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# Enzymatic Synthesis of Selenocysteine in Rat Liver†

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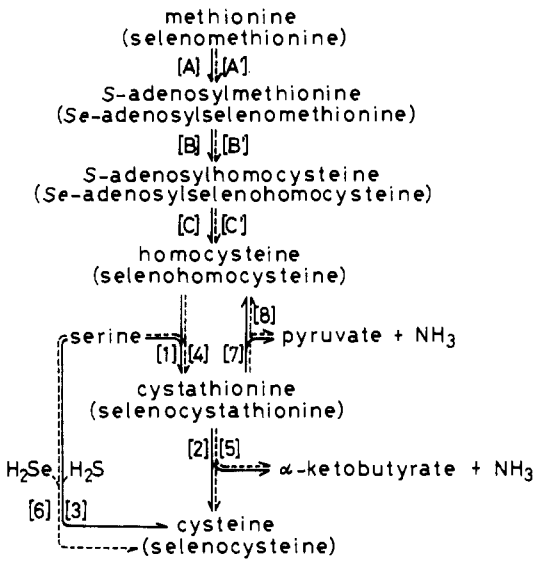
ABSTRACT: We have investigated selenocysteine (2-amino-3-hydroselenopropionic acid) synthesis with cystathionine β-synthase (EC 4.2.1.22) and cystathionine γ-lyase (EC 4.4.1.1) of rat liver. When selenohomocysteine and serine were incubated with cystathionine β-synthase, selenocystathionine was formed at a rate of 69% of that of cystathionine synthesis. Cystathionine γ-lyase catalyzed α,γ elimination of selenocystathionine to yield α-ketobutyrate, selenocysteine, and $NH_3$. The reaction rate was about 3 times higher than that of cystathionine elimination. Cystathionine β-synthase, however, did not catalyze direct formation of selenocysteine from serine and $H_2Se$. Thus, selenocysteine is synthesized from selenohomocysteine and serine through selenocystathionine by coupling of cystathionine β-synthase and cystathionine γ-lyase reactions. We confirmed this synthetic pathway also with a mixture of both enzymes and with a homogenate of rat liver.

Various selenium-containing amino acids occur in nature and play important physiological roles (Shrift, 1973; Stadtman, 1980). Several microbial and mammalian proteins, in particular enzymes, contain selenium as an essential component (Stadtman, 1980). Three of them have been shown to contain a selenocysteine (2-amino-3-hydroselenopropionic acid) residue in their polypeptide chains: selenoprotein A of glycine reductase complex from *Clostridium sticklandii* (Cone et al., 1976), formate dehydrogenase of *Methanococcus vannielii* (Jones et al., 1979), and glutathione peroxidase of rat liver (Forstrom et al., 1978) and bovine erythrocyte (Ladenstein et al., 1979). Evidence has been obtained for the participation of a selenocysteine residue in the catalytic processes (Forstrom et al., 1978; Ladenstein et al., 1979). There are two possible pathways for synthesis of the selenocysteine residue of glutathione peroxidase, posttranslational incorporation of selenium to the residue, as suggested by Sunde & Hoekstra (1980), and direct incorporation of selenocysteine (Hawkes et al., 1979). However, little attention has been paid to the biosynthesis of selenocysteine.

Selenocysteine can be formed from *O*-acetylserine and $H_2Se$ by *O*-acetylserine (thiol)-lyase (EC 4.2.99.8) of selenium accumulator and nonaccumulator plants (Ng & Anderson, 1978) and of *Paracoccus denitrificans* (Burnell & Whatley, 1977). Selenomethionine has been demonstrated in wheat and some other grains, but there are few reports of the occurrence of selenocysteine and selenocystine (Olson et al., 1970). In mammalian tissues, selenocysteine synthesis has been assumed to be analogous to that of cysteine (Scheme I), but no evidence has been obtained.

We here present the evidence that selenocysteine is produced enzymatically by coupling of reactions 4 and 5, but not by reaction 6, with cystathionine β-synthase (EC 4.2.1.22) and cystathionine γ-lyase (EC 4.4.1.1) of rat liver.

