DHA as a neuroprotective subtance

Polyunsaturated fatty acids (PUFA) play an important role as an antioxidant in the whole human body but more specifically in the brain. A systematic study on the computational aspects of these lipids started before the OTKA T046861 grant was awarded [THEOCHEM **666-667**, (2003) 445-449]. The overall project had 4 Topics.

- Topic 1 Fundamental Thermodynamics of Molecular Conformational Changes
- Topic 2 Conformational information of fatty acid
- Topic 3 Conformational information of phospholipids
- Topic 4 Free radical reactions with PUFA and PUFA models

Topic 1 Simple organic molecules were investigated in computing continues thermodynamic functions along conformational changes. These included a variety of compounds from hydrocarbons to peptides as their felxibility is expected to be analogous to that of PUFA as well as to lipids.

The conformational changes of ethane and disubstituted ethanes, $X-CH_2-CH_2-X$, where X were hydrogen isotopes of higher masses, -F atoms and $-CH_3$ groups, were investigated by CBS-4M and MP2/6-311G(d,p) computational levels. Thermodynamic functions such as ΔH, ΔG and S were computed along the approximate reaction coordinate of conformational changes. The lowest frequency associated with the rotation about the central C-C bond was omitted along the whole reaction path. The thermodynamic functions were continuous in the case when only $3N-7$ internal degrees of freedom were taken in consideration. Identical results were obtained using projected frequency calculations in the surrounding of rotational transition states.

The effect of the mass and electronic difference on the entropy of the two key compounds ($X=F$ and $X=CH_3$) was separated and assessed. The periodicity of reaction coordinates determined the periodicity of the thermodynamic functions. The second derivatives of the potential energy functions had a shape similar to the entropy functions.

The enthalpy was plotted as a function of entropy and rotational reaction coordinate. The concept of “entropy funnel” used by protein chemists was recognized even in the cases of the simple compounds investigated. We concluded that the bottom of the entropy funnel could correspond to the gauche conformers, in accordance to the concept of protein folding.

Various folded molecular structures contain different amount of information. The relative amount of information may be related to relative entropy or entropy change. The conformational entropy change for n-butane has been computed as the function of rotation around the central C-C bond. It appears that the g^+ or g^- conformers contain about 16% more information than the *anti*-structure. Furthermore, the *syn* conformation with the two groups eclipsed contained about 42% more information than the fully staggered *anti* orientation. The conformational entropy function calculated from $3N$-7 internal degrees of freedom was found to be a continuous function.

Topic 2 The conformational intricacies of fatty acids including PUFA, as well as their model compounds were studied. Interestingly enough their felxibility was similar to that of peptides as could be judged from the similarity of their conformational potential energy surfaces. DHA is the most prominent member of the PUFA family.

The conformational properties of ω-3 type of polyunsaturated fatty acid (PUFA) chains and their fragments were studied using Hartree Fock (RHF/3-21G) and DFT (B3LYP/6-31G(d)) methods. Comparisons between a unit (**U**) fragment of the PUFA chain and a mono *N*-Ac-glycine-NHMe residue show that both structures have the same sequence of $sp^2 - sp^3 - sp^2$ atoms. The flexibility of PUFA originates in the internal rotation about the above pairs of σ bonds. Therefore, potential energy surfaces (PES-s) were generated by a scan around the terminal dihedral angles (ϕ_{t1} and ϕ_{t2}), as well as the ϕ_1 and ψ_1 dihedrals of both 1U congeners (Me-CHCH-CH_2-CHCHMe and MeCONH-CH_2-CONHMe) at the RHF/3-21G level of theory. An interesting similarity was found in the flexibility between the *cis* allylic structure and the *trans* peptide models. A flat landscape can be seen in the *cis* **1U** (hepta-2,5-diene) surface, implying that several conformations are expected to be found in this (PES).

An exhaustive search carried out on the **1U** and **2U** models revealed that straight chain structures such as *trans* and *cis* beta ($\phi_1 \approx \psi_1 \approx 120^o$; $\phi_2 \approx \psi_2 \approx -120^o$), *trans* and *cis* extended ($\phi_1 \approx \psi_1 \approx \phi_2 \approx \psi_2 \approx 120^o$) can be formed at the lowest energy of both isomers. However, forming helical structures, such as *trans* helix ($\phi_1 \approx -120^o$, $\psi_1 \approx 12^o$; $\phi_2 \approx -120^o$, $\psi_2 \approx 12^o$) or *cis* helix $\phi_1 \approx -130^o$ $\psi_1 \approx 90^o$ $\phi_2 \approx -145^o$ $\psi_2 \approx 90^o$, will require more energy. These six conformations, found in **2U,** were selected to construct longer chains such as **3U**, **4U**, **5U** as well as **6U**, in order to obtain the thermochemistry of secondary structures. The variation in the extension or compression of the chain length turned out to be a factor of two between the helical and non-helical structures. The inside diameter of the "tube" of *cis* helix turned out to be 3.5 Å after discounting the internal H-atoms.

Thermodynamic functions were computed at the B3LYP/6-311+G(2d,p)//B3LYP/6-31G(d). The *cis-trans* isomerization energy of 1.7 ± 0.2 kcal mol^{-1} unit^{-1} for all structure pairs indicates that the conformer selection was consistent. A folding energy of 0.5 ± 0.1 kcal mol^{-1} unit^{-1} has been extracted from the energy comparison of the helices and most extended non-helical structures. The entropy change associated with the folding ($\square S_{\mathrm{folding}}$) is decreases faster with the degree of polymerization (n) for the *cis*- than for the *trans*-isomer. As a consequence, the linear relationships between ($\square G_{\mathrm{folding}}$) and n for the *cis*- and *trans*-isomer crossed at about n = 3. This suggested that the naturally occurring *cis*-isomer less ready to fold than the *trans*-isomer since a greater degree of organization is exhibited by *cis*-isomer during the folding process.

The result of this work leads to the question within the group additivity rule: could the method applied in our study of the folding of polyallylic hydrocarbons be useful in investigating the thermochemistry of protein folding?

Polyunsaturated fatty acids (**PUFA**) like **S**teari**D**onic **A**cid (**SDA**;18:4 n-3) **E**icosa**P**entaenoic **A**cid (**EPA** 20:5 n-3), and **D**ocosa**H**exenoic **A**cid (**DHA** 22:6 n-3) and its chain fragment models were studied at B3LYP/6-31G(d) levels of theory. Significant conformations for the *cis* and *trans* isomers were selected to obtained the thermodynamic functions (ΔH, ΔS, ΔG) for the *cis-trans* isomerization and for the folding using B3LYP/6-311+G(2d,p)//B3LYP/6-31G(d) level of theory.

The structural analysis shows that there are significant differences in thermodynamic function of the *trans*- and *cis*-**PUFA**'s. The *trans-cis* isomerization energy values reinforce the consistency and the relative accuracy of theoretical model calculations.

The observed flexibility of naturally *cis*-**PUFA**s could be explained by a very special "smooth basin" PES of the motif of sp^2-sp^3-sp^2 hybrid states as reported previously (J. Phys. Chem. A. **2005**, *109*, 520-533). We assumed that intrinsic thermodynamic functions may describe this flexible folding process. The folding enthalpy as well as the folding entropy suggests that there is a new role of the *cis*-**PUFA**s in membranes: these *cis*-isomers may have a strong influence on membrane stability, and permeability. The average length of the cis helix and beta PUFA was approximated. The difference between the lengths of these two structures is approximately 10Å.

Topic 3 The results and experience obtained from the first two topics were used to construct simple phospholipids. The relative orientations of the two fatty acids in a phospholipid had to be studied to see if the nearly parallel arrangement within the lipid bilayer is enforced by nearest neighbour interaction or if such a geometry is an intrinsically stable structure.

The theoretical method as well as the conformational selections have already been made. The interaction with the head group as well es the interactions of paralel chains are calculated. The energy as well as enthalpy contribution to the build up a thermodinamically correct membran bilayer can now be estimate.

Topic 4 Free radicals and their reactions with PUFA within the lipid bilayer are a biologically very important reactions. Vitamin E is one of the most effective free radical scavenger in the lipid bilayer [THEOCHEM 666-667 (2003) 439-449]. The generation of free radicals and their transformation [THEOCHEM 731 (2005) 57-60] as well as their reactions with allylic C-H bonds, which are always present is PUFA, has been studied in details.

The allylic H-atom abstraction reaction plays a more dominant role, especially at lower temperature, than addition reactions. An stable π- or reactant-like as well as a late product-like complexes were found on the potential energy surface of allylic H-abstraction using first principle computation. Different computational methods including *ab initio* HF, MP2, QCISD, QCISD(T) CCSD and CCSD(T), as well as density functional B3LYP, BH&HLYP methods combined with the 6-31G(d) split valence basis set, have been used to verify the importance of allylic H-abstraction.

The deviations from the CCSD(T) reference surface show that the BH&HLYP, QCISD, CCSD and QCISD(T) surfaces are in excellent agreement with each other; maximum deviations of the complete potential energy surfaces are less than 10.0, 5.5, 6.5 and 1.0 kJ / mol, respectively. These methods closely reproduce the geometrical parameters for the structures of the stationary points. A modest basis set effect was observed in the case of the BH&HLYP method. In contrast to that, the standard HF, MP2, and B3LYP methods show remarkable discrepancies in geometries of the hydrogen abstraction transition state. The BH&HLYP method turns out to be a fast and accurate method for obtaining realistic geometries for allylic H-abstractions.

Based on higher level single point calculations (QCISD(T)/6-311+G(3df,2p)), a new activation enthalpy value: $\Delta^{\ddagger}H^{\circ} = 0.3 