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Formation of 7-*cis*- and 13-*cis*-Retinal Pigments by Irradiating Squid Rhodopsin†

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ABSTRACT: Squid rhodopsin was irradiated with orange light (>530 nm) at various temperatures from −190 to 10 °C until a photo-steady-state mixture was formed. Then the chromophoric retinals were extracted from the photo-steady-state mixtures and their isomer composition was analyzed by high-performance liquid chromatography. In the case of a photo-steady-state mixture formed at −85 °C, large peaks in the chromatogram were found at the positions of both 7-*cis*- and 13-*cis*-retinals. Each peak was further identified by synthesizing the pigments from these retinals with cattle opsin or apobacteriorhodopsin. Both 7-*cis*- and 13-*cis*-retinals were also extracted from a photo-steady-state mixture formed by irradiation at −40, at 0, or at 10 °C. These isomers were scarcely detected in a photo-steady-state mixture formed by irradiation at −190 °C, though 9-*cis*-retinal was found as a major constituent in this mixture. Irradiation of lumirhodopsin at −190 °C, however, produced 7-*cis*-retinal pigment. These findings suggest that bathorhodopsin may have a conformation to prevent the formation of 7-*cis*-retinal from the all-trans form and that this particular conformation may be relaxed by the conversion of bathorhodopsin to lumirhodopsin.

Squid rhodopsin has 11-*cis*-retinal as its chromophore (Hubbard & St. George, 1958). Upon absorbing light it converts into the all-trans form and the conformation of rhodopsin changes through several kinds of intermediates, hypsorhodopsin (Shichida et al., 1979), bathorhodopsin (Yoshizawa & Wald, 1964), lumirhodopsin (Kropf et al., 1959), and LM-rhodopsin (Tokunaga et al., 1975; Shichida et al., 1978). Depending on pH, the final product is acid or alkaline metarhodopsin (Hubbard & St. George, 1958). Each intermediate is stable at its characteristic temperatures (Shichida et al., 1978, 1979). The excitation of visual cells is believed to be caused by one of the intermediates.

Besides this primary action of light, there is a secondary photoprocess which leads to the formation of isorhodopsin, a stable isomeric form of rhodopsin. Isorhodopsin can be produced by irradiation of rhodopsin with orange light at liquid nitrogen temperatures (Yoshizawa & Wald, 1964). Recently, the chromophore of isorhodopsin was identified as 9-*cis*-retinal in cattle and frog rhodopsin systems by means of high-performance liquid chromatography (LC)[1] (Kawamura et al., 1978). Isorhodopsin can also be synthesized from authentic 9-*cis*-retinal with cattle opsin (Hubbard & Wald, 1952–1953).

Since all the mono-*cis*-retinals can be produced by simply irradiating *all-trans*-retinal in polar organic solvents (Denny & Liu, 1977; Maeda et al., 1978b), it is of interest to test the possibility that 7-*cis*- and 13-*cis*-retinals can be formed at the retinal binding site of rhodopsin. Maeda et al. (1978a) previously observed the formation of a 7-*cis*-retinal pigment by irradiating cattle rhodopsin at −75 °C.

The present report gives data on the isomeric composition of retinals which can be extracted from photo-steady-state mixtures formed by irradiating squid rhodopsin at various temperatures where the different intermediates are stable.

Materials and Methods

Chemicals. All the solvents used were reagent grade of Nakarai Chemicals, Ltd., Kyoto, Japan. Petroleum ether (bp 30–50 °C) and diethyl ether were refined as described by Rotmans & Kropf (1975).

Preparation of Microvilli (Rhodopsin). A microvilli fraction from squid (*Todarodes pacificus*) retina was prepared by a slight modification of a method described previously

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[1] Abbreviation used: LC, high-performance liquid chromatography.