Scheme I: Biosynthetic Pathways of Cysteine (–) and Selenocysteine (---)[a]




[a] Cystathionine β-synthase catalyzes reactions 1, 3, 4, and 6; cystathionine γ-lyase catalyzes reactions 2, 5, 7, and 8. Reaction A or A' is catalyzed by ATP:L-methionine *S*-adenosyltransferase of rabbit liver (Greene, 1969). Reaction B or B' is catalyzed by various transmethylation systems (Mudd & Cantoni, 1957; Bremer & Natori, 1960; Pan & Tarver, 1967). Reaction C is catalyzed by *S*-adenosylhomocysteine hydrolase. No evidence has been obtained for reaction C'.

## Experimental Procedures

*Materials.* DL-Selenomethionine and DL-selenocystine were purchased from Sigma, L-homocysteine, L-cystathionine, dithiothreitol, and heptafluorobutyric acid were from Nakarai Chemicals, Kyoto, Japan, and aluminum selenide was from Alfa Division—Ventron. *Se*-Benzyl-L-selenohomocysteine was synthesized from dibenzyl diselenide and L-α-amino-γ-

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0006-2960/81/0420-4492$01.25/0 

bromobutyric acid (Baddiley & Jamieson, 1954) by a modification of the method of Jakubke et al. (1968). L-Selenohomocystine was synthesized from *Se*-benzyl-L-selenohomocysteine with sodium in liquid ammonia according to the method of Painter (1947). L-Selenocystathionine was synthesized from *Se*-benzylselenohomocysteine and β-chloroalanine by the method of Zdanski (1968). Selenohomocysteine and selenocysteine were prepared from the corresponding diselenides by reduction with 2 mol of dithiothreitol per mol of diselenide in 40 mM Tricine–NaOH buffer (pH 8.0) in a stream of $N_2$ at room temperature (about 20 °C) for 10 min. $H_2Se$ was prepared from aluminum selenide by the method of Ng & Anderson (1978). Cystathionine β-synthase was purified from rat liver according to the method of Kimura & Nakagawa (1971) except that steps after the DEAE-cellulose column chromatography were omitted. The specific activity of the enzyme for cystathionine synthesis (reaction 1) was 0.16 unit/mg of protein under the conditions described below. Crystalline cystathionine γ-lyase of rat liver was prepared by the method of Matsuo & Greenberg (1958). Alanine dehydrogenase (EC 1.4.1.1) (Ohshima & Soda, 1979) and leucine dehydrogenase (EC 1.4.1.9) (Ohshima et al., 1978) were purified to homogeneity from cell-free extracts of *Bacillus sphaericus* (IFO 3525) and *Bacillus megaterium* (ICR 1390), respectively.

*Enzyme Assay.* All the reactions were performed at 37 °C in sealed tubes in which air was displaced by $N_2$. Boiled enzyme was substituted for the native enzyme in a blank.

*(1) β-Replacement Reactions Catalyzed by Cystathionine β-Synthase.* The rate of reaction 4 was determined with the system (0.5 mL) composed of 1.0 μmol of L-serine, 1.5 μmol of L-selenohomocysteine, 3.8 μmol of dithiothreitol, 50.0 μmol of Tricine–NaOH buffer (pH 8.6), 5.0 nmol of pyridoxal 5′-phosphate (pyridoxal-P), and 60 μg of enzyme (specific activity 0.16 μmol $mg^{-1}$ $min^{-1}$). After incubation for 30 min, the reaction was stopped by addition of 0.1 mL of 50% trichloroacetic acid. An aliquot of the reaction mixture was analyzed on a Hitachi amino acid analyzer (see below) to determine selenocystathionine.

The incubation mixture for reactions 3 and 6 contained 20 μmol of L-serine, 20 μmol of $H_2S$ (or $H_2Se$), 10 nmol of pyridoxal-P, 150 μmol of Tricine–NaOH buffer (pH 8.6), and 0.12 mg of enzyme (specific activity 0.16 μmol $mg^{-1}$ $min^{-1}$) in a final volume of 1.0 mL. After 10 min, the reaction was terminated by addition of 1.0 mL of 1 N HCl, and the remaining $H_2S$ (or $H_2Se$) was released by heating the mixture at 100 °C for 20 min. Cysteine (or selenocysteine) formed was determined by amino acid analysis after derivatization as described below.

