Structural basis of the phospholipid acyltransferase enzyme substrate specificity: a computer modeling study of the phospholipid acceptor molecule

Heather-Marie P. Wilson,* Waldemar Neumuller,† Hansjorg Eibl,† William H. Welch, Jr.,* and Ronald C. Reitz[1],*

Department of Biochemistry,* University of Nevada, Reno, NV 89557, and Max-Planck Institute for Biophysical Chemistry,† D-3400 Gottingen, Germany

Abstract The activity of the 1-acyl-*sn*-glycero-3-phosphocholine acyltransferase enzyme (E.C. 2.3.1.??) was measured with three radically different acceptor substrates: 1-palmitoyl-*sn*-glycero-3-phosphocholine (P-*sn*-G3PC), 1-palmitoyl-*sn*-glycero-2-phosphocholine (P-*sn*-G2PC), and 1-hexadecyl-*sn*-glycero-3-phosphocholine (He-*sn*-G3PC). It was found that the enzyme had similar activity with P-*sn*-G3PC, the natural acceptor substrate, and with P-*sn*-G2PC. The enzyme showed no detectable activity toward He-*sn*-G3PC. These results are much different than would be expected from simple examination of the structures. Computer-assisted molecular modeling was done to study the geometrical configurations and to focus upon the similarities and differences of the three substrate acceptor molecules. Three bond distances were selected as important for enzyme recognition: the distance between the oxygen of the acceptor hydroxyl group and *1)* the phosphorus; *2)* the nitrogen; and *3)* the oxygen bridge to the hydrocarbon chain. There were striking similarities for the bond distances of two of the three acceptor substrates, P-*sn*-G3PC and P-*sn*-G2PC. These were the two molecules that were shown to have activity with the enzyme. The bond distances found for the enzymically inactive acceptor substrate, He-*sn*-G3PC, differed significantly from P-*sn*-G3PC and P-*sn*-G2PC. Therefore, this latter molecule probably does not fit into the active site of the enzyme. The modeling data are also consistent with the experimental observation that He-*sn*-G3PC is not an inhibitor.—**Wilson, H-M., P., W. Neumuller, H. Eibl, W. H. Welch, Jr., and R. C. Reitz.** Structural basis of the phospholipid acyltransferase enzyme substrate specificity: a computer modeling study of the phospholipid acceptor molecule. *J. Lipid Res.* 1995. **36:** 429–439.

Supplementary key words lysophospholipid substrates • lysophosphatidylcholine • 1-palmitoyl-*sn*-glycero-3-phosphocholine • 1-palmitoyl-*sn*-glycero-2-phosphocholine • 1-hexadecyl-*sn*-glycero-3-phosphocholine

Phospholipid acyltransferases are important enzymes because they catalyze the remodeling of fatty acids in the *sn*-1 and *sn*-2 positions of tissue phosphoglycerides (1). Although there appears to be a small amount of acyltransferase activity in plasma membranes, studies have shown that phospholipid acyltransferases can be found predominantly as integral membrane proteins in the endoplasmic reticulum (2–4). The optimum in vitro temperature at which acyl transfers occur is 30–35°C (5). It has been shown that there are several acyltransferases with different functions. Phospholipid acyltransferases use, as substrates, many different unsaturated acyl-CoAs and 1-monoacyl-*sn*-glycero-3-phosphocholine. Recently, Neumuller et al. (6) and W. Neumuller and H. Eibl (unpublished results) have shown that 1-monoacyl-*sn*-glycero-2-phosphocholine can act as a receptor molecule much like the natural acceptor substrate, the glycerol-3-phosphocholine series.

Much research has been done on the specificity of the donor substrate, acyl-CoA, using either 1-monoacyl-*sn*-glycero-3-phosphocholine or 2-monoacyl-*sn*-glycero-3-phosphocholine as acceptor substrates (7–9). In general, there are at least two groups of acyltransferase enzymes. One group transfers saturated fatty acids into the 1 position of 2-monoacyl-*sn*-glycero-3-phosphocholine. The second group transfers unsaturated fatty acid into the 2 position of 1-monoacyl-*sn*-glycero-3-phosphocholine (2). Using acyl-CoAs with different acyl chain lengths or different degrees of unsaturation, studies have been able to show that acyltransferases can discriminate between the acyl groups of different acyl-CoA substrates (7–9). Therefore, not only do the enzymes discriminate against acyl-CoAs in which the acyl group contains differing degrees of unsaturation, they have the ability to distinguish differences in the chain length of the acyl groups as well as the placements of the double bonds within the chain. Thus, there is much information involving the specificities of the phos-

Abbreviations: P-*sn*-G3PC, 1-palmitoyl-*sn*-glycero-3-phosphocholine; P-*sn*-G2PC, 1-palmitoyl-*sn*-glycero-2-phosphocholine; He-*sn*-G3PC, 1-hexadecyl-*sn*-glycero-3-phosphocholine; RMS, root mean square; PUFA, polyunsaturated fatty acid; MNDO, semi-empirical quantum mechanics.

[1]To whom correspondence should be addressed.

Downloaded from www.jlr.org by guest, on June 18, 2012

ASBMB JOURNAL OF LIPID RESEARCH

ASBMB JOURNAL OF LIPID RESEARCH

pholipid acyltransferases for different acyl-CoA substrates. On the other hand, there has been little research done on the specificity of the acceptor substrates, the 1-monoacyl- and 2-monoacyl-*sn*-glycero-3-phosphocholines. Neumuller et al. (6) have shown that replacing the acyl group at the 1-position with an alkyl group results in very low acyltransferase rates when either 18:1-CoA or 20:4n-6-CoA were used as the acyl donor substrate. These data suggest that an acyl group rather than an alkyl group in the 1-position of the lysophospholipid acceptor molecule may be an important structural feature of the acceptor molecule in its interaction with the acyltransferase enzyme but do not provide an explanation for this requirement.

Three different acceptor substrates were made and tested for enzymatic activity with different acyl-CoAs. The three substrates were: 1-palmitoyl-*sn*-glycero-3-phosphocholine (P-*sn*-G3PC), 1-palmitoyl-*sn*-glycero-2-phosphocholine (P-*sn*-G2PC), and 1-hexadecyl-*sn*-glycero-3-phosphocholine (He-*sn*-G3PC). In addition to the enzymic testing, these molecules were subjected to computer-assisted modeling. The studies reported in this paper were designed to determine the geometric similarities and differences among these substrates in an effort to understand the mechanism of substrate discrimination observed with the enzyme.

MATERIALS AND METHODS

Radioactive P-*sn*-G3PC and He-*sn*-G3PC were obtained from Amersham Buchler, Braunschweig, Germany. The 1-acyl-*sn*-glycero-2/3-PC was synthesized by procedures already described (10–12). The acyl-CoA derivatives were synthesized according to Reitz et al. (7). Conditions for the preparation of rat liver microsomes and for the assay of the acyltransferase have been described (6).

