only a few major peaks. More than 80% of its molecular species at 39 and 15 °C were accounted for by the e34 peak, which consisted of a mixture of species each containing one alkyl ether side chain paired with an acyl side chain in combinations of chain lengths totalling 34 carbon atoms. The monoalkyl monoacyl derivatives were always eluted from the column ahead of their diacyl analogues but overlapped diacyl derivatives having one carbon less. Thus, C_{e32} and C_{e34} peaks contained C_{31} and C_{33} carbon peaks, respectively. In AEPL, the amounts of C_{31} and C_{33} were less than 2% of C_{e32} and C_{e34} peaks, respectively, as determined by GC–MS, and hence were treated as negligible components. Except for a slight increase in the C_{e34} peak and C_{34} peak in 15 °C ciliary AEPL, the distribution patterns in 39 and 15 °C fractions were similar.

In contrast to AEPL, ciliary PE exhibited major temperature-induced differences, the most noteworthy being the significantly higher population of molecular species having odd carbon numbers, e.g., C_{29}, C_{31}, and C_{33}, in 39 °C cilia, while PE isolated from 15 °C cilia contained elevated levels of certain even carbon numbers, namely, C_{30}, C_{32}, and C_{34}.

Table II: Molecular Species Composition of Ciliary Phospholipids[a]

carbon no.	molecular species	2-(aminoethyl)-phosphonolipid 39 °C	2-(aminoethyl)-phosphonolipid 15 °C	phosphatidylethanolamine 39 °C	phosphatidylethanolamine 15 °C	phosphatidylcholine 39 °C	phosphatidylcholine 15 °C
26	14:0/12:0	–	–	2.5	4.0	1.2	0.8
27	13:0/14:0	–	–	0.9	tr	4.7	tr
	15:0/12:0	–	–	1.7	tr	2.3	tr
28	16:0/12:0	–	–	3.6	3.8	tr	–
	14:0/14:0	–	–	1.4	2.2	3.1	15.5
	16:1/12:0	–	–	2.0	tr	–	–
29	15:0/14:0	–	–	2.1	0.6	1.0	tr
	13:0/16:0	–	–	1.5	tr	5.6	0.7
30	14:0/16:0	–	–	4.5	9.0	7.7	12.2
	14:0/16:1	tr	tr	4.1	8.7	1.9	3.8
	18:1/12:0	–	tr	3.2	–	tr	tr
	14:0/16:2	–	–	0.9	tr	–	tr
31	15:0/16:0	–	–	4.9	1.7	–	–
	14:0/17:1	–	–	7.3	1.6	–	–
	15:0/16:1	–	–	0.8	0.8	–	–
	15:0/16:2	–	–	0.7	tr	–	–
e32[b]	e16:0/16:0	tr	tr	–	–	9.0	0.8
	e16:0/16:1	3.6	2.1	–	–	8.6	2.2
	e16:0/16:2	0.7	0.8	–	–	–	–
	e16:1/16:T[c]	0.9	tr	–	–	–	–
32	15:0/17:0	–	–	–	–	1.1	–
	14:0/18:0	0.6	–	–	–	0.6	–
	15:0/17:1	–	–	–	–	3.2	1.1
	14:0/18:1	0.9	0.9	7.2	12.7	1.7	3.6
	14:0/18:2	1.6	1.5	6.6	6.6	5.2	3.2
	14:0/18:3	0.8	0.6	2.2	tr	–	1.3
	16:T/16:T	1.3	0.9	11.2	14.6	21.7	8.0
33	16:0/17:0	–	–	5.3	2.0	–	–
	15:0/18:1	–	–	2.2	2.4	–	–
	16:0/17:1	–	–	1.2	3.5	–	–
	15:0/18:2	–	–	2.5	3.0	–	–
	16:1/17:1	–	–	3.9	–	–	–
e34[b]	e16:0/18:0	–	–	–	–	1.0	–
	e16:0/18:1	11.8	6.8	–	–	3.8	6.2
	e16:0/18:2	35.6	20.3	–	–	5.4	6.5
	e16:0/18:3	27.9	45.6	–	–	2.1	18.7
	e16:1/18:T[d]	9.4	27.3	–	–	1.2	3.3
34	16:0/18:T	3.3	1.2	10.7	13.3	3.4	4.9
	16:1/18:T	tr	0.8	2.1	7.1	tr	–
	16:2/18:T	–	tr	tr	0.9	–	–