The velocity of cystathionine synthesis (reaction 1) was determined as follows. The reaction was performed for 10 min in the assay mixture composed of 10 μmol of L-serine, 10 μmol of L-homocysteine, 10 nmol of pyridoxal-P, 100 μmol of Tricine–NaOH buffer (pH 8.6), and 0.12 mg of enzyme (specific activity 0.16 μmol $mg^{-1}$ $min^{-1}$) in a final volume of 1.0 mL. Cystathionine produced was determined by amino acid analysis.

*(2) β- and γ-Elimination Reactions Catalyzed by Cystathionine γ-Lyase.* The assay mixture of reactions 2, 5, 7, and 8 contained 3 μmol of L-cystathionine (or L-selenocystathionine), 50 μmol of potassium phosphate buffer (pH 7.5), 5 nmol of pyridoxal-P, and 1.5 μg of enzyme. The reaction was performed at 37 °C for 5 min. A total amount of α-keto acids was measured with 3-methyl-2-benzothiazolone hydrazone hydrochloride (MBTH) (Soda, 1968). α-Ketobutyrate and pyruvate formed were converted to α-aminobutyrate and alanine with leucine dehydrogenase and alanine dehydrogenase (Ohshima & Soda, 1979), respectively, and determined by amino acid analysis. The dehydrogenase reaction was performed for 5 min in the reaction mixture (1.0 mL) containing 40 μmol of $NH_4Cl$, 80 μmol of Tricine–NaOH buffer (pH 8.6), 0.8 μmol of NADH, 0.07 unit of alanine dehydrogenase (or 0.08 unit of leucine dehydrogenase, or both), and 0.3 mL of the cystathionine γ-lyase reaction mixture incubated as described above.

*(3) Selenocysteine Synthesis by Coupling of Reactions 4 and 5.* The reaction mixture (0.5 mL) contained 5 nmol of pyridoxal-P, 40 μmol of Tricine–NaOH buffer (pH 8.0), 1.5 μmol of L-serine, 1.5 μmol of L-selenohomocysteine, 10 μmol of dithiothreitol, 0.06 unit of cystathionine β-synthase (9.6 μg) and 0.12 unit of cystathionine γ-lyase (19.3 μg). It was incubated for 10, 20, and 30 min. When rat liver homogenate was used, cystathionine β-synthase and cystathionine γ-lyase were replaced by the homogenate (4 mg as protein) dialyzed against 0.01 M potassium phosphate buffer (pH 7.2) containing $2 \times 10^{-5}$ M pyridoxal-P. The homogenate (4 mg) contained 0.02 unit of cystathionine β-synthase and 0.08 unit of cystathionine γ-lyase. Selenocysteine was determined by amino acid analysis after derivatization as described below.

*Alkylation of SH or SeH Groups.* Sulfur and selenium amino acids formed by the elimination reactions were determined after S-methylation and Se-ethylation with methyl iodide and ethyl iodide, respectively. The reaction mixture (1.3 mL) containing 2 μmol of dithiothreitol, 200 μmol of methyl iodide or ethyl iodide, and 0.3 mL of the enzyme reaction mixture was incubated at room temperature (about 20 °C) for 1 h. Both amino acids were alkylated quantitatively under these conditions.

*Analytical Methods.* Amino acid analysis was performed with a Hitachi 835 high-performance amino acid analyzer on a 4 × 150 mm column according to the manual of the analyzer. Gas chromatography–mass spectrometry was conducted with a Shimadzu-LKB 9000 gas chromatography–mass spectrometer as follows. Amino acids were derivatized to the *N*-heptafluorobutyryl (HFB) *n*-propyl ester according to the method of Jönsson et al. (1973). Selenol groups of selenohomocysteine and selenocysteine were methylated with methyl iodide in the same manner as described above prior to derivatization to the HFB *n*-propyl ester. Gas chromatography was run on a glass column (3 mm × 1 m) packed with 3% SE 52 on Chromosorb W (60–80 mesh, acid washed and silanized). Helium was used as a carrier gas at a rate of 30 mL/min. The column was programmed from 100 to 270 °C at a rate of 5 °C/min. Ionization voltage, acceleration voltage, and trap current were 22 eV, 3.5 kV, and 60 μA, respectively. The ion source was kept at 290 °C.

