Molecular Modeling Studies of Triacylglycerols

Zhen-Yi Yan,* Stephen D. Huhn, Lawrence P. Klemann, and Michael S. Otterburn

Nabisco Foods Group, 200 DeForest Avenue, East Hanover, New Jersey 07936

The methods of molecular mechanics and semiempirical quantum mechanics have been used to investigate the steric and electrostatic features for a group of lipid-like molecules. Included in this group were trilaurin (LLL), tridecyl 1,2,3-propanetricarboxylate (TPT), and a family of short and long acid triacylglycerol molecules (SALATRIM). Computational studies on all of the molecules in the gas phase indicated that the hydrophobic interactions between the fatty acid chains were dominant in determining the molecular conformation. Analysis also showed that the triacylglycerols (LLL and SALATRIM) have similar molecular electrostatic potentials (EP). This electronic similarity suggests that one might expect similar reactivity toward lipase-mediated hydrolysis. A very different EP map was found for TPT, a compound which has each ester linkage molecularly reversed. TPT appears to be resistant to lipolysis, as evidenced by the near total lack of digestibility of this type of lipid in published *in-vivo* studies conducted in rodents.

INTRODUCTION

Molecular computational techniques have the potential to add to our understanding of chemical reactions, even those mediated by natural enzymes. Susceptibility to enzymatic reactions is generally characterized by substrate specificity which includes molecular size, conformation, and electrostatic features. These factors all contribute to molecular recognition and reactivity. Molecular recognition in sweet and salt taste perception has been investigated by computational modeling (Venanzi and Venanzi, 1990; Venanzi *et al.*, 1991, 1992). Also, correlations among molecular conformation, electrostatic potential, and activity have been successfully employed in drug research (Boyd, 1990; Weinstein *et al.*, 1981). It is reasonable to expect that new and useful information would be uncovered by a computational study focused on natural lipids and lipid-like systems.

Lipids found in plants and animals include esters based on glycerol. Fatty acids containing 16–22 carbons predominate in these molecules whose hydrolysis, *in vivo*, is catalyzed by natural enzymes collectively known as lipases. The nature of the lipase-mediated hydrolysis is generally believed to proceed stepwise from triglyceride, through diglyceride, and finally to monoglyceride species (Brady *et al.*, 1990; Mattson and Volpenhein, 1964; Winkler *et al.*, 1990; Zubay, 1988). Much is known about the hydrolysis of natural long-chain acid triacylglycerols, including the relative site specificity of the most common lipases (Borgstrom, 1986; Brockerhoff and Jensen, 1974; Carey *et al.*, 1983; Mattson and Volpenhein, 1964; Patton, 1981). Triacylglycerols made up solely of short acid chain acids (two to six carbons) have also been studied, and as a class these molecules tend to experience more rapid lipolysis compared to their long-chain counterparts (Brockerhoff and Jensen, 1974; Mattson and Volpenhein, 1964; Patton, 1981). Although a through explanation of this rate enhancement is lacking, differences in steric size, molecular polarizability, diffusivity, *etc.*, offer a variety of platforms for the rationalization of what has been reported.

Triacylglycerols containing both long and short acid chains offer compositions that could add to our understanding of the effects of acid chain length on both structure and reactivity (Hayes, 1994; Klemann *et al.*, 1994). Such compositions, while being available for investigation for some time, have recieved little attention (Bauer, 1954a,b; Bauer and Lange, 1949; Reuge, 1955). We have selected this group of triacylglycerols for the focal point of this study. In recognition of their interesting structural attributes, we have applied the acronym, SALATRIM, to this group of short and long acid triglyceride molecules.

To further investigate the role potentially played by the molecular electrostatic potential on lipid structure and properties, we also included a so-called retro-fat in our study. The specific compound modeled, tridecyl 1,2,3-propanetricarboxylate (TPT), while possessing the same three-carbon backbone as trilaurin, has the oxygen and carbon atoms of each ester group assembled in a reverse order compared with a natural fat. This structural feature places TPT in a family of synthetic lipids that appear to be completely indigestible according to the results of published animal studies (Fulcher, 1987; Hamm, 1984, 1985). The lack of digestibility shown by molecules like TPT infers a concomitant lack of hydrolytic reactivity in the presence of endogenous lipases. Whether this lack of apparant reactivity is due to the steric, electronic, or conformational differences is unknown. Molecular mechanics and semiempirical quantum mechanics provide a tool that has seen little application in the area of lipid chemistry. The opportunity to open a new scientific door on this potentially fertile area of investigation motivated our present study.