Computer modeling

Three acceptor substrate molecules, P-*sn*-G3PC, P-*sn*-G2PC, and He-*sn*-G3PC, were constructed as molecular models using the SYBYL computer packages 5.51 and 6.0 (Tripos Associates) running on ESV50 and PS390 platforms (Evans and Sutherland). The conformational structures of the three molecules created were minimized using molecular mechanics. The acyl chain used on all three substrates was 16:0. Because P-*sn*-G3PC is the natural acceptor molecule in the enzyme system and the best substrate of the three, the assumption was made that the global minimum configuration of the P-*sn*-G3PC should be the template molecule.

Systematic and Monte Carlo search

A systematic search of the various conformations was done by identifying three to six rotatable bonds (indicated by arrows in **Fig. 1**) important in positioning the potential sites of interaction between substrate and enzyme (Fig. 1a). The conformational space of the molecules was explored by systematic rotations in 30° increments. The specified angle increments were started at the absolute torsional angle of zero. At the end of each 30° rotation, the molecule was minimized. Those conformers that contained any distance(s) that were less than the sum of van der Waals radii of the interacting atoms were immediately rejected. In addition, random searches were done. Seven rotatable bonds for P-*sn*-G2PC and six rotatable bonds for P-*sn*-G3PC and He-*sn*-G3PC were identified to which the computer arbitrarily assigned torsional angles in a Monte Carlo fashion and the structures were minimized (Fig. 1b). These searches sample the conformational space around the groups of interest; however, neither search guarantees the coverage of all of the conformational space of the molecule due to the selection of only certain bonds for rotation.

Root mean square fit

Root mean square (RMS) fit comparisons were done to compare the molecules of the lowest global energy for the three substrates. This rigid-body fit was used to compare the position of the phosphorus, the nitrogen, and the oxygen of the oxygen bridge to the acyl chain, and the acceptor hydroxyl group of one molecule to the counterparts in another molecule. The computer does this by moving the second molecule over the template molecule until it finds the minimum RMS fit without altering the internal coordinates of either molecule.

Distance maps

Three distances were selected to be of particular interest to the enzyme in substrate recognition. These distances were between the oxygen of the acceptor hydroxyl group and *1)* the phosphorus; *2)* the nitrogen; *3)* the oxygen bridge to the hydrocarbon chain (Fig. 1c). In the future, these will be referred to as Distance 1 (D1), Distance 2 (D2), and Distance 3 (D3), respectively (Fig. 1). Distance maps were generated to limit the number of conformations to those that had distances which matched the template molecule's conformers. Again, P-*sn*-G3PC was the template molecule used to generate the premiere distance map. Four bonds were chosen and rotated at 10 degree increments on the template molecule (Fig. 1c). After each rotation, the molecular structure was minimized and the resulting conformer was recorded. From the data generated, a three-dimensional distance map was created. Each point on the distance map represents at least one conformer, and the locations of the points on the map are dependent on the lengths of the three distances. For the template molecule, P-*sn*-G3PC, there can be as many data points as there are conformers. However, due to degeneracy (when more than one con-