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Table I: Molar Composition of Retinal Isomers Extracted from Squid Rhodospin[a] Irradiated at Various Temperatures

	temp (°C)	molar ratio[b] 13-cis[c]	11-cis	9-cis	7-cis	all-trans
unirradiated		1 ± 1	96 ± 1	0	0	3 ± 1
irradiated with 437-nm light	−190	2 ± 1	24 ± 1	27 ± 1	0	47 ± 2
irradiated with >530-nm light	−190	3 ± 1	11 ± 6	84 ± 6	0	2 ± 1
	−85	19 ± 1	21 ± 4	12 ± 2	40 ± 2	8 ± 2
	−40	14 ± 1	18 ± 3	4 ± 1	46 ± 3	18 ± 2
	0	7 ± 1	38 ± 3	3 ± 1	21 ± 1	31 ± 3
	10	8 ± 1	47 ± 2	2 ± 0	12 ± 1	31 ± 2

[a] About 0.4 $A_{480\,nm}$ unit for each experiment. [b] Averages of the successive four extracts. [c] Di-cis isomers of 13-*cis*-retinal may be included in this fraction. However, the amount of retinals in this fraction was calculated as if all the material in this fraction is 13-*cis*-retinal.

Table II: Molar Composition of Retinal Isomers Extracted from the Photoproducts Obtained by Irradiating the Mixture Containing Lumirhodopsin or Bathorhodopsin[a]

	irradiation		molar ratio[b] 13-cis[c]	11-cis	9-cis	7-cis	all-trans
sample irradiated with 437-nm light at −196 °C			5 ± 1	19 ± 2	33 ± 4	0	43 ± 3
sample warmed to	>560 nm	15 s	3 ± 0	24 ± 3	32 ± 1	13 ± 0	28 ± 1
−78 °C, then recooled to	>530 nm	2 s	4 ± 0	24 ± 3	35 ± 1	10 ± 0	27 ± 2
−196 °C (containing lumi-	>530 nm	1 min	7 ± 1	10 ± 1	66 ± 2	4 ± 0	13 ± 1
rhodopsin) followed by	>530 nm	5 min	9 ± 1	7 ± 1	70 ± 3	4 ± 0	11 ± 1
irradiation	>530 nm	30 min	5 ± 1	5 ± 1	76 ± 1	5 ± 0	9 ± 2
sample kept at −196 °C	>560 nm	15 s	3 ± 0	44 ± 2	50 ± 2	0	3 ± 1
(containing bathorhodopsin)	>530 nm	2 s	4 ± 0	38 ± 3	53 ± 3	0	5 ± 0
followed by irradiation	>530 nm	30 min	6 ± 1	8 ± 2	82 ± 4	0	4 ± 1

[a] See the legend of Figure 2. [b] Averages of successive four extracts. [c] Di-cis isomers of 13-*cis*-retinal may be included in this fraction. However, the amount of retinals in this fraction was calculated as if all the material in this fraction is 13-*cis*-retinal.

(Shichida et al., 1978). The outer limbs of photoreceptor cells were detached from retinas by gently shaking the bisected eyes in 0.1 M phosphate buffer, pH 6.8 (buffer P). The outer limbs were then floated on 40% sucrose by centrifuging at 10000*g* for 30 min. The supernatant was diluted with 3 volumes of buffer P and centrifuged at 10000*g* for 1 h. The precipitate was suspended in buffer P. Next flotation on 38% sucrose and centrifugation of the diluted supernatant were repeated twice. The final precipitate was washed successively by centrifugation at 14000*g* for 40 min with buffer P, distilled water, 0.1 M sodium carbonate buffer, pH 10.0 (buffer C), and distilled water. These successive washings were repeated five times. The precipitate finally obtained consisted of two layers, a semitransparent upper layer, presumably rhabdomal membranes (microvilli), and relatively dense lower layer, presumably the cores or basal parts of the outer segments.