[a] Values are expressed as mole percent and are from a single analysis from a pooled sample of each phospholipid. tr represents values <0.5%; (–) means not detected. [b] The small e represents the presence of an alkyl ether linkange at the *sn*-1 position. [c] 16:T represents the total of 16:0, 16:1, and 16:2. [d] 18:T represents the total of 18:0, 18:1, 18:2, and 18.3.

The distribution of carbon numbers in ciliary PC was intermediate to that of AEPL and PE. Like AEPL, PC had sizable amounts of monoalkyl monoacyl peaks (C_{e32} and C_{e34}). The proportion of C_{e32} was higher in PC from 39 °C cilia while C_{e34} was higher in PC from 15 °C cilia. In addition to this difference, the PC from 39 °C cilia had comparatively higher proportions of C_{26}, C_{27}, C_{29}, and C_{32}, whereas that from 15 °C cilia contained elevated levels of C_{28}, C_{30}, and C_{34}.

Specific Fatty Acid Pairings of Phospholipid Molecular Species at High and Low Temperatures. Each chromatographically resolved peak, representing lipids having a similar carbon number, was shown by a more detailed analysis of the mass spectral data to be a mixture of many molecular species differing in their degree of unsaturation or specific fatty acid composition. These values are assembled in Figure 1 and Table II.