METHODS

QUANTA/CHARMM software (Brooks *et al.*, 1983) for molecular modeling was obtained under license from Molecular Simulations, Inc. SPARTAN software for molecular orbital calculations was obtained from Wavefunctions, Inc. Both software packages ran on an IBM RS/6000 Model 550 platform and were used to investigate the structures of seven selected lipid-type molecules. The flow chart in Figure 1 provides an overview of the sequence of calculations that were performed. More detailed descriptions of these calculations are provided below.

Model Building. The compounds selected for this investigation and their respective abbreviations are summarized in Table 1. Initial structures were built using interactive computer graphics and the molecular modeling facility of SPARTAN. Standard bond lengths, bond angles, and all-trans fatty acid chains were used to generate a crude structure after the appropriate atom types (*i.e.*, sp^3 hybridized carbon atoms,

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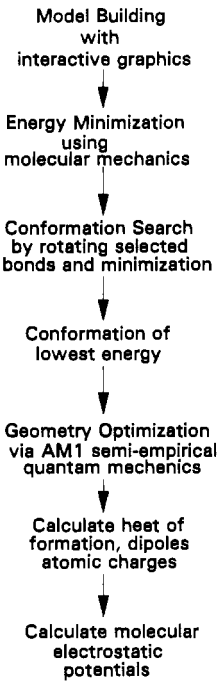

Figure 1. Flow chart of calculations using QUANTA/CHARMM molecular modeling and SPARTAN molecular orbital software.

Table 1. Compounds Investigated by Computer Modeling

compound	abbrev
trilaurin	LLL
1,2-diacetyl-3-stearoylglycerol	AAS
1,3-diacetyl-2-stearoylglycerol	ASA
1,2-dipropionyl-3-stearoylglycerol	PPS
1,3-dipropionyl-2-stearoylglycerol	PSP
1,2-dibutyryl-3-stearoylglycerol	BBS
tridecyl 1,2,3-propanetricarboxylate	TPT

carbonyl oxygen atoms, *etc.*) and connectivity between these atoms were selected. Energy minimization was then performed on the structure using molecular mechanics (Clark *et al.*, 1989). This partially refined structure was then in a local minimum energy well.

Conformational Search. To determine the lowest energy conformation for a given structure, a selected set of C–C and C–O bonds were systematically rotated. Following this exercise, the new conformer has its energy computationally reminimized to relax the structure. This process of searching conformational space is computationally intensive as the number of structures to be calculated increases exponentially with the number of rotations within each bond and the number of bonds being rotated. The relative orientations of the three fatty acid chains are mainly determined by the five dihedral angles within the glycerol backbone. Therefore, in this calculation five bonds, the two carbon–carbon bonds and the three carbon–oxygen bonds in the glycerol backbone, were rotated in steps of 120° producing 243 unique conformations for each molecule. After each rotation, the conformation was relaxed via energy minimization. The lowest energy state of these 243 conformations yielded a global minimum that was used in further calculations.

Geometry Optimization. The next series of calculations performed were geometry optimizations *via* semiempirical quantum mechanics with the AM1 parameters implemented in SPARTAN. At this simple quantum mechanical level all of the electrons were assigned to orbital functions and the individual contributions of each electron to each orbital were computed. Next, small distance perturbations were applied to atomic centers of the molecule, and the energy was recalculated until the changes in the energy gradient were less than 1.0E-04 a.u. and could be disregarded. At this point the structure had converged on the final geometry.

Calculating Atomic Charges and Molecular Properties. The atomic point charges for the optimized geometry of each molecule were calculated from the molecular electrostatic potentials using the method of AM1 semiempirical quantum mechanics. The heats of formation and total dipoles for the lowest energy conformations of each molecule were also obtained from AM1 calculations.