In order to isolate the microvilli fraction, the upper layer of the precipitate was centrifuged in a linear sucrose gradient (25–45 %) in buffer P at 105000*g* for 8 h. The microvilli fraction was clearly separated from the other parts. To remove a screening pigment (ommochrome) contaminated in microvilli fraction, washing by suspending and centrifuging at 24000*g* for 1 h in buffer C and in distilled water were repeated three times. The microvilli fraction thus obtained is completely free from the other retinal-containing pigment, retinochrome (Hara & Hara, 1965). The microvilli fraction was suspended in buffer P and mixed with 2 volumes of glycerol.

Irradiation of Rhodopsin. For the selection of wavelengths to irradiate rhodopsin, a glass cut-off filter, Toshiba VO 55, which transmits light of wavelengths longer than 530 nm, or Toshiba VO 58, which transmits light of wavelengths longer than 560 nm, or an interference filter of Toshiba which transmits light of 437 nm was used.

In the experiments of Figure 1 and Table I, the sample was put into the optical cell which was mounted in a specially designed glass cryostat described by Yoshizawa (1972). An opal glass was placed just behind the cell. The temperature of the sample was monitored with a copper–constantan thermocouple attached to a sample cell holder made of deoxygenated copper. A xenon lamp (Ushio, 500 W) was used as a light source for irradiation. Absorption spectra were measured with a Hitachi Type 323 recording spectrophotometer. The formation of a photo-steady-state was assured by the measurements of the spectra after successive irradiations.

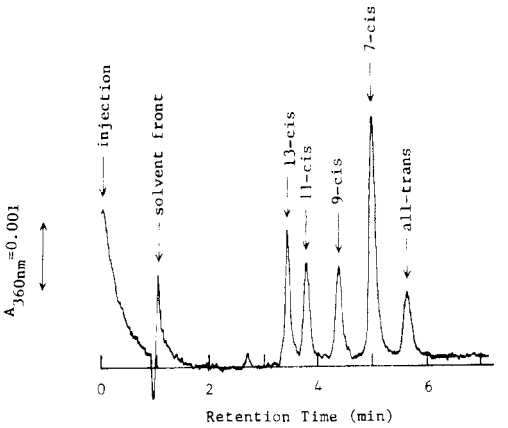

FIGURE 1: An LC pattern of retinals extracted from the products obtained by irradiating squid rhodopsin (about 0.4 A_{480nm} unit) with >530-nm light at −85 °C.

In the experiments of Figure 2 and Table II, 0.5 mL of the sample was put into test tubes and irradiated in the bath containing liquid nitrogen with light from a 1-kW tungsten lamp.

Extraction of Retinals. Retinals were extracted from the squid microvilli according to the method of Pilkiewicz et al. (1977) with slight modifications. The sample in the optical

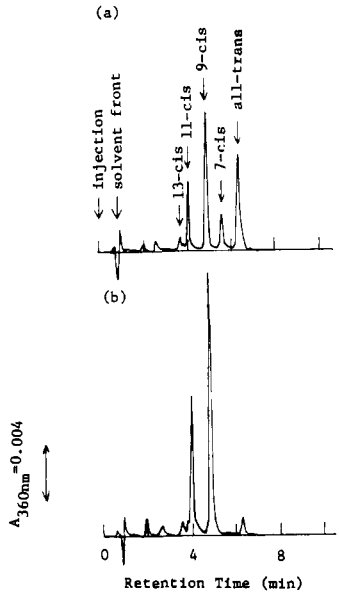

FIGURE 2: LC patterns of retinals extracted from the products obtained by irradiating the mixtures containing (a) lumirhodopsin and (b) bathorhodopsin with >530-nm light at −196 °C for 2 s. These mixtures were prepared as described in the text; 0.6 A_{480nm} unit of squid rhodospin was used as the starting material for each experiment.