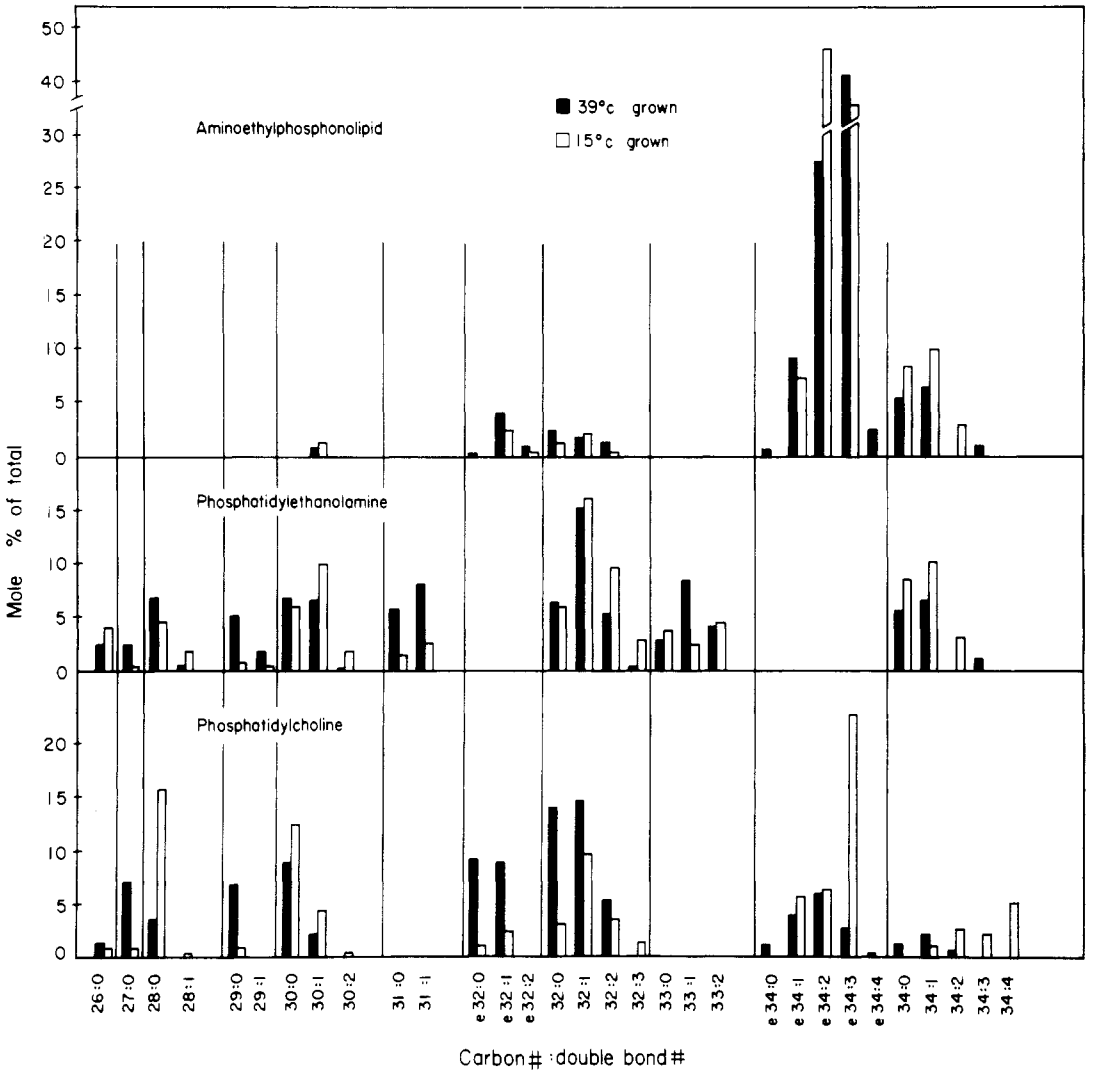

FIGURE 1: Relative abundance of M − 57 ions in the *t*-BDMS derivatives of ciliary phospholipids from *Tetrahymena* grown at 39 or 15 °C. The prefix e appearing before some carbon numbers indicates the presence of an alkyl ether side chain replacing one acyl group.

Figure 1 was compiled by using the values obtained from the mass spectrometer by selected ion retrieval of the specific M − 57 ion fragments, i.e., the fragments representing the total mass of each *t*-BDMS derivative minus the *tert*-butyl group (Myher et al., 1978; Satouchi & Saito, 1979). These figures might be expected to provide a quantitative impression of the molecular species groupings resolved by degree of unsaturation as well as carbon number. However, because the highly unsaturated fatty acids (such as 18:2 and 18:3) are more prone to fragment under the ionization conditions employed than are saturated fatty acids (Myher et al., 1978; Dickens & Thompson, 1982), the uncorrected M − 57 data as presented in Figure 1 are only semiquantitative. Nevertheless, they are of considerable value in making relative comparisons of change between the 39 and the 15 °C lipids.

By applying the corrections for differential fragmentation sensitivity described elsewhere (Dickens & Thompson, 1982) and analyzing other fragments characteristic of each peak, we were able to compile a table of corrected values showing how much of each molecular combination was present in a particular sample (Table II). The fatty acid pairings shown in the table list first the fatty acid believed to occur predominantly in the *sn*−1 position, as judged from a number of positional specificity studies using *Tetrahymena* [see Dickens & Thompson (1982) for discussion]. These corrected figures are in substantial agreement with the raw data in Figure 1 and also with the fatty acid composition reported earlier (Ramesha & Thompson, 1982).

Comparison of Ciliary Phospholipid Molecular Species from 39 and 15 °C Grown Cells. In interpreting the molecular species compositional changes in the ciliary lipids, it is important to bear in mind that the relative proportion of certain phospholipid classes differed greatly at high and low temperature. Ciliary phospholipids of 15 °C grown cells had a markedly reduced level of both phosphatidylcholine and phosphatidylethanolamine as compared with those of 39 °C cells (Ramesha & Thompson, 1982). Examination of the molecular species distributions (Table II) indicates that the decline in these two phospholipid classes was caused primarily by a very selective reduction of certain molecular species.