Electrostatic Potential. The molecular electrostatic potential (EP) was calculated within the QUANTA/CHARMM computational program. EP values were determined by the Coulombic interaction between a positive point charge and the static charge distribution of the desired molecule. The atomic point charges and the molecular geometry for EP calculations were obtained from the previous semiempirical AM1 calculations.

RESULTS AND DISCUSSION

Conformational Analysis. If a molecule is to participate in a reaction at a catalytic center, such as an active site in an enzyme, it must have an energetically accessible compatible molecular conformation. In this work, conformational searches were performed for seven lipid and lipid-like molecules. For each molecule, the lowest energy conformation of 243 candidates was further optimized *via* semiempirical AM1 calculations. Figure 2 shows the molecular structures in their lowest energy conformations after geometry optimizations.

Inspection of the lowest energy conformations for all seven molecules suggests that, in a noncondensed phase, it is energetically more favorable for the hydrophobic fatty acid chains to be on a common side of the glycerol backbone, as seen in Figure 2. These conformations are comparable to that proposed for triacylglycerol species in lipid membranes on the basis of ^{13}C NMR studies (Hamilton, 1989). It is therefore clear that hydrophobic interactions between the fatty acid chains dominate the lowest energy conformations in both the gas and the condensed phases.

Table 2 lists respective heats of formation and total dipoles for the lowest energy conformation of each molecule. The heat of formation for ASA (−377.024 kcal/mol) was slightly higher than that for AAS (−377.138 kcal/mol). The same trend was observed for the pair of PSP and PPS, with values for heat of formation at −389.154 and −389.168 kcal/mol, respectively. These results indicate that the asymmetric structures of AAS and PPS are lower in energy compared to the respective symmetric structures of ASA and PSP.

The heat of formation values also show that the length of the fatty acid chain within a triglyceride plays an important role in determining the overall molecular energy. The energy was found to decrease about 12 kcal/mol going from AAS to PPS and to decrease an additional 14 kcal/mol going from PPS to BBS. The decrease in energy with increasing chain length was responsible for the lower energies determined for both trilaurin and tridecyl 1,2,3-propanetricarboxylate.

Electrostatic Potential Analysis. Chemical reactivity, however, certainly depends not only on molecular size and shape but also on electronic factors. For a chemical reaction to occur, complimentary electrostatic potential patterns must be present before the nucleophilic attack of the substrate is possible. Therefore, electronic effects, which may be completely independent of steric factors, also need to be properly taken into account in any realistic treatment of chemical reactivity and selectivity. One measure of electronic preference is provided by the molecular electrostatic potential. We calculated the molecular electrostatic potential surfaces for trilaurin, the SALATRIM molecules, and tridecyl 1,2,3-propanetricarboxylate listed in Table 1. At each point on the three-dimensional grid, the interaction energy between a positive