cell was washed out with 4.5 mL of ice-cold water. After the addition of 0.05 mL of glacial acetic acid and 5 mL of dichloromethane, the sample was vigorously mixed by ten times injection from a syringe with a needle (no. 15). Ten milliliters of petroleum ether was then added, and the solution was mixed well. After centrifugation at 3000*g* for 10 min, the upper layer composed of mainly dichloromethane and petroleum ether was removed with a Pasteur pipet. To this mixture was added a small amount of solid anhydrous sodium sulfate. The supernatant fluid was decanted and completely evaporated under a stream of nitrogen gas. The residue thus obtained was dissolved in 25 μL of heptane and then subjected to LC analysis. The second and the successive extractions were repeated by mixing 5 mL of dichloromethane and 10 mL of petroleum ether with the residual water phase. The extraction processes were repeated five times. The amount of the retinals in one cycle of the extraction process was usually several percent of the total expected amount in the starting material. However, no significant changes were noticed in the molar ratios among successive extracts (see standard deviations in Tables I and II). All the manipulations were done below 4 °C and under dim red light.

LC Analysis. A Shimadzu-Du Pont LC-1 system equipped with a Zorbax SIL column (4.0 × 150 mm) was used. Solvent was petroleum ether:diethyl ether, 88:12 (v/v). Flow rate was 2 mL/min (at a pressure of 40 kg/cm^2). Temperature was 30 °C. The chromatogram was recorded by a Shimadzu spectrophotometric detector, SPD-1, at 360 nm. Each retinal peak was identified by a comparison of its retention time with those of authentic isomers, which were prepared by irradiating *all-trans*-retinal with white light (Denny & Liu, 1977; Maeda et al., 1978b) and then purified in the same LC system from the mixture. The molar amount of each retinal was determined by comparing the area under the peak integrated by a Shimadzu digital integrator (ITG-4A) with that obtained from the authentic sample, whose concentration was calculated from the known molar extinction coefficient (13-*cis*-retinal, 38 800 at 363 nm; 11-*cis*-retinal, 26 400 at 362.5 nm; 9-*cis*-retinal, 39 700 at 362 nm; *all-trans* retinal, 44 300 at 366.5 nm in hexane (Hubbard et al., 1971); or 7-*cis*-retinal, 44 100 at 359 nm in heptane (DeGrip et al., 1976)). This method gave almost the same retinal ratio as that determined by the cut-and-weigh method.

Results

The isomeric composition of the chromophoric retinals which were extracted from squid microvilli irradiated with orange light at various temperatures was analyzed by LC (Table I). When starting such experiments, it is necessary to check for possible contamination of free retinals or acid metarhodopsin in the microvilli preparation before irradiation. As shown in Table I, line 1, the amount of 11-*cis*-retinal, presumably due to rhodopsin, was estimated at 96% of the total retinal. The residual amount (4%) of retinals consisted of 3% of all-trans, less than 1% of 13-cis, and traces of other forms.

Next, retinals were extracted from various photo-steady-state mixtures containing spectrophotometrically known photoproducts of rhodopsin. A large amount of bathorhodopsin, a presumed all-trans intermediate, was prepared by irradiating squid rhodopsin with blue light (437 nm) at −190 °C. As expected, *all-trans*-retinal along with 9-*cis*- and 11-*cis*-retinals were found as the major constituents in the extract of retinals (Table I, line 2). The molar fraction of 13-*cis*-retinal in the extract was 2% and no 7-*cis*-retinal was found. The molar composition of retinal isomers obtained by the present LC analysis is slightly different from that obtained spectrophotometrically (Shichida et al., 1978) probably owing to the fact that the retinals were extracted from microvilli, while spectra were measured on digitonin-extracted rhodopsin.

Irradiation of rhodopsin at liquid nitrogen temperatures with orange light (>530 nm) produces a photo-steady-state mixture composed of a large amount of isorhodopsin with small amounts of bathorhodopsin and rhodopsin (Yoshizawa & Wald, 1964). As seen in Table I, line 3, 9-*cis*-retinal was predominant in the extract from this photo-steady-state mixture. This result is consistent with our previous findings on the chromophoric composition of frog and cattle isorhodopsins (Kawamura et al., 1978). The amount of 13-*cis*-retinal was very small and no 7-*cis*-retinal was found.