## Results

*Synthesis of Selenocystathionine with Cystathionine β-Synthase (Reaction 4).* The activity of cystathionine β-synthase to catalyze the selenocystathionine formation (reaction 4) was examined with the reaction system described above. The product was identified as selenocystathionine by amino acid analysis and gas chromatography–mass spectrometry (Table I). It emerged at the same retention time as the authentic selenocystathionine. The mass spectrum of the HFB *n*-propyl ester of the product (Figure 1) showed characteristic selenium isotope pattern at *m/e* 746 ($M^+$), 533 (M − 213), and 420 (M − 326) and coincided with that of authentic selenocystathionine. Main fragment ions are assiged as shown in Figure 1. Fragment ions at *m/e* 298 and 252 are explained

Table I: Identification of Products of Cystathionine β-Synthase and Cystathionine γ-Lyase Reactions

| reaction | products | identified form[a] | retention time (min): amino acid analysis | retention time (min): GC of HFB *n*-propyl ester | mass spectrum of HFB *n*-propyl ester (*m/e*) |
|---|---|---|---|---|---|
| 4 | selenocystathionine | selenocystathionine | 49.3 | 26.5 | 746[b] ($M^+$) |
| 5[c] | selenocysteine | *Se*-ethylselenocysteine | 50.8 | | |
| | | *Se*-methylselenocysteine | | 10.0 | 421[b] ($M^+$) |
| | α-ketobutyrate | α-aminobutyrate | 47.0 | | |
| 8[c] | selenohomocysteine | *Se*-ethylselenohomocysteine | 63.5 | | |
| | | selenomethionine | | 12.7 | 435[b] ($M^+$) |
| | pyruvate | alanine | 46.0 | | |
| 2 | cysteine | *S*-methylcysteine | 36.9 | | |
| | α-ketobutyrate | α-aminobutyrate | 47.0 | | |
| 7 | homocysteine | methionine | 53.0 | | |
| | pyruvate | alanine | 46.0 | | |

[a] Derivatization was carried out as described under Experimental Procedures. [b] These values are for $^{80}Se$. Satellite ion peaks due to other isotopes of selenium ($^{76}Se$, $^{77}Se$, $^{78}Se$, and $^{82}Se$) are also found. [c] Experimental conditions are the same as those described in the text except that 1 μmol of L-selenohomocysteine and 18 μg of cystathionine γ-lyase were used.

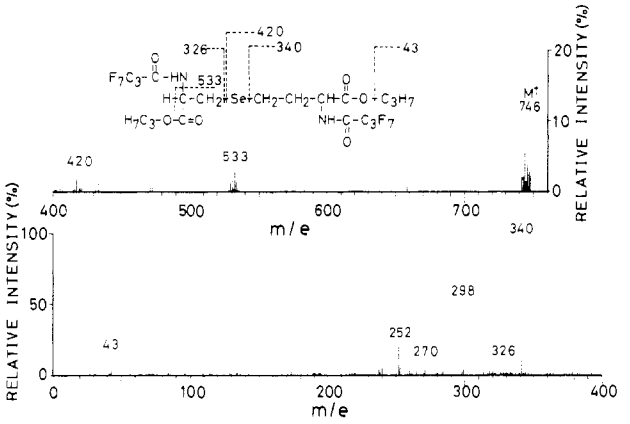


FIGURE 1: Mass spectral fragmentation of HFB *n*-propyl ester of selenocystathionine produced enzymatically by reaction 4.

by loss of $C_3H_6$ and $C_3H_7OCHO$, respectively, from the fragment at *m/e* 340. The mass spectral fragmentation of the selenocystathionine derivative was given here for the first time. Kinetic parameters for selenocystathionine synthesis (reaction 4) were compared with those for cystathionine synthesis (reaction 1) (Table II). Selenohomocysteine was found to be as susceptible as homocysteine. The ratio of the $V_{max}$ value for reaction 4 to that for reaction 1 was 0.69. L-Homocysteine inhibits the selenocystathionine synthesis in the presence of 5 mM L-serine as a competitive inhibitor for L-selenohomocysteine, and L-selenohomocysteine also inhibits the cystathionine synthesis in the same manner. The $K_i$ values obtained are substantially consistent with the $K_m$ values (Table II). This shows that both reactions 4 and 1 are carried out at the same active site.