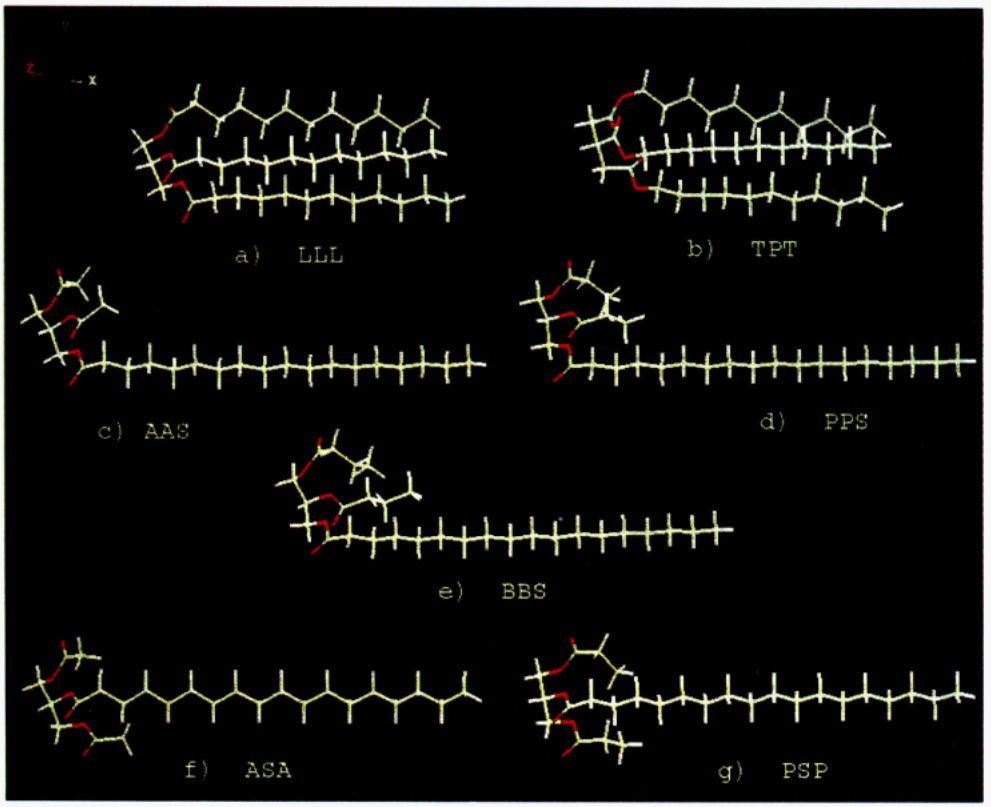

Figure 2. Molecular structures in their lowest energy conformations for (a) trilaurin; (b) tridecyl 1,2,3-propanetricarboxylate; (c) AAS; (d) PPS; (e) BBS; (f) ASA; (g) PSP.

Table 2. Heat of Formation and Total Dipole for the Lowest Energy Conformers from Semiempirical Quantum Mechanics Calculations

molecule	heat of formation (kcal/mol)	dipole (D)
ASA	−377.024	662.17
AAS	−377.138	689.04
PSP	−389.154	631.41
PPS	−389.168	642.40
BBS	−403.177	630.01
LLL	−474.917	602.21
TPT	−469.434	560.87

point charge located at that position and the static charge distribution of the molecule was calculated. Points of equal energy were connected and displayed with the QUANTA program to produce the results given in Figures 3–7. The electrostatic potential maxima, *i.e.*, the region with the energy above 150 kcal/mol, and minima, *i.e.*, where the energy is below −150 kcal/mol, were plotted in yellow and red, respectively.

Figure 3 shows the molecular electrostatic potential for trilaurin. In the glycerol backbone region, an electrostatic potential maximum (in yellow) was seen at the position 1 methylene and was almost zero at positions 2 and 3. Three EP minima (in red) with their centers at the glycerol oxygen were found next to the backbone carbons. The three largest EP maxima are located further away from the backbone region.

The SALATRIM molecules have the same structure about the glycerol backbone region as that of a natural triacylglycerol, such as trilaurin. The structural difference of SALATRIM molecules from trilaurin is the length of the three fatty acid chains. Figures 4–6 show that SALATRIM molecules have electrostatic potential patterns of the same location and magnitude as trilaurin for the respective EP minima and maxima. All of the electrostatic potentials, including those not shown here, exhibit a local maximum potential at the position 1 carbon of the glycerol backbone and show that three EP minima are adjacent to the glycerol backbone carbons. It appears that the length of the fatty acid fragments on a glycerol backbone has no effect on the molecular electrostatic potentials; therefore, SALATRIM is expected to bind to the lipase active site with the same electrostatic interactions as the natural triacylglycerols.

To answer the question as to whether the molecular EP patterns differ for lipid molecules that are known to differ in terms of their reactivity toward hydrolysis by specific lipases, the molecular EP of tridecyl 1,2,3-propanetricarboxylate (TPT) is shown in Figure 7 and compared with that of trilaurin. The difference in the molecules is the location of the carbonyl group with respect to the three-carbon backbone. In trilaurin (Figure 3) an oxygen atom separates each carbon atom of the glycerol backbone from the carbonyl group of a fatty acid chain. Conversely, a reverse ester is formed in TPT so that each carbon atom of the backbone is directly connected to the carbonyl group which then bonds to an oxygen atom. This difference in structure between TPT and a triacylglycerol leads to significantly different molecular electrostatic potential patterns for the two molecules. First, in the backbone region, TPT gives two negative electrostatic potential minima for the backbone methylenes at positions 1 and 3, in contrast to the case of trilaurin which has a positive electrostatic potential at the position 1 methylene. Second, three EP maxima were found next to the backbone carbons of TPT, in contrast to trilaurin, where three EP minima appeared. As the third difference in the EP patterns, the three largest EP maxima for trilaurin are located away from the central glycerol backbone region compared to their relative center locations for TPT.

The molecular electrostatic potential patterns can be further analyzed from the atomic point charges in Table 3. These values of atomic charges were calculated from the coefficients of the molecular orbitals for the individual

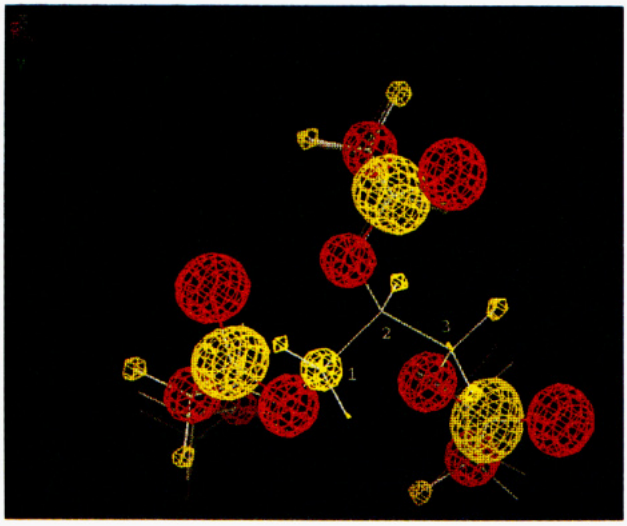

Figure 3. Molecular electrostatic potential for trilaurin with the regions of the energy above 150 kcal/mol in yellow and the regions of the energy below −150 kcal/mol in red.

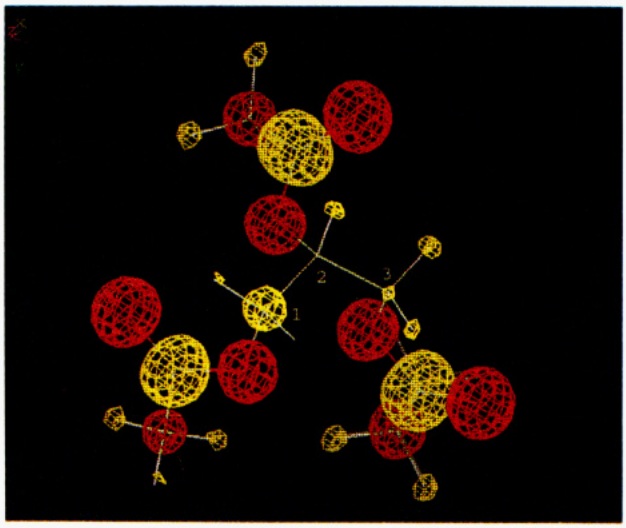

Figure 4. Molecular electrostatic potential for AAS. Yellow, 150 kcal/mol; red, −150 kcal/mol.

atoms with the method of AM1 semiempirical quantum mechanics. Since they are dependent on the electric field and local molecular environment, atomic charges provide the basis for electrostatic recognition in a hydrolytic reaction. Comparison of the charges for trilaurin, the SALATRIM molecules, and TPT shows that the largest differences in charge are for carbon atoms around the carbonyl groups of those molecules, which are the possible reactive sites for hydrolysis. In the case of TPT, there is a large negative charge on each of the backbone carbons relative to those for trilaurin, AAS, PPS, and BBS. The atomic charges on the α carbons of fatty acid chains are all negative in the trilaurin, AAS, PPS, and BBS molecules, while the corresponding carbons are positively charged in TPT. These differences in charges clearly show that the SALATRIM molecules are electrostatically very similar to trilaurin, while TPT is electrostatically very different from a natural triacylglycerol.

Generally, it is working hypothesis that the molecular electrostatic potential pattern of a substrate molecule determines its compatibility with the electrostatic potential pattern of an enzyme. Trilaurin and SALATRIM molecules are susceptible to hydrolysis reactions, apparently because their electrostatic potential patterns are

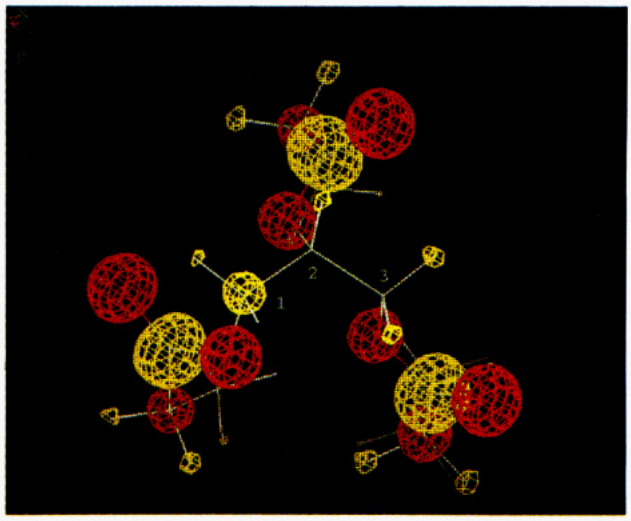

Figure 5. Molecular electrostatic potential for PPS. Yellow, 150 kcal/mol; red, −150 kcal/mol.

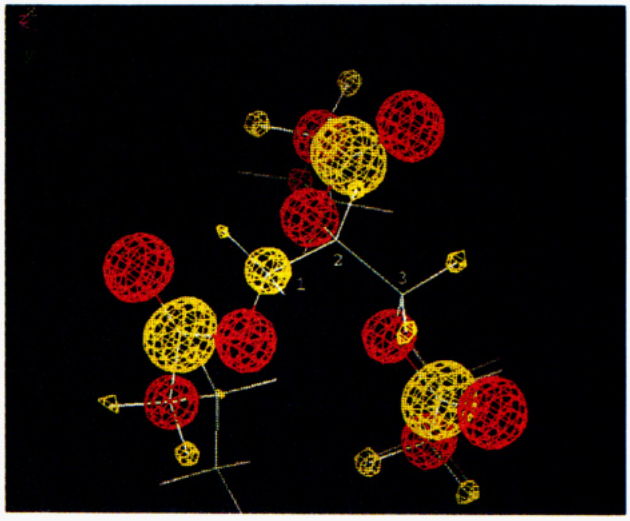

Figure 6. Molecular electrostatic potential for BBS. Yellow, 150 kcal/mol; red, −150 kcal/mol.

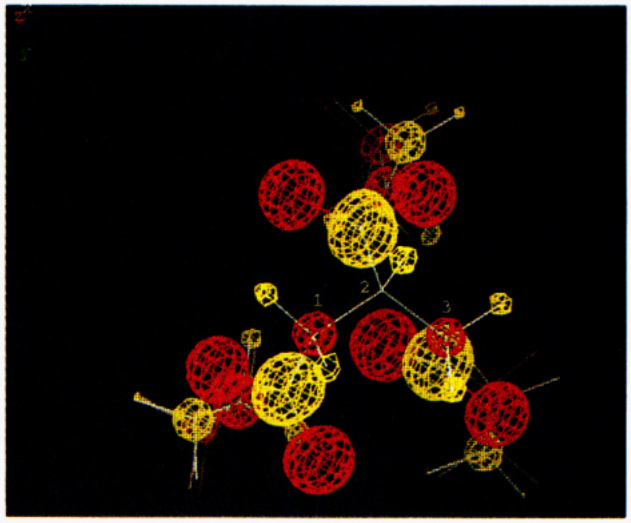

Figure 7. Molecular electrostatic potential for trilauryltricarballylate. Yellow, 150 kcal/mol; red, −150 kcal/mol.

complementary to that at the active site of the hydrolysis enzyme. Conversely, tridecyl 1,2,3-propanetricarboxylate is not reactive toward lipolysis. The location and size of