Downloaded from www.jlr.org by guest, on June 18, 2012

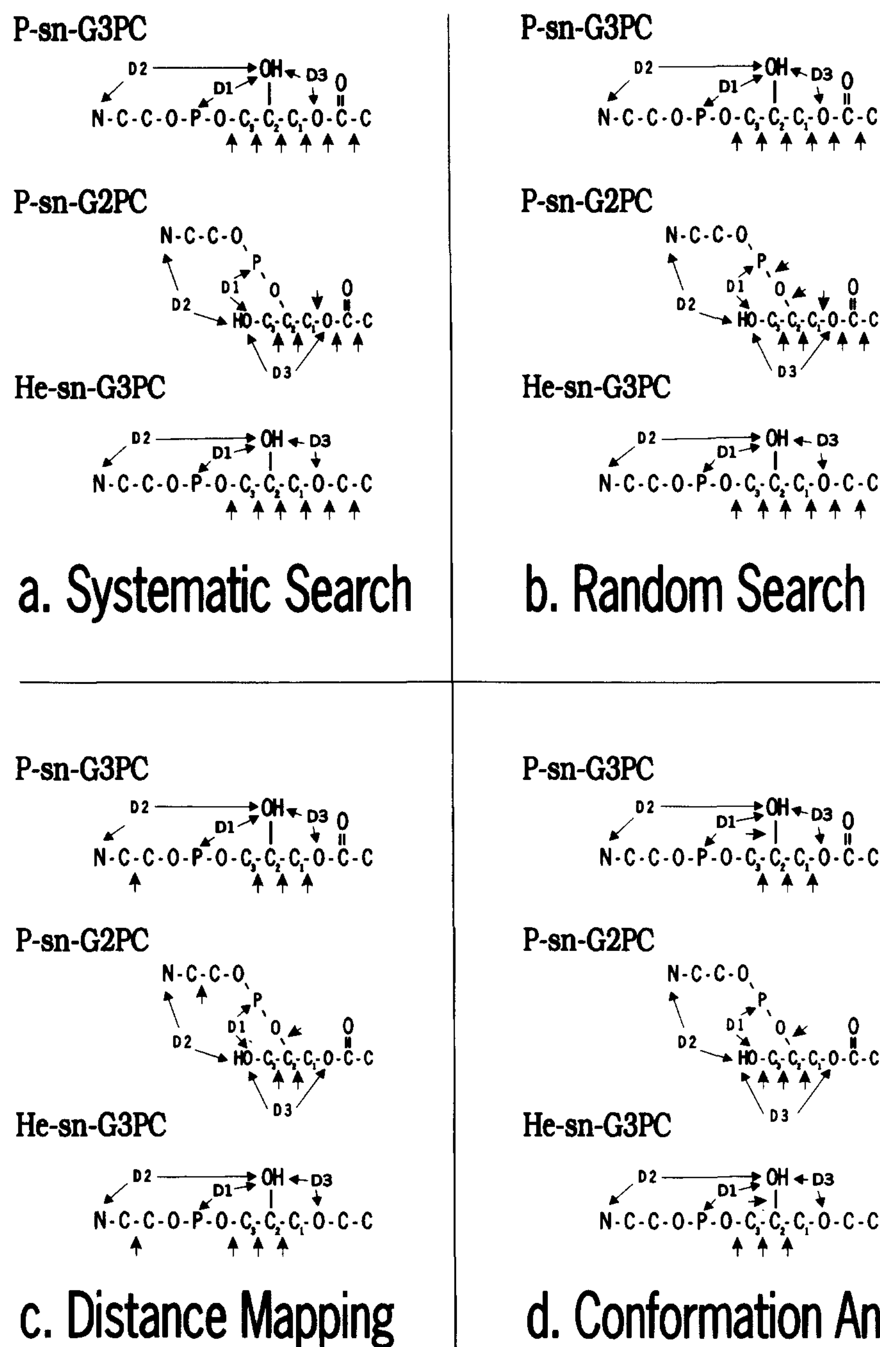


Fig. 1. Structures of acceptors used for modeling. a. Bonds and distances used for systematic search. Three acceptor substrate molecules, P-*sn*-G3PC, P-*sn*-G2PC, and He-*sn*-G3PC, were constructed as molecular models using the SYBYL computer packages 5.51 and 6.0 (Tripos Associates) running on ESV50 and PS390 platforms (Evans and Sutherland). The conformational structures of the three molecules created were minimized using molecular mechanics. The acyl chain used on all three substrates was 16:0. The P-*sn*-G3PC was used as the template molecule. D1, D2, and D3 refer to the three distances that are being measured and represent the distances between the oxygen of the acceptor hydroxyl group and the phosphorus (D1); the nitrogen (D2); the oxygen bridge to the hydrocarbon chain (D3). The bonds being rotated at 30° increments are indicated by small arrows. At the end of the 30° rotation, the molecule was minimized, and those conformers containing any distance(s) less than the sum of van der Waals radii of the interacting atoms were rejected. b. Bonds and distances used for random search. In this search, the computer randomly assigned torsional angles, indicated by the small arrows, in a Monte Carlo fashion and the structured was minimized. c. Bonds and distances used for distance mapping. Four bonds were chosen and rotated (small arrows) at 10° increments. After each 10° rotation, the molecule was minimized and the resulting conformer was recorded and a three-dimensional distance map was created. The details of the distance mapping are found in the Materials and Methods Section. d. Bonds and distances used for conformational analysis. Predetermined distances (D1–D3) were constrained to ±0.2 Å of the template molecule. Four rotatable bonds were chosen (small arrows) and rotated at 10° increments. After each rotation, the resulting conformer was minimized, and those molecules whose distances fell within the ranges of ±0.2 Å were recorded. At the end of each search, the minimum energy and the number of total conformations that met all requirements were recorded.

Downloaded from www.jlr.org by guest, on June 18, 2012

ASBMB JOURNAL OF LIPID RESEARCH

former have all three distances in common), many conformers can share the same data point because they have the same measured distances even though their overall conformation is different. The second distance map, using P-*sn*-G2PC as the molecule of interest, was created by selecting four rotatable bonds identical to those chosen for the template molecule. These bonds were rotated at 10 degree increments. This time, however, the distance map was constrained to the first distance map. That is, only those data points of P-*sn*-G2PC that can find a match with another data point in the distance map for P-*sn*-G3PC were allowed in the second distance map. A third distance map was generated using He-*sn*-G3PC. This was set up like the previous two distance maps. However, the distance map for He-*sn*-G3PC was constrained to the map generated by P-*sn*-G2PC. Distance maps have the advantage of providing a means by which the data from a search can be rapidly visualized.

Distance constraint analyses

In another conformational analysis, predetermined distances of the substrates were constrained to ± 0.2 Å of the template molecule, the P-*sn*-G3PC conformer with the lowest global energy. Four rotatable bonds in each molecule (P-*sn*-G3PC, P-*sn*-G2PC, He-*sn*-G3PC) were chosen (arrows, Fig. 1d) and rotated at 10° increments. After each rotation, the resulting conformer was minimized. Those molecules whose distances fell within the allowed ranges of ± 0.2 Å were recorded. At the end of each search, the minimum energy and the number of total conformations that met all requirements were recorded. This analysis allowed those conformers to be identified whose dimensions were within the distance range of P-*sn*-G3PC. This search served the purpose to determine whether P-*sn*-G2PC or He-*sn*-G3PC could be oriented into conformations that contained those distances believed to be vital for the phospholipid acyltransferase activity.

Molecular dynamics

To determine how the three substrates might behave at approximately physiologic temperature, the three molecules were subjected to molecular dynamics at 300°K. The dynamics search was used to study the configurational space. In molecular dynamics, the motions of the molecular system are studied by integrating the classical Newtonian equations of motion when given a potential energy function and an associated force field. The dynamics analysis was used because it was able to cover a different area of conformational space compared to the other methods and because all molecular bonds are allowed to rotate in this procedure. Further, the systematic searches give strain energies, analogous to enthalpic information, while molecular dynamics gives information analogous to free energy. The calculations of motion were done at discreet intervals of 1 fs. The molecules were allowed 30 ps of simulation time to thermally equilibrate. The final conformers of each substrate were subjected to molecular dynamics for an additional 60 ps of simulation time with recordings made at 60 fs intervals to give 1001 conformers per substrate. These results were analyzed by creating scatter graphs showing the oscillations of Distance 1, Distance 2, and Distance 3 (data not shown). These oscillations were around the mean distance and indicated that the molecules had fully equilibrated with the thermal bath. These three distance ranges were then visualized via histograms (see Figs. 3, 4, and 5).

Energy calculations

Minimum energies were determined for substrates constrained to the determined distances (D1–D3, Fig. 1) of the template molecule, again P-*sn*-G3PC. The constraints were removed and the molecular conformation was minimized a second time. The energy of the resulting molecules was used in comparison with the energy of the global minimum to determine an equilibrium constant (K_{eq}) between the P-*sn*-G3PC-like conformation and the conformation at the global minimum. The equation used to calculate K_{eq} was $\Delta SE = -RT\ln K_{eq}$ where $R = 1.9872$ cal × K^{-1} mol^{-1}, $T = 298°K$, and ΔSE = the difference in strain energy between ground state and constrained molecule. In this calculation, it is assumed that the entropy of all conformers is the same.

Semi-empirical quantum mechanics

The MOPAC suite of quantum mechanics programs included with the Sybyl package were used to evaluate the electron density at the oxygen atom of the acceptor hydroxy group.

Force field calculations

The SYBYL (Tripos) force field was used for the calculations (13). We have obtained good consistency with experimental data for ryanodyne (14) and for oligosaccharides (E. Ottensen, T. R. Kozel, and W. H. Welch, Jr., unpublished results). We also have found a good correlation between strain energy differences calculated using the SYBYL force field and the differences in enthalpy calculated using semi-empirical quantum mechanics (MNDO).

RESULTS

Enzymatic studies

In nature, it is 1,2-diacyl-glycerol-3-phosphocholine that is the natural product of transacylation, and earlier data (6, and W. Neumuller and H. Eibl, unpublished results) suggested that if the 1-position ester was replaced by an ether, acylation of the 2-position was significantly reduced. In an attempt to understand the importance of

Downloaded from www.jlr.org by guest, on June 18, 2012

ASBMB JOURNAL OF LIPID RESEARCH

the substituants at the three positions of the acceptor substrate for the acyltransferase reaction, catalytic activities of phospholipid acyltransferase with P-*sn*-G3PC and P-*sn*-G2PC have been compared to the enzyme's activities with He-*sn*-G3PC (**Table 1**). As can be seen, the enzyme had activities that were negligible when He-*sn*-G3PC and He-*sn*-G2PC were the acceptor molecules. However, the acylation activities of the P-*sn*-G3PC and P-*sn*-G2PC were similar. A priori one would have expected the ether analog to react better with the enzyme than the positional isomer in which the phosphocholine had been moved from the 3-position to the 2-position. This suggests that either the enzyme is not very discriminating or that the conformations of the two monoacyl molecules are similar. The question we asked was why did the enzyme exhibit activity with P-*sn*-G3PC and P-*sn*-G2PC while no activity was recorded when He-*sn*-G3PC was used as the acceptor substrate? Several possibilities exist including conformational and chemical reactivity. To resolve this question molecular modeling has been used. A geometrical approach was applied in the modeling searches for different conformers. The rationale was that the geometry of a molecular structure plays an important role in a compound's reactivity with other molecules, for example, an enzyme.