*Elimination of Selenocystathionine Catalyzed by Cystathionine γ-Lyase.* Cavallini et al. (1962) reported that cystathionine γ-lyase catalyzes α,β elimination of L-cystine in addition to α,γ elimination of L-cystathionine. However, we have found that the α,β elimination of L-cystathione (reaction 7) proceeds much more slowly (<3%) than the α,γ elimination (reaction 2). When L-selenocystathionine was used as a substrate, the enzyme catalyzed both α,γ elimination (reaction 5) and α,β elimination (reaction 8) to yield α-ketobutyrate, pyruvate, selenohomocysteine, selenocysteine, and $NH_3$. Selenohomocysteine and selenocysteine were identified by amino acid analysis and gas chromatography–mass spectrometry after derivatization as described above (Table I). Pyruvate and α-ketobutyrate were converted to alanine and α-aminobutyrate, followed by identification with an amino acid analyzer (Table I). Table III summarizes kinetic data for α,γ and α,β elimination reactions catalyzed by cystathionine γ-lyase. The ratio of velocity of α,β elimination to that of α,γ elimination of L-selenocystathionine was 0.5. Cystathionine γ-lyase can eliminate further both the amino acids formed from selenocystathionine and cystathionine by elimination reactions, though slowly. All the selenium amino acids were

Table II: Kinetic Parameters for Cystathionine β-Synthase[a]

| | $V_{max}$ (μmol mg$^{-1}$ min$^{-1}$) | $K_m$ (mM) | $K_i$ (mM) |
|---|---|---|---|
| selenocystathionine synthesis (reaction 4) | 0.11 | | |
| L-serine | | 0.3 | |
| L-selenohomocysteine | | 2.5 | 3.4 |
| cystathionine synthesis (reaction 1) | 0.16 | | |
| L-serine | | 1.2 | |
| L-homocysteine | | 1.2 | 0.85 |

[a] The reaction mixture contained 12 μmol of dithiothreitol, 80 μmol of Tricine–NaOH buffer (pH 8.6), 10 nmol of pyridoxal-P, 0.015 unit of cystathionine β-synthase, and various amounts of L-serine and L-selenohomocysteine (or L-homocysteine) in a final volume of 1.0 mL. Substrate concentrations used were 0.40, 0.53, 0.80, and 1.60 mM L-serine and 1.5, 2.0, 3.0, and 5.0 mM L-selenohomocysteine for reaction 4, and 0.6, 0.8, 1.2, and 2.4 mM L-serine and 0.8, 1.0, 1.6, and 3.2 mM L-homocysteine for reaction 1. The $V_{max}$ and $K_m$ values were calculated from the secondary plots of intercepts obtained from the double-reciprocal plots.

Table III: Kinetic Parameters for Cystathionine γ-Lyase

| substrates | type of reaction | $K_m$ (mM) | $V_{max}$ (μmol mg$^{-1}$ min$^{-1}$) |
|---|---|---|---|
| L-selenocystathionine | α,γ elimination (reaction 5) | 1.7 | 19 |
| | α,β elimination (reaction 8) | | 9.5 |
| L-cystathionine | α,γ elimination (reaction 2) | 1.3 | 6.2 |
| | α,β elimination (reaction 7) | | 0.18 |
| L-selenohomocysteine | α,γ elimination | 1.4 | 0.62 |
| L-homocysteine | α,γ elimination | 2.9 | 0.25 |
| DL-selenocysteine | α,β elimination | 1.2[a] | 0.51 |
| L-cysteine | α,β elimination | 1.1 | 0.17 |

[a] The value was estimated on the assumption that only the L form is active, because D-cysteine was not a substrate.

decomposed 2.5–3 times more rapidly than the corresponding sulfur analogues (Table III).

*Synthesis of Selenocysteine by Coupling of Cystathionine β-Synthase and Cystathionine γ-Lyase Reactions.* We examined whether selenocysteine is synthesized from selenohomocysteine and serine through selenocystathionine by coupling of reactions 4 and 5. The products were identified by amino acid analysis and gas chromatography–mass spectrometry as described above. Selenocysteine was formed at a rate of 3 nmol/min under the conditions described under Experimental Procedures. We observed the formation of selenocysteine also with rat liver homogenate. The reaction rate was 0.3 nmol/min under the conditions used. Cysteine was synthesized in the analogous reaction system in which L-selenohomocysteine was replaced by L-homocysteine. The reaction rate was 12 nmol/min with the mixture of both the purified enzymes and 5 nmol/min with the rat liver homogenate.