Table 3. Selected Atomic Point Charges for Triacylglycerols and Trilauryltricarballylate

atom position	molecule				
	TPT	LLL	AAS[a]	PPS[a]	BBS[a]
glycerol					
carbon-1	−0.3444	0.2254	0.2513	0.2379	0.2729
carbon-2	−0.1181	0.0268	0.0024	0.0254	0.0128
carbon-3	−0.2838	0.0659	0.0659	0.0134	−0.0096
carbonyl					
carbon-1	0.8116	0.8946	0.7579	0.8172	0.8578
carbon-2	0.7707	0.8853	0.8496	0.8034	0.8487
carbon-3	0.7628	0.9031	0.8923	0.8410	0.8315
α					
carbon-1	0.2671	−0.4650	−0.3678	−0.3627	−0.4638
carbon-2	0.2595	−0.4684	−0.4924	−0.3556	−0.4484
carbon-3	0.2250	−0.4764	−0.5280	−0.4746	−0.4757

[a] The steroyl chain is located at position 3 in AAS, PPS, and BBS.

EP minima and maxima on TPT are apparently not compatible with the electrostatic requirements of the hydrolytic enzyme. The results of electrostatic potential calculations, therefore, suggest that the SALATRIM molecules should be as reactive in hydrolysis as the natural triglyceride. These results correlate very well with experimental data which show that SALATRIM molecules are susceptible to lipase-mediated hydrolysis (J. R. Hayes, 1993, unpublished results).

CONCLUSIONS

Computational chemistry techniques can determine unique physical properties of a molecule, including its size, shape, and electrostatic potential. These properties, when presented in graphical form, can rapidly distinguish the similarities and differences in a group of molecules. A key range of parameters is then identified by correlating experimental results on natural lipids and digestibility, *e.g.*, caloric availability. Once this range is known, computational chemistry can be used to measure and predict the susceptibility of new lipid or lipid mimetic molecules to similar types of enzymatic hydrolysis reactions.

The conformational and electrostatic properties of a natural triacylglycerol, a family of triacylglycerols bearing short- and long-chain saturated fatty acids, and tridecyl propanetricarboxylate (TPT) were studied by using semiempirical quantum mechanics and molecular mechanics. To determine the relative orientations of three fatty acid chains with respect to the glycerol backbone of a molecule, 243 initial conformations for each molecule were considered in our calculations. The energy of each conformation was evaluated and minimized by the method of molecular mechanics. The geometry of the lowest energy conformation was further optimized *via* semiempirical quantum mechanics with the AM1 parameters, and the atomic point charges were calculated for the final geometry of a molecule. Then the molecular electrostatic potentials were computed from the optimized molecular geometry and atomic point charges. The results were compared among the seven molecules and interpreted in terms of the relationship among the molecular electrostatic potentials, atomic charges, and hydrolytic reactivities.

The optimized geometries of all seven molecules in this study indicated that the hydrophobic interactions between the fatty acid chains dominate the lowest energy structure and, therefore, it is energetically more favorable for the hydrophobic fatty acid chains to be on a common side of the glycerol backbone in vacuum. The heat of formation values from AM1 calculations further supported the importance of the fatty acid chain length in determining molecular energy.

Analysis of molecular electrostatic potentials showed that SALATRIM molecules have electrostatic potentials similar to those of the natural triacylglycerol, trilaurin, and, therefore, the length of fatty acid chains has no apparent effects on the molecular EP. The results indicate that the electrostatic component that is consistent with hydrolytic reactivity of natural triacylglycerols is also present in SALATRIM molecules, in agreement with the experimental results.

Although the overall size and shape of trilaurin and TPT are comparable as seen in Figure 2, the electrostatic potentials for these two lipids would seem to confer either a compatibility or a noncompatibility with the active site of a lipolytic enzyme. This enzyme–substrate compatibility is expected, at least in part, to explain the ability of a lipase to recognize and bring about the hydrolysis of triacylglycerol compounds, while leaving compounds like TPT unchanged. Therefore, we believe that computational investigations involving lipids and lipid-like materials can provide a unique perspective for understanding complex processes such as lipid hydrolysis and digestion.

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Received for review August 25, 1992. Revised manuscript received February 1, 1993. Accepted April 17, 1993.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts,* December 15, 1993.