A retinal extract was also prepared from the photo-steady-state mixture formed by irradiating rhodopsin with orange light (>530 nm) at −85 °C, where lumirhodopsin is stable (Kropf et al., 1959). The LC pattern of the extract is shown in Figure 1. In this case, large peaks were found at exactly the same positions as those of authentic 7-*cis*- and 13-*cis*-retinals. The component at the 7-*cis*-retinal position was mixed with cattle opsin. A pigment was formed, λ_{max}(450 nm) of which is identical with that produced from authentic 7-*cis*-retinal (DeGrip et al., 1976; Maeda et al., 1978b). The 13-*cis*-retinal fraction was also mixed with apobacteriorhodopsin (Oesterhelt & Schuhmann, 1974; Maeda et al., 1977), which was provided by Dr. F. Tokunaga. A pigment with λ_{max} at 550 nm, which is characteristic of 13-*cis*-bacteriorhodopsin, was produced, indicating that the retinal in this fraction contains the 13-cis isomer. But the possible presence of di-cis isomers of 13-*cis*-retinal cannot be excluded. For the sake of convenience, the amount of retinals in this fraction was calculated on the assumption that all the material in this fraction is 13-*cis*-retinal. The molar composition of the retinals is shown in Table I, line 4.

The composition of retinal was also analyzed from a photo-steady-state mixture irradiated with orange light (>530 nm) at −40 °C where LM-rhodopsin is stable (Shichida et al., 1978). The formation of 7-*cis*- and 13-*cis*-retinals was observed (Table I, line 5). Rhodopsin was also irradiated with orange light (>530 nm) at 0 °C or at 10 °C where acid

metarhodopsin is stable (Hubbard & St. George, 1958; Table I, lines 6 and 7). 7-*cis*- and 13-*cis*-retinals, though somewhat smaller in molar fraction, were also found in the photo-steady-state mixture. In a series of the experiments described above, significant amounts of 7-*cis*- and 13-*cis*-retinals were found by irradiation at all the temperatures above −85 °C.

The failure to produce 7-cis isomers by irradiation at −190 °C could result from the presence of specific barriers around the retinal binding site in bathorhodopsin, which is stable at −190 °C. As the 7-cis isomer was produced at the temperatures where lumirhodopsin is stable, an experiment was designed to determine whether the 7-*cis*-retinal isomer can be produced from lumirhodopsin by irradiation at liquid nitrogen temperatures or not.

Two tubes of the mixture which contained bathorhodopsin along with rhodopsin and isorhodopsin, but did not contain 7-*cis*-retinal pigment, were prepared by irradiation of rhodopsin with 437-nm light at −196 °C for 1 h. One was warmed in a bath of dry ice–acetone (about −78 °C) to convert bathorhodopsin into lumirhodopsin and then recooled to −196 °C. The other was kept at −196 °C. Thus, the former contained lumirhodopsin and the latter contained bathorhodopsin in exactly the same molar quantity. Both were then irradiated with light of wavelengths longer than 530 nm for 2 s or with light of wavelengths longer than 560 nm for 15 s. The retinals were then extracted. Figure 2 shows the LC patterns of these extracts. The 7-*cis*-retinal peak obviously emerged in the photoproducts from lumirhodopsin but not in the photoproducts from bathorhodopsin. The latter contained a much larger amount of 9-*cis*-retinal.

The molar compositions of these samples were compared with those of the starting material and of the products irradiated for longer durations (Table II). More than half of the lumirhodopsin was converted into the 7-*cis*-retinal pigment at early stages of irradiation. On further irradiation, 7-*cis*-retinal decreased, while 9-*cis*-retinal increased at the expense of *all-trans*-, 11-*cis*-, and 7-*cis*-retinals. Thus, at liquid nitrogen temperatures, the 7-*cis*-retinal pigment can be produced transiently from lumirhodopsin but cannot be produced at all from bathorhodopsin. It can be concluded that the retinal binding site of lumirhodopsin allows photoisomerization of *all-trans*-retinal into 7-*cis*-retinal even at liquid nitrogen temperatures. 13-*cis*-Retinal formation from lumirhodopsin was not noticed at these low temperatures.

Discussion

On irradiation of squid rhodopsin at temperatures between −85 and 10 °C, a considerable fraction of its 11-*cis*-retinal chromophore can eventually be isomerized into 7-cis and 13-cis (and possibly its di-cis) forms, whereas these retinals were not detected in the photo-steady-state mixture irradiated at liquid nitrogen temperatures. Roughly speaking, at higher temperatures the amount of 9-*cis*-retinal is very small under the conditions where 7-*cis*-retinal can be formed. The possibility that 7-cis and 13-cis isomers were produced from *all-trans*-retinal by thermal isomerization in the process of extraction was eliminated by the fact that a large amount of all-trans isomer was found in the preparation with small amounts of 7-cis and 13-cis isomers (less than 2% and 5% for 7-*cis*- and 13-*cis*-retinals, respectively, to the amount of *all-trans*-retinal) (Table I, line 2).

7-*cis*-Retinal pigment was formed from lumirhodopsin even at liquid nitrogen temperatures, whereas 7-*cis*-retinal was not formed at all from bathorhodopsin. This fact could be interpreted to mean that the specific conformation around the retinal binding site of bathorhodopsin inhibits the formation of 7-*cis*-retinal and such inhibition may be relieved by the conversion of bathorhodopsin into lumirhodopsin. This is an indication of the conformational changes of the protein part of the pigment in the process of the conversion of bathorhodopsin to lumirhodopsin. The 7-*cis*-retinal formed from lumirhodopsin at liquid nitrogen temperatures, however, cannot survive in the light, presumably because the isomerization of retinal at liquid nitrogen temperatures may no longer allow the protein conformation corresponding to the lumi state. As a result, the 7-*cis*-retinal pigment presumably changes to bathorhodopsin, which eventually changes to isorhodopsin, 9-*cis*-retinal pigment.

Differences between bathorhodopsin and lumirhodopsin might be inferred from the conformational difference of retinal isomers produced from these respective intermediates by irradiation. It should be noted that both 7-*cis*- and 13-*cis*-retinal isomers have cis conformation at the ends of the polyene side chain and the plane of the polyene side chain of 7-*cis*-retinal is almost perpendicular to the plane of the Ionone ring. Thus, 7-*cis*-retinal in rhodopsin would need some space outside the region usually occupied by 11-*cis*- and 9-*cis*-retinals, assuming that the Ionone ring does not move from its binding site during the photoisomerization process. In lumirhodopsin, the protein moiety might be flexible enough to accommodate the formation of 7-*cis*-retinal or *all-trans*-retinal.

Suzuki et al. (1976) reported that a photoproduct, P_{465}, formed by irradiating squid rhodopsin with orange light (>530 nm) at −40 °C. They argued from their spectrophotometric experiments that P_{465} has a cis-type retinal other than 9-*cis*- and 11-*cis*-retinal. We infer from the present experiments that P_{465} is a photoproduct having either 7-*cis*- or 13-*cis*-retinal as a chromophore or is a mixture of the products having both these retinals as chromophores.

Do the 7-*cis*- and 13-*cis*-retinal pigments produced by irradiation at low temperatures belong to a class of stable photoproducts together with isorhodopsin? A preliminary experiment indicates that the pigments formed at −85 °C changed their conformation irreversibly upon warming and in a different manner from that of *all-trans*-lumirhodopsin. Thus, they should be the unstable entities, that is, intermediates in the dark reaction.

7-*cis*-Retinal was also found in the retinal extracts from cattle rhodopsin irradiated at −75 °C (Maeda et al., 1978a). Rhodopsin from other sources is also expected to form 7-*cis*-retinal upon irradiation at dry ice–acetone temperatures or above. In this respect the data on lumirhodopsin or other intermediates should be carefully evaluated.

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Gel to Liquid-Crystalline Phase Transitions in Water Dispersions of Saturated Mixed-Acid Phosphatidylcholines†

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ABSTRACT: Mixed-acid saturated lecithins containing myristate, palmitate, and stearate chains have been synthesized by phospholipase A_2 digestion of the appropriate single-acid lecithin, followed by reacylation of the lysolecithin with the desired fatty acid anhydride. Variable amounts of acyl migration were found to occur during the syntheses. The positional isomers 1-myristoyl-2-palmitoyl-*sn*-glycero-3-phosphorylcholine and 2-myristoyl-1-palmitoyl-*sn*-glycero-3-phosphorylcholine as well as 1-palmitoyl-2-stearoyl-*sn*-glycero-3-phosphorylcholine and 2-palmitoyl-1-stearoyl-*sn*-glycero-3-phosphorylcholine have been made. The thermotropic behavior of these lipids dispersed in excess water has been studied by differential scanning calorimetry. Positional isomers of mixed-acid saturated lecithins containing acids whose chain lengths differ by two methylene units are found to have different gel to liquid-crystalline transition temperatures and enthalpies. For each isomeric pair of mixed-acid lecithins, the one which had the longer chain in the sn-1 position of glycerol was found to have the lower transition temperature of the two. A partial phase diagram has been prepared by use of the palmitoyl and myristoyl mixed-acid lecithins so as to obtain extrapolated transition temperatures for the pure lecithins without acyl migration. The gel to liquid-crystalline transition temperatures of water dispersions of 2-myristoyl-1-palmitoyl-*sn*-glycero-3-phosphorylcholine and 1-myristoyl-2-palmitoyl-*sn*-glycero-3-phosphorylcholine were found to be 27.2 and 35.5 °C, respectively. A model which proposes slight differences in chain packing in the bilayer in the gel state is presented as a possible explanation for the observed differences in the thermotropic behavior of the positional isomers.

Water dispersions of phosphatidylcholines containing a single type of saturated fatty acyl residue have been studied extensively as models for biological membranes. A considerable body of information is now available from a number of physical techniques on the thermotropic phase changes which occur in aqueous suspensions of these simple lipids alone and of their binary mixtures (Chapman, 1975; Lee, 1977a,b). On the other hand, mixed-acid lecithins as single species have received only scant study in model membranes (Phillips et al., 1972; DeKruyff et al., 1973; Barton & Gunstone, 1975; Seelig & Waespe-Šarčevič, 1978), although they comprise by far the majority of naturally occurring lecithins.

Here we report on the thermal characteristics of lecithins in which myristoyl, palmitoyl, and stearoyl chains have been mixed. Although thermal data is available on dispersions of mixtures of single-acid lecithins containing these chains (Ladbrooke et al., 1968; Phillips et al., 1970; Shimshick & McConnell, 1973; Chapman et al., 1974; Mabrey & Sturtevant, 1976), no systematic study of mixtures of these chains in the same phosphatidylcholine molecule has been carried out previously. Small modifications within a given molecule—in the position of double bonds, in the methylation of the head-group nitrogen, and in the types of glycerol–hydrocarbon chain links—have been observed to change the gel to liquid-crystalline transition temperature of phospholipids in water dispersions (Abramson, 1970; Vaughan & Keough, 1974; Barton & Gunstone, 1975). Here we report another intramolecular structural change which has a significant effect on the thermal phase transition of a phospholipid, but in this case no new types of bonds or chemical groups are introduced. We observe that in a mixed-acid saturated lecithin, when dispersed in water, the gel to liquid-crystalline phase transition temperature is determined by the distribution of the two acyl

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