*Possibility of Selenocysteine Formation by Reaction 6 with Cystathionine β-Synthase.* Braunstein et al. (1971) reported that cystathionine β-synthase of chicken liver catalyzes synthesis of cysteine from serine and $H_2S$ (reaction 3). We have found that the rat liver enzyme also catalyzes the reaction at a rate of 12% of cystathionine synthesis (reaction 1). However, attempts were made to demonstrate the selenocysteine formation from L-serine and $H_2Se$ (reaction 6) under the conditions described under Experimental Procedures by amino acid analysis and gas chromatography–mass spectrometry without success. The enzyme was not inactivatd when incubated at 37 °C for 30 min in the reaction mixture containing 20 mM $H_2Se$. We also could not observe the α,β elimination of selenocysteine with the enzyme.

## Discussion

The evidence presented here shows that selenocysteine is synthesized from serine and selenohomocysteine through selenocystathionine by coupling of reactions 4 and 5, but not at all from serine and $H_2Se$ (reaction 6). This is probably due to low reactivity of selenide as a substituent donor in replacement reactions as demonstrated with *O*-acetylserine (thiol)-lyase (Ng & Anderson, 1978; Burnell & Whatley, 1977). We have reported that L-methionine γ-lyase (EC 4.4.1.11) of *Pseudomonas putida* (IFO 3738) catalyzes the α,γ elimination of selenomethionine and also γ-replacement reactions of L-methionine and its derivatives with selenols (Esaki et al., 1979). Selenomethionine is a better substrate than methionine in the α,γ elimination whereas selenols are less reactive substituent donors than thiols in the γ-replacement reactions. This is compatible with the present results for cystathionine β-synthase and cystathionine γ-lyase; selenols are more reactive as a leaving group in eliminations than thiols but less effective as a substituent donor in replacement reactions. According to the general mechanism for β- and γ-replacement reactions catalyzed by pyridoxal-P enzymes, a nucleophilic addition of a substituent donor (e.g., thiols and selenols) occurs in the unsaturated intermediates (α-aminoacrylate pyridoxaldimine and α-ketobutenoate pyridoximino quinoid intermediates) derived from the substrates (Snell & DiMari, 1970; Davis & Metzler, 1972; Walsh, 1979). Selenols once added to the unsaturated intermediate probably are eliminated again more readily than thiols.

The present results provide the first proven mechanism for enzymatic synthesis of selenocysteine in mammals. Selenocysteine probably is synthesized in vivo from selenomethionine contained in a diet (Olson et al., 1970) through selenohomocysteine in the analogous sequence to the sulfur counterparts (Scheme I, A′ → B′ → C′). However, selenocysteine synthesis from selenohomocysteine and serine proceeded much slower than cysteine synthesis under the same conditions with the mixture of purified cystathionine β-synthase and cystathionine γ-lyase and also with rat liver homogenate. In these coupling systems, various factors affect the rate of synthesis such as side reactions catalyzed by cystathionine γ-lyase (e.g., β elimination of selenocystathionine and γ elimination of selenohomocysteine) and competitive inhibition between substrate amino acids. We have found that the low rate of selenocysteine synthesis with rat liver homogenate is attributable to the degradation of selenocysteine catalyzed by a novel enzyme contained in the homogenate.[1] The enzyme acts exclusively on L-selenocysteine to produce $H_2Se$. Stadtman (1980) also has reported a cleavage of selenocysteine by *Clostridium sticklandii* cells. Selenomethionine and selenocysteine are toxic for animals, and $H_2Se$ is the most toxic selenium compounds so far studied (Martin, 1973). Hydrogen selenide probably is produced from selenomethionine administered through selenocysteine. Thus, lack of specificity of the enzymes acting on the biosynthetic pathway of cysteine from methionine is physiologically important in selenium toxicity.

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[1] We have purified the enzyme (selenocysteine reductase) to homogeneity from pig liver. We will describe elsewhere the distribution of enzyme in mammalian tissues and the purification and the enzymological properties of the enzyme.