In the case of PC, decreases in certain alkyl ether containing molecular species, especially e16:0/16:0 and e16:0/16:1, and in diacyl combinations containing two C_{16} fatty acids were most noticeable, followed by slightly less pronounced changes in some species having odd chain components, particularly 14:0/13:0, 14:0/15:0, and 12:0/15:0. Other molecular species either sustained little change or increased in relative amounts. But since the level of ciliary PC in 15 °C grown cells was so greatly reduced below that found in 39 °C, even these latter species were present in somewhat lower absolute amounts.

The response pattern of ciliary PE to low-temperature exposure was totally different. A change in ether-linked species

could not be expected, since alkyl ethers were not present in PE, but the prevalent combinations of two C_{16} fatty acids did not decrease relative to other species. Sizable decreases were noted in several molecular species, however, especially among those having odd C_m e.g., 16:0/15:0, 14:0/17:1, and 16:0/17:0.

The striking decrease in ciliary PC and PE of 15 °C grown cells was offset by (1) a small rise in the level of AEPL and (2) a larger increase in ceramide-containing phospholipids (Ramesha & Thompson, 1982). The rise in AEPL could be accounted for mainly by a higher level of e16:1/18:T. Although evidence has been found for the metabolic transfer of diglyceride moieties from PC to AEPL (Thompson, 1969), the rise of e16:1/18:T in ciliary AEPL was not correlated with a fall in that species within the PC class. However, as mentioned above, the slight rise of e16:1/18:T (from 1% to 3%) in ciliary PC actually reflects a net decrease of that species along with many others, since the content of PC in cilia plummeted from 15% in 39 °C grown cells to 3% in 15 °C grown cells. Thus, a direct transfer of some e16:1/18:T moieties from PC to AEPL cannot be ruled out. But there is clearly no sign that the PC molecular species which show the greatest decline (both a relative and an absolute decrease) furnished diglycerides for AEPL synthesis.

In general, we can conclude that each of the three major ciliary phosphoglycerides responded to chilling in a manner that was both different in its details from that exhibited by the other two and independent of major input from the other two.

Differences between the Phospholipid Molecular Species in Ciliary and Microsomal Membranes. Because most phospholipid biosynthesis occurs in microsomal membranes, it is to be expected that the molecular species patterns of each ciliary phosphoglyceride would be strongly influenced by the composition of that lipid class in microsomes. From a qualitative point of view, this was found to be true [Figure 1 and Table II of this paper and Figure 2 and Table IV of Dickens & Thompson (1982)]. However, the two organelles, even when derived from cells grown at the same temperature, showed significant molecular species differences at the quantitative level. Moreover, the changes in ciliary phospholipid molecular species induced by low temperature were in some cases very dissimilar to those being effected during the same period in microsomal membranes. For example, ciliary PC 14:0/16:0 was a major constituent, increasing from 8% at 39 °C to 12% at 15 °C, but in microsomes, it was only 2% at 39 °C and showed no significant increase. Likewise, ciliary PC 14:0/14:0 rose from 3% at 39 °C to 15% at 15 °C while this species rose insignificantly from 0.5% to 3% in microsomes.

The same lack of predictability was observed in PE. In microsomes, the major C_{30} species, 14:0/16:1, decreased from 10% at 39 °C to 8% at 15 °C, while in cilia this species, also one of the two major components, increased from 4% at 39 °C to 9% at 15 °C. Microsomal 14:0/16:0 and 14:0/18:1 remained unchanged (2% and 4%, respectively) whereas in cilia 14:0/16:0 increased from 4% at 39 °C to 9% at 15 °C and 14:0/18:1 increased from 7% at 39 °C to 13% at 15 °C.

Even in AEPL, which was the phospholipid class least susceptible to change in both organelles, the cilia responded differently and in some cases more conspicuously than microsomes. Thus, the e16:1/18:T group of molecular species maintained a constant microsomal level of 10% at both 39 and 15 °C but changed from 9% in 39 °C cilia to 17% in 15 °C cilia. Also, the e16:0/18:3 species, which at 48% of the total AEPL was essentially unaffected by temperature in microsomes, rose from 28% in 39 °C cilia to 46% in 15 °C cilia. This latter sharp rise in cilia was offset by a loss of e16:0/18:1 and e16:0/18:2. It is worth emphasizing that as a rule the extent of molecular species change was considerably greater in cilia than in microsomes despite the fact that the majority of enzymes for lipid modification are considered to be more accessible to microsomal lipids.

Conclusions

Although there is still considerable room for refinement of GC–MS as a quantitative tool for lipid molecular species analysis, the technique is sensitive enough to support a number of important conclusions regarding *Tetrahymena* lipid metabolism. These conclusions modify our views on how cells respond to environmental stress.

Under steady-state growth conditions, the different phosphoglyceride classes of *Tetrahymena* microsomal membranes each maintains a molecular species composition that is very characteristic and significantly different from that of the other related lipid classes. Although the equivalent phospholipid classes in ciliary membranes each retains the same recognizable characteristics of their microsomal counterparts, there are reproducible differences in the molecular species patterns which are large enough to modify membrane physical properties. The molecular species differences between microsomal and ciliary lipids are every bit as striking as are the differences in fatty acid composition and serve to emphasize the structural individuality of the different organelles. This also suggests that during dissemination of lipids from microsomes to other organelles such as cilia, there is selection of specific phospholipid species based not only on their polar head group but also on their fatty acid makeup.

During acclimation of the cells to low temperature, the response of each phosphoglyceride class follows a relatively predictable course involving an increase in fatty acid unsaturation. Accompanying this trend are previously unrecognized alterations in molecular species distribution which in their magnitude overshadow the observed fatty acid changes (Ramesha & Thompson, 1982). The exact pattern of molecular species redistribution is distinctive for each lipid class but leads in all cases to a compositional reorganization appreciably greater than would be predicted on the basis of changes in fatty acid composition alone. The fact that ciliary structural responses to low temperature are in amost all respects more pronounced than those of microsomes leaves little doubt that the former changes are more than a passive consequence of localized microsomal response. Selective lipid transport from the microsomes or enzymatic retailoring of phospholipids at some extramicrosomal site might well be involved.

Unfortunately, there are as yet few other literature reports containing data comparable to those described here. Miller et al. (1976) have described quantitative differences in microsomal phospholipid molecular species classes of intestinal mucosa isolated from goldfish acclimated to 30 or 6 °C. Detailed information on fatty acid pairing was not reported. Thus, the relative importance of stress-induced phospholipid molecular species redistribution in other organisms cannot be assessed. However, if the degree of molecular species change observed in *Tetrahymena* is at all typical, it would seem highly desirable to monitor this type of response in other systems.

It is not possible to determine on the basis of the initial findings reported here just how much influence changes in phospholipid molecular species per se can have on ciliary membrane physical properties. Fluorescence depolarization analyses of individual phospholipid classes isolated by prepa-

rative TLC and reconstituted mixtures containing one or more components from a different temperature regime or from microsomes are now feasible. Such studies should be useful in establishing the relative contributions of molecular species changes vs. changes in the overall fatty acid composition in defining the ciliary membrane's characteristic physical behavior.

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References

Dickens, B. F., & Thompson, G. A., Jr. (1982) *Biochemistry* (first of three papers in this issue).

Fukushima, H., Martin, C. E., Iida, H., Kitajima, Y., Thompson, G. A., Jr., & Nozawa, Y. (1976) *Biochim. Biophys. Acta 431*, 165.

Fukushima, H., Nagao, S., Okano, Y., & Nozawa, Y. (1977) *Biochim. Biophys. Acta 488*, 442.

Miller, N. G. A., Hill, M. W., & Smith, M. W. (1976) *Biochim. Biophys. Acta 455*, 644.

Morré, D. J., & Ovtracht, L. (1977) *Int. Rev. Cytol., Suppl. No. 5*, 61.

Myher, J. J., Kuksis, A., Marai, L., & Yeung, S. K. F. (1978) *Anal. Chem. 50*, 557.

Nozawa, Y., & Thompson, G. A., Jr. (1971) *J. Cell Biol. 49*, 722.

Ramesha, C. S., & Thompson, G. A., Jr. (1982) *Biochemistry* (second of three papers in this issue).

Satouchi, K., & Saito, K. (1979) *Biomed. Mass Spectrom. 6*, 396.

Thompson, G. A., Jr. (1969) *Biochim. Biophys. Acta 176*, 330.

Thompson, G. A., Jr., & Nozawa, Y. (1977) *Biochim. Biophys. Acta 472*, 55.

Wirtz, K. W. A., Devaux, P. F., & Bienvenue, A. (1980) *Biochemistry 19*, 3395.

Zilversmit, D. B., & Hughes, M. E. (1976) *Methods Membr. Biol. 7*, 211.

Effects of Lipids on Acetylcholine Receptor. Essential Need of Cholesterol for Maintenance of Agonist-Induced State Transitions in Lipid Vesicles†

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ABSTRACT: The effects of lipids on the maintenance of characteristic functional properties of the acetylcholine receptor during the course of reconstitution into lipid vesicles were studied by following the kinetics of agonist-induced state transitions. The requirements for successful preservation of these properties could be dissected into two components: (a) adequate nature and concentration of lipids during detergent solubilization; (b) correct lipid environment during reincorporation into lipid vesicles by the cholate dialysis procedure. Optimal bulk lipid concentrations and lipid:cholate ratios for preserving state transitions during solubilization were studied by using both crude soybean lipids and pure synthetic phospholipids. The latter class of lipids was found to be unsuitable substitutes for the crude soybean lipids, irrespective of their polar head group and/or fatty acyl chain, even when detergent:lipid ratios as high as 1:1 (w/w) were employed. Addition of cholesteryl hemisuccinate was able to make up this deficiency, attaining preservation of acetylcholine receptor state transitions at cholate:steroid ratios of about 6:1 (w/w). The presence of steroid decreased the amount of protein solubilized. The correct choice of lipid type was also essential to the reincorporation step, and higher concentrations of lipid were required—about 20 mg/mL for soybean lipids. Pure phospholipids at similar concentrations, however, were unable to maintain the state transitions. Again, steroid (40–46% cholesteryl hemisuccinate/mol of total lipid) provided the adequate conditions at the reincorporation stage and enhanced the amount of protein reincorporated into the vesicles. A large (70–90%) percentage of the receptor was reincorporated with the correct vectorial sidedness. No specificity could be detected for the phospholipid polar head or alkyl chain in relation to any of these findings. The effect of the protein on the physical state of the lipids in the reconstituted vesicles was studied by diphenylhexatriene fluorescence depolarization. The results may be interpreted as a disordering of the acyl chains in the gel state and an ordering in the liquid-crystalline state in the presence of protein, accompanied by shifts in the transition temperatures of the pure phospholipids to lower values.

The acetylcholine receptor (AChR)[1] is a multimeric protein complex of five subunits, two of which appear to be almost identical (α) and the other three (β, γ, δ) partially homologous (Raftery et al., 1980). Affinity labels react covalently with the α chain, suggesting that this polypeptide is related totally or partially to the cholinergic recognition site [see the review in Karlin (1980)]. The function of the other subunits remains unknown, although an involvement of the δ chain in the binding of noncompetitive blockers was suggested from affinity

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[1] Abbreviations: AChR, acetylcholine receptor; DMPC, L-β,γ-dimyristoyl-α-lecithin; DMPA, 1,2-dimyristoyl-*sn*-glycero-3-phosphate; DMPG, 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol; PMPC, 1-palmitoyl-2-myristoyl-*sn*-glycero-3-phosphocholine; SOPC, 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPE, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine; NP buffer, 100 mM NaCl, 0.1 mM phenylmethanesulfonyl fluoride, and 10 mM sodium phosphate buffer, pH 7.4; DPH, 1,6-diphenyl-1,3,5-hexatriene.

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