Global minima

The global minima of the three molecules, P-*sn*-G3PC, P-*sn*-G2PC, and He-*sn*-G3PC were found as a result of the Monte Carlo and systematic computer searches. These strain energies were similar for P-*sn*-G3PC and He-*sn*-G3PC, while that for the P-*sn*-G2PC was considerably lower.

Distance mapping

Distance maps for all three substrates were generated (data not shown), using Distance 1, Distance 2, and Distance 3 of the template P-*sn*-G3PC as the determining factors (see Fig. 1). The distance map for P-*sn*-G3PC consisted of 3,346 data points that represented 36,070 conformations. The distance map for P-*sn*-G2PC, constrained to that of P-*sn*-G3PC, had a total of 395 data points that represented 1,210 conformers. The distance map generated for He-*sn*-G3PC and constrained to the map created for P-*sn*-G2PC, had 21,257 possible conformers which were represented by 395 data points. Therefore, all three compounds can assume conformations with essentially the same distances between nitrogen, phosphorus, acyl group and acceptor hydroxyl group. In the conformational analysis searches, the following number of conformations were found: P-*sn*-G3PC = 4,527; P-*sn*-G2PC = 502; He-*sn*-G3PC = 1,409. This analysis also demonstrated that the acceptors could assume the same conformation. These two searches served the purpose of showing that both P-*sn*-G2PC and He-*sn*-G3PC could get into a conformation with the same or similar distances as those found for P-*sn*-G3PC.

TABLE 1. Activity of phospholipid acyltransferase with several acceptor molecules

Substrate	P-*sn*-G3PC	P-*sn*-G2PC	He-*sn*-G3PC	He-*sn*-G2PC
		nmol/min/mg protein		
16:0	18	24	NA	NA
18:1n-9	69	52	NA	NA
18:2n-6	118	164	NA	NA
18:3n-3	72	50	NA	NA
18:3n-6	76	113	NA	NA
20:1n-9	NA	9	NA	NA
20:2n-6	25	9	NA	NA
20:3n-3	NA	<3	NA	NA
20:3n-6	157	61	NA	NA
20:4n-6	136	60	NA	NA

Assay conditions were as previously described (6); NA, no activity.

Molecular dynamics

Although all acceptors could assume the same conformation, the energies of these conformations may be greatly different. The next step was to find the average distances for the three bonds selected as being important in the various conformations of the three molecules at 300°K (D1, D2, and D3). Molecular dynamics provides a useful means to access this question. This was done to determine whether the distances contributed to the similarities and/or differences between the three acceptor substrates. Histograms were created from data collected during molecular dynamics analyses (see Figs. 2-4). The histograms show the grouping of conformers found for each of the three molecules. In **Figure 2,** it can be observed that the P to OH bond distance was similar for the two acyl derivatives, and that for the alkyl isomer was significantly longer. In **Figure 3,** again the histograms show that the N to OH bond distances for the two acyl isomers were similar, while the alkyl isomer was longer. **Figure 4** shows that the bond distance of the O-bridge to the OH shorter for the alkyl isomer compared to the two acyl isomers. The data used to create these histograms were then used to construct **Table 2** which presents the mean bond distances for each isomer. This table is a quantitative measure of the data visualized in the histograms in Figs. 2-4.

Conformational equilibria

The distances D1-D3 of the three molecules, P-*sn*-G3PC, P-*sn*-G2PC, and He-*sn*-G3PC, were constrained to the values found in the global minimum of P-*sn*-G3PC and the strain energies of the P-*sn*-G3PC-like conformers were compared to the strain energies of the global minimum of each phospholipid as described in Materials and Methods. The K_{eq} values were found and are shown in

JOURNAL OF LIPID RESEARCH ASBMB

Downloaded from www.jlr.org by guest, on June 18, 2012

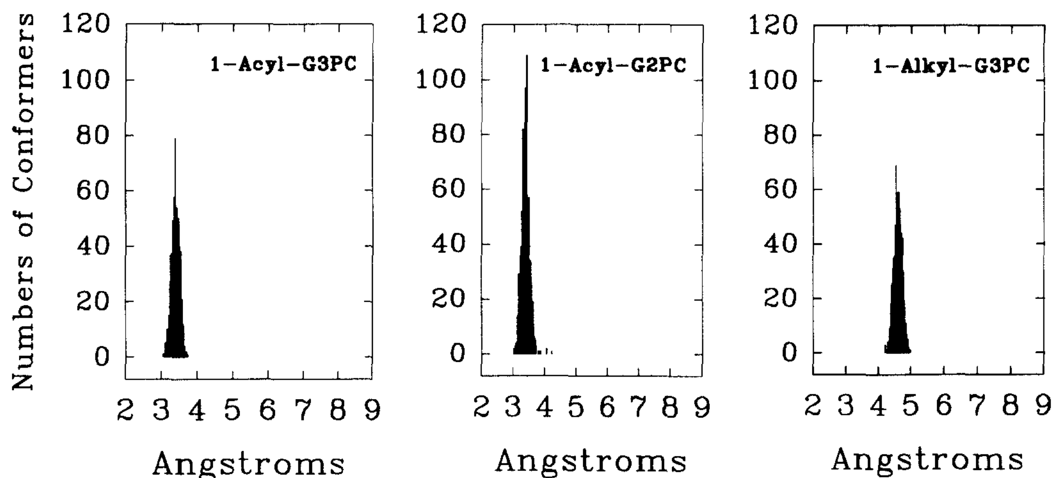


Fig. 2. Distribution of phosphorus to hydroxyl oxygen distances. This histogram shows the numbers of conformers found by molecular dynamics and the respective bond distances for each of the three acceptor molecules. This bond distance was figured at 300°K to more closely approach physiologic temperature.

Table 3. These data show that if the P-*sn*-G2PC molecule is constrained into the global minimum for the normal substrate, P-*sn*-G3PC, for every one P-*sn*-G2PC in the P-*sn*-G3PC-like conformation, there are about two P-*sn*-G2PCs in the global minimum. When the alkyl isomer was compared, for every one He-*sn*-G3PC in the P-*sn*-G3PC-like conformation, there are 1,042 conformers in the global minimum conformation. Therefore, there is little energetic reason that P-*sn*-G2PC cannot mimic the P-*sn*-G3PC conformation, whereas, there is a strong energetic prohibition against He-*sn*-G3PC acquiring the P-*sn*-G3PC conformation.

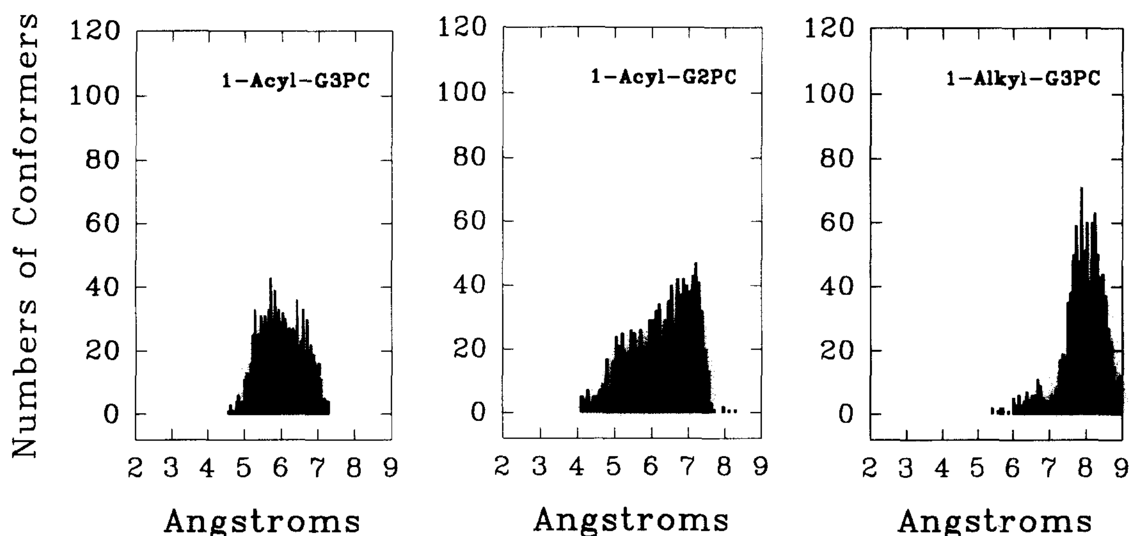


Fig. 3. Distribution of nitrogen to hydroxyl oxygen distances. This histogram shows the numbers of conformers found by molecular dynamics and the respective bond distances for each of the three acceptor molecules. Conditions for this figure were as described for Fig. 2.

Downloaded from www.jlr.org by guest, on June 18, 2012

ASBMB JOURNAL OF LIPID RESEARCH

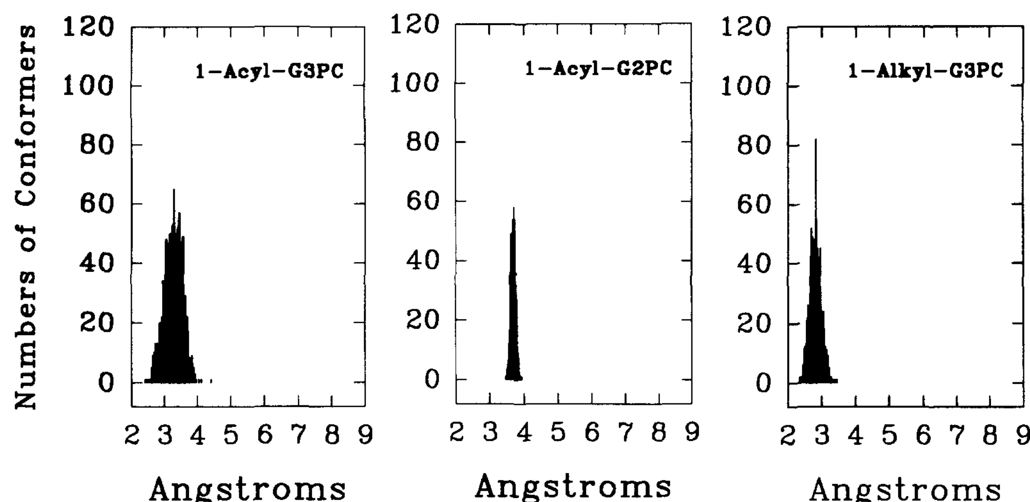


Fig. 4. Distribution of oxygen-bridge to hydroxyl oxygen distances. This histogram shows the numbers of conformers found by molecular dynamics and the respective bond distances for each of the three acceptor molecules. Conditions for this figure were as described for Fig. 2.

Family analysis

The conformational equilibria predicted from the calculated strain energies neglects entropic factors. To evaluate the predicted conformational equilibria, family analysis was applied to the molecular dynamics data (which include entropic information). One thousand conformations were generated for each of the putative substrates. The three distance measurements (Table 2, Figs. 1-4) were gridded at 0.1 Å, and formed the basis of a family analysis for each molecule. The numbers of families generated varied between 20 and 60. As expected, the vast majority of conformers (>900) were found in one family. No family of either P-*sn*-G3PC or P-*sn*-G2PC overlapped with any family of He-*sn*-G3PC. This is consistent with the strain energy calculations which predict that less than one He-*sn*-G3PC in 1000 will be in a P-*sn*-G3PC-like conformation. Therefore, a consideration of entropic factors does not materially alter the strain energy result. Both P-*sn*-G2PC and P-*sn*-G3PC were found to belong to the same family.

Conformational overlap also was evaluated by a simple comparison of the 1000 conformations recorded in the dynamics analyses. The vast majority of conformers fell within plus or minus two standard deviations of the average for the three measured distances (see Table 2). For example, the average plus or minus two standard deviations for P-*sn*-G2PC contained 916 conformers. Examination of the same data revealed 199 P-*sn*-G2PC conformers within two standard deviations of the average distances for P-*sn*-G3PC. Therefore, the strain energy estimate of the equilibrium of P-*sn*-G2PC between a P-*sn*-G3PC-like conformation and the global minimum is supported by molecular dynamics. The same analysis for He-*sn*-G3PC found no conformational overlap with P-*sn*-G3PC.

TABLE 2. Bond length distances

	P to OH Distance 1	N to OH Distance 2	O-Bridge to OH Distance 3
		Å	
P-*sn*-G3PC	3.41 ± 0.31	6.36 ± 0.85	3.70 ± 0.076
P-*sn*-G2PC	3.40 ± 0.11	6.05 ± 0.58	3.32 ± 0.29
He-*sn*-G3PC	4.62 ± 0.13	7.99 ± 0.61	2.84 ± 0.17

Data calculated from dynamics analyses done at 300°K as described in Materials and Methods, and represent the average distance ± 1 standard deviation; n, 1001.

TABLE 3. Calculated strain energies and K_{eq} for the three acceptor molecules

	Global Minimum	Constrained Conformer	Energy Change	K_{eq}
		kcal/mol		
P-*sn*-G3PC	−22.821	−22.821	0	1/1.00
P-*sn*-G2PC	−57.572	−57.245	0.327	1/1.74
He-*sn*-G3PC	−21.265	−17.149	4.115	1/1,041.94

Enthalpies of the global minimum conformations and their respective K_{eq} at 298°K. The K_{eq} = (global minimum)/(P-*sn*-G3PC-like conformers).

Downloaded from www.jlr.org by guest, on June 18, 2012

ASBMB JOURNAL OF LIPID RESEARCH

Quantum mechanics (reactivity at the acceptor OH-group)

Semi-empirical quantum mechanics (MNDO) indicated that the charge density on the acceptor hydroxyl oxygen was essentially the same for all three analogs. The MOPAC charges for P-*sn*-G3PC, P-*sn*-G2PC and the He-*sn*-G3PC were −0.328 (−0.400), −0.334 (−0.403), and −0.334 (−0.406), respectively. The values in parentheses are the Mullikin charges.

Kinetic parameters

In order to relate our modeling studies back to the acyltransferase activity, we determined the K_M and V_{Max} values for each substrate. As shown in **Table 4**, the K_M value for P-*sn*-G2PC was slightly higher than that for the natural substrate, P-*sn*-G3PC, and the V_{Max} was slightly lower. No measurable activity was found for the He-*sn*-G3PC molecule, and this molecule did not serve as an inhibitor. The K_M for P-*sn*-G2PC and the failure of He-*sn*-G3PC to act as an inhibitor fit what would be predicted from the K_{eq} values determined and shown in Table 3. The elevated K_M for P-*sn*-G2PC is consistent with the prediction that a sizeable fraction of the substrate is not in the proper conformation to bind to enzyme. The failure to He-*sn*-G3PC to serve as either substrate or inhibitor is consistent with the prediction that less than one molecule in 1000 is in the proper conformation to bind to enzyme.

DISCUSSION

Using four acyl acceptor substrates and ten acyl-CoA substrates, the acyltransferase activity of the phospholipid acyltransferase enzyme was measured. When comparing the four acyl acceptor substrates, it was observed that the enzyme activity toward P-*sn*-G3PC and P-*sn*-G2PC was similar to most of the ten different acyl-CoAs, while the other two acceptor substrates, He-*sn*-G3PC and He-*sn*-G2PC, were not active as either acceptors or inhibitors with any of the acyl-CoAs. It is important to note that the rearrangement of the phosphocholine from the *sn*-3 position to the *sn*-2 position did not appreciably alter the acyltransferase activity of the enzyme; however, simply changing the ester linkage at the 1-position to an ether linkage resulted in a dramatic loss of enzymatic activity. This raised the question regarding how the stereochemistry of these acceptor molecules differs such that the acyltransferase enzyme does not recognize the ether compound as being an appropriate substrate.

TABLE 4. Kinetic constants for acyltransferase activity

	K_M	Relative V_{Max}	V/K
	μM		
P-G3PC	0.3	1.0	0.40
P-G2PC	0.5	0.67	0.16
He-G3PC	none	none	none

The acyltransferase activities were determined as previously described (6). The V_{Max} for the P-G3PC was 120 nmol/min per mg. He-G3PC had no measurable activity, nor did it serve as an inhibitor.

Computer molecular modeling, which allows a comparison of similarities and differences, was used to study the geometrical configurations of three acceptor substrate molecules. Only three of the four substrates were chosen for study: P-*sn*-G3PC, P-*sn*-G2PC, and He-*sn*-G3PC. We chose P-*sn*-G3PC, the natural substrate of the phospholipid acyltransferase enzyme, as the template molecule for comparison with P-*sn*-G2PC and He-*sn*-G3PC. The computer molecular modeling studies done for diglycerides (15) are of particular interest because these studies can be compared to the P-*sn*-G3PC, the P-*sn*-G2PC, and the He-*sn*-G3PC substrates. The acyl portion of the three acceptor substrate molecules has attributes similar to the acyl chains of the diglycerides. Also, the discussion of how the two fatty acids of the diglycerides pack together has relevance to the product molecules of the phospholipid acyltransferase enzyme. It has been shown that there are two types of *sn*-2 chain sequences that can affect the width and thickness of diglycerides as well as the orientation of the *sn*-1-carbonyl dipoles by infiuencing the axial rotation of the *sn*-1 chain (15). The two types of *sn*-2 sequences, as described by Applegate and Glomset (15), are: diglycerides that contain *sn*-2 chains with proximal polyene segments; and diglycerides that contain *sn*-2 chains with proximal saturated segments. The products of the acyl-CoA:phospholipid acyltransferase enzyme reaction would be in the category of diglycerides that contain the *sn*-2 chains with proximal polyene segments.

The first task in the conformational analysis was to find the global minimum of each molecule. This was done through a combination of the systematic and random searches. Rotatable bonds of interest were chosen and rotated at selected 30° increments (systematic) or randomly assigned torsional angles (random). From these searches, the global minimum strain energies of the three molecules were found. When comparing global minimum energies, it is important to keep in mind that because these are molecular mechanics calculations the comparisons should be made only between conformers of the same molecule.

Three distances between important molecular landmarks were chosen for comparison. These distances were between the oxygen of the hydroxyl group (the acyl acceptor position) and *1)* the phosphorus; *2)* the nitrogen; *3)* the oxygen bridge to the acyl chain (Distances 1, 2, and 3, respectively, Fig. 1). These four atoms and three distances were chosen for several reasons. The phosphate and the nitrogen on the phosphocholine group were chosen as im-

Downloaded from www.jlr.org by guest, on June 18, 2012

ASBMB JOURNAL OF LIPID RESEARCH JLR

portant atoms due to their charges. These charges should be important for their interaction with the enzyme in the Michaelis complex. The oxygen of the oxygen bridge at position 1 was selected as being important because it *1)* defines the glycerol backbone and *2)* suggests whether the substitution of the ester for an ether group resulted in a conformational shift of the bridging oxygen.

The distance maps showed that the conformers of P-*sn*-G2PC and He-*sn*-G3PC could be orientated so that the three distances would be common with the template molecule, P-*sn*-G3PC. The conformational analysis defined only those conformers whose three distances were within ±0.2 Å of the template molecule's three bond lengths. Like the distance maps, the conformational analyses found many conformers of each molecule that had their three lengths similar to those of the template molecule. Therefore, all can obtain the same geometrical conformation. The question now became whether these conformations are populated at physiological conditions.

The studies up to this point were done at a simulated temperature of 0°K. The dynamics analyses allowed a study of the behavior and geometrical characteristics of the acceptor substrate molecules at 300°K. The histograms generated from the dynamics analyses gave a pictorial view of the differences and similarities between the three molecules. There was close similarity for the distances between the oxygen of the hydroxyl group and the phosphorus (Distance 1) for only the P-*sn*-G3PC and P-*sn*-G2PC, the two acceptor substrates that were active with the enzyme. These data strongly indicate that Distance 1 must be important for substrate recognition by the enzyme. Distance 2, the bond length from the oxygen of the hydroxyl group to the nitrogen, also showed some marked similarities between P-*sn*-G3PC and P-*sn*-G2PC. Referring to Fig. 4, the conformations of P-*sn*-G3PC and P-*sn*-G2PC generally occupied the same distance ranges. The majority of conformations for He-*sn*-G3PC once again occupied a distance range higher than those found for the ester molecules, indicating that the bond distance from the oxygen of the hydroxyl group to the nitrogen was also important for enzyme recognition. The third distance range (shown in Fig. 5) indicates that all the bond lengths for the conformers of P-*sn*-G2PC fall under the curve for the distance range of the P-*sn*-G3PC molecule. While there was some overlapping between the P-*sn*-G3PC and the He-*sn*-G3PC molecules, the majority of conformers for the He-*sn*-G3PC fall outside the curve of P-*sn*-G3PC. This distance also could have some influence on the enzyme's activity with the three substrates.

The dynamics analysis was interesting and useful because it allowed the observation of the molecule's behavior and movements at 300°K. Observation of the molecules in motion showed that the hydrogen of the hydroxyl group formed a hydrogen bond with the phosphate moiety in both the P-*sn*-G3PC and the P-*sn*-G2PC derivatives for long periods of time. Because of the longer observed distance for Distance 1 compared to those distances found for P-*sn*-G3PC and P-*sn*-G2PC, there should be a lack of hydrogen bonding in the He-*sn*-G3PC. This suggests that hydrogen bonding serves the purpose of minimizing the movement and rotation of the hydroxyl group, thus allowing the enzyme to acylate the *sn*-2 or *sn*-3 positions of P-*sn*-G3PC and P-*sn*-G2PC, respectively, with ease.

With respect to potential hydrogen bond effects, the loss of the carbonyl oxygen when the 1-ester is changed to the 1-ether could eliminate a hydrogen bond acceptor within the active site of the enzyme. Such a hydrogen bond could contribute to binding at the active site of the enzyme and, therefore, play a role in the reactivity of the ester acceptor molecule. Because there are no data available concerning the amino acids of the active site or the configuration of the active site, one can only speculate regarding the role of this oxygen in catalysis. Certainly there are many other substrate–enzyme interactions, and it is doubtful this particular H-bond is critical. After searching the available databases, none of the phospholipid acyltransferases have been sequenced; therefore, no direct structural information on the mammalian enzymes is available at this time.

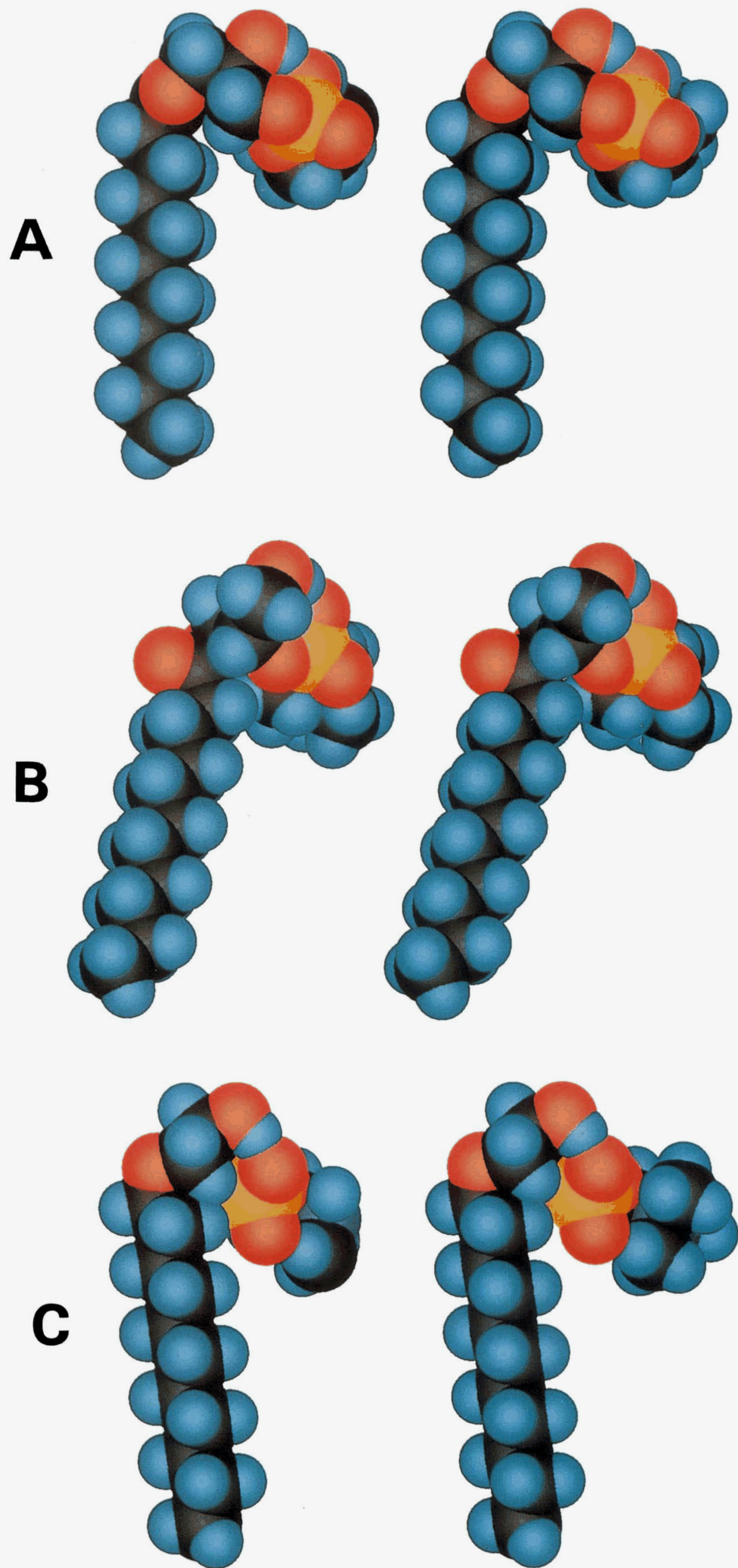
The results of Applegate and Glomset (15) predict that the introduction of polyunsaturated fatty acid (PUFA) into the *sn*-2-position promotes an ordered packing arrangement in a lipid bilayer. It may be that this ordered chain packing plays an important role in the increased activity of the phospholipid acyltransferase towards PUFA when the 1-acyllysophosphatidylcholine is the acceptor substrate; however, it is beyond the scope of this investigation to consider this possibility at this time.

Although all three substrates can attain the same conformation, as shown by the interatomic distances defined in this paper, the probability of the three substrates having the same conformation is small. This conclusion is based on the following criteria. First, little difference in strain energy between the global minimum of the P-*sn*-G2PC molecule and the dominant conformation of P-*sn*-G3PC, the natural in vivo substrate, was observed, while the ether derivative had a global minimum energy of 4 kcal/mol lower than the enzymatically active (P-*sn*-G3PC-like) conformation. This difference is so large that very little He-*sn*-G3PC would exist in a conformation resembling the natural substrate. Second, molecular dynamics (which includes entropic contributions) indicated that He-*sn*-G3PC lacks the flexibility to attain the dominant P-*sn*-G3PC conformation. Third, the differences in strain energy between the conformations of the model phospholipids used in this study were confirmed by semi-empirical quantum mechanics (MNDO). The conclusion is supported by the observation that He-*sn*-G3PC is a poor substrate as well as a poor inhibitor, indicating that this molecule does not bind to the enzyme.

The difference in reactivity between substrates may be

Downloaded from www.jlr.org by guest, on June 18, 2012

ASBMB JOURNAL OF LIPID RESEARCH JLR



Downloaded from www.jlr.org by guest, on June 18, 2012

ASBMB
JOURNAL OF LIPID RESEARCH

the result of differences in electron density at the acceptor hydroxyl group. Inspection of the molecule suggests that this explanation is unlikely. Semi-empirical quantum mechanics (MNDO, 100× precision) calculated less than a 1% difference in both the MOPAC and Mullikin charge at the acceptor oxygen atom of the three substrates. Further, while a difference in electron density might explain why the ether is a poor substrate, such a difference would not explain why the ether is not an inhibitor.

The carbonyl oxygen atom of the ester at the 1-position may be important as a hydrogen-bond acceptor in the enzyme-substrate complex. Because the energy of a hydrogen bond could be as high as 7 kcal/mol, such a mechanism cannot be eliminated. While an interaction with the carbonyl oxygen may occur, it is only one of many interactions that could occur with the substrate, including the stronger ionic interactions. Therefore, one would not expect that the carbonyl oxygen atom alone would account for the drastic loss of enzymic activity with the substrate. A comparison of the three molecules in a stereo diagram (Fig. 5) illustrates the greater nitrogen to acceptor oxygen distance in the ether compared to the two ester compounds (Table 2). **Figure 5** illustrates the resulting more compact polar head group of the esters as compared to the ether. We propose that the conformational difference results in steric repulsions preventing significant binding of the ether to the enzyme active site. Therefore, the steric factors revealed in the modeling study seem to be the most likely explanation for the failure of the ether substrate to react.

There are other distances, in addition to those we have studied, that may also be of importance to the enzyme. In particular, the acyl chain is likely one of the major points of attachment. However, as was observed during the dynamics analysis, the acyl chain showed excessive rotational curling- and lengthening-type motions that would make it difficult to chose specific bonds within the chain to study. The fatty acid chain flexibility suggests that chain conformation is probably not an important explanation for the present data. Solvation energy may be an important factor, but it was not considered here because the enzyme binding site is not known. The present study provides a sufficient reason for the lack of reactivity of the ether analog. While the use of other model substrates will be helpful in testing the suggestions put forward in the communication, proof most likely will come only when the crystal structure of this enzyme is elucidated.

Fig. 5. Stereo space-filling models of the global minima for each compound. The three molecules discussed in this paper are shown in stereo space-filling representations of their respective global minima. Fig. 5A is P-*sn*-G3PC, Fig. 5B is P-*sn*-G3PC, and Fig. 5C is He-*sn*-G3PC. The molecules have been aligned using a rigid-body, least-squares fit of the acceptor oxygen, the bridging oxygen, the phosphorous atom, and the nitrogen atom of each molecule (the atoms used to define the molecular distances and the conformations of each phospholipid, see Fig. 1). The molecules have been rotated so that the acceptor hydroxyl group is at the top of each molecule and the acyl side chain is in approximately the same position. The phosphocholine group is to the right in all cases.

Supported in part by the University of Nevada Molecular Modeling/Graphics Core Facility.

Manuscript received 18 May 1994 and in revised form 16 September 1994.

REFERENCES

1. Lands, W. E. M., M. L. Blank, L. J. Nutter, and O. S. Privett. 1966. A comparison of acyltransferase activities in vitro with the distribution of fatty acids in lecithins and triglycerides in vivo. *Lipids.* **1:** 224-229.
2. Lands, W. E. M., and P. Hart. 1966. The control of fatty acid composition in glycerolipids. *J. Am. Oil Chem. Soc.* **43:** 290-295.
3. Okuyama, H., W. E. M. Lands, F. D. Gunstone, and J. A. Barve. 1972. Selective transfers of *trans*-ethylenic acids by acyl coenzyme A: phospholipid acyltransferases. *Biochemistry.* **11:** 4392-4398.
4. Eibl, H., E. E. Hill, and W. E. M. Lands. 1969. The subcellular distribution of acyltransferase which catalyze the synthesis of phosphoglycerides. *Eur. J. Biochem.* **9:** 250-258.
5. Jezyk, P., and W. E. M. Lands. 1968. Specificity of acyl-CoA:phospholipid acyltransferases: solvent and temperature effects. *J. Lipid Res.* **9:** 525-531.
6. Neumuller, W., E. A. M. Fleer, C. Unger, and H. Eibl. 1987. Enzymatic acylation of ether and ester lysophospholipids in rat liver microsomes. *Lipids.* **22:** 808-812.
7. Reitz, R. C., W. E. M. Lands, W. W. Christie, and R. T. Holman. 1968. Effects of ethylenic bond position upon acyltransferase activity with isomeric *cis,cis*-octadecadienoyl coenzyme A thiol esters. *J. Biol. Chem.* **243:** 2241-2246.
8. Reitz, R. C., M. El-Sheikh, W. E. M. Lands, I. A. Ismail, and F. D. Gunstone. 1969. Effects of ethylenic bond position upon acyltransferase activity with isomeric *cis*-octadecenoyl coenzyme A thiol esters. *Biochim. Biophys. Acta.* **176:** 480-490.
9. Okuyama, H., W. E. M. Lands, W. W. Christie, and F. D. Gunstone. 1969. Selective transfer of cyclopropane acids by acyl coenzyme A:phospholipid acyltransferase. *J. Biol. Chem.* **244:** 6514-6519.
10. Eibl, H., and P. Wooley. 1988. Synthesis of enantiomerically pure glycerylesters and ethers. II. Methods employing the precursor 3,4-isopropylidene-D-mannitol. *Chem. Phys. Lipids.* **47:** 47-53.
11. Eibl, H., and P. Wooley. 1988. A general synthetic method for enantiomerically pure ester and ether lysophospholipids. *Chem. Phys. Lipids.* **47:** 63-68.
12. Wooley, P., and H. Eibl. 1988. Synthesis of enantiomerically pure phospholipids including phosphatidylserine and phosphatidylglycerol. *Chem. Phys. Lipids.* **47:** 55-62.
13. Clark, W., R. D. Cramer, and N. Van Opdenbosch. 1989. Validation of the general purpose Tripos 5.2 force field. *J. Computational Chem.* **10:** 982-1012.
14. Welch, W., S. Ahmad, J. A. Airey, K. Gerzon, R. A. Humerickhouse, H. R. Besch, Jr., L. Ruest, P. Deslongchamps, and J. L. Sutko. 1994. Structural determinants of high-affinity binding of Ryanoids to the vertebrate skeletal muscle ryanodine receptor: a comparative molecular field analysis. *Biochemistry.* **33:** 6074-6085.
15. Applegate, K. R., and J. A. Glomset. 1991. Effect of acyl chain unsaturation on the conformation of model diacylglycerols: a computer modeling study. *J. Lipid Res.* **32:** 1635-1654.

Downloaded from www.jlr.org by guest, on June 18, 2012

ASBMB JOURNAL OF LIPID RESEARCH JLR