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# Dependence of Acyl Chain Packing of Phospholipids on the Head Group and Acyl Chain Length†

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ABSTRACT: The temperature dependencies of the factor group splitting of the infrared-active $CH_2$-scissoring bands of a series of fully hydrated gel phase diacyl phospholipids were determined. It is shown that, in all cases, the acyl chains are packed in an orthorhombic subcell and that the degree of rigidity of the subcell increases with increasing chain length. It is also demonstrated that the subcell in 3-*sn*-phosphatidylcholines differs from that found in 3-*sn*-phosphatidylethanolamines and 3-phosphatidic acids.

While the gel phases of model membranes such as 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine (DPPC)[1] and 1,2-dipalmitoyl-3-*sn*-phosphatidylethanolamine (DPPE) may be generally categorized as solidlike with the acyl chains in predominantly trans conformations, they in fact exhibit subtle but complex thermotropic behavior. The pretransition, $T_{pre}$, of 3-*sn*-phosphatidylcholines has recently been characterized as a particular solid–solid phase transition, similar to a transition found in *n*-alkanes (Cameron et al., 1980a; Janiak et al., 1976, 1979). Between $T_{pre}$ and the chain melting phase transition, $T_m$, the acyl chain packing is hexagonal. Immediately below $T_{pre}$, the packing is orthorhombic (Figure 1) with a high degree of torsional and librational motion about the long axes of the acyl chains. As the temperature is reduced, the rates and amplitudes of motion about the long axes decrease (Davis, 1979; Marsh, 1980), and at low temperatures (−50 °C), the orthorhombic packing is quite rigid (Cameron et al., 1980b). Further, it has recently been reported (Chen et al., 1980) that incubation of phosphatidylcholines at 0 °C for long periods (1–3 days) results in a third exothermic transition near 15–20 °C.

Gel phase thermotropic behavior dependent on both the chain length and the head group has been previously reported. In a series of homologous phosphatidylcholines, the difference in temperature between $T_{pre}$ and $T_m$ decreases as the chain length is increased (Mabrey & Sturtevant, 1976). There is also a dependence of $T_{pre}$ on whether the chain length is odd or even (Silvius et al., 1979), similar to differences observed in homologous series of fatty acids and other terminally substituted *n*-alkanes (Malkin, 1952).

Differences related to the head group are 2-fold. First, the pretransition has only been observed in phosphatidylcholines and in the closely related phosphatidylsulfocholines (Tremblay & Kates, 1981). Second, in phosphatidylethanolamines, $T_m$ is generally observed some 10–30 °C higher than the $T_m$ of the corresponding phosphatidylcholine. Indications of progressive changes in the acyl chain packing at reduced temperatures have also been observed in X-ray studies of DPPE bilayers (Harlos, 1978). In addition, X-ray studies of DPPE and DPPC (Janiak et al., 1976, 1979; Harlos & Eibl, 1980; McIntosh, 1980) have shown substantial differences in the bilayer thicknesses. This has been attributed to a tilting of the acyl chains of DPPC but not those of DPPE, the tilt resulting from the relatively large volume occupied by the phosphatidylcholine head group compared to that occupied by the phosphatidylethanolamine head group (McIntosh, 1980).

Infrared spectroscopy provides an extremely sensitive method for monitoring changes in the acyl chain packing of phospholipids. In the orthorhombic subcell, common to solid polyethylene, *n*-alkanes, and terminally substituted *n*-alkanes, the interchain interactions result in factor group splitting of the $CH_2$-scissoring band at 1468 $cm^{-1}$ and the $CH_2$-rocking band at 720 $cm^{-1}$ (Snyder, 1961, 1979). This factor group

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[1] Abbreviations used: DLPC, 1,2-dilauryl-3-*sn*-phosphatidylcholine; DMPC, 1,2-dimyristoyl-3-*sn*-phosphatidylcholine; DPPC, 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine; DHPC, 1,2-diheptadecanoyl-3-*sn*-phosphatidylcholine; DSPC, 1,2-distearoyl-3-*sn*-phosphatidylcholine; DBPC, 1,2-dibehenoyl-3-*sn*-phosphatidylcholine; DPPE, 1,2-dipalmitoyl-3-*sn*-phosphatidylethanolamine; DPPA, 1,2-dipalmitoyl-3-*sn*-phosphatidic acid; FT-IR, Fourier-transform–infrared spectroscopy.

0006-2960/81/0420-4496$01.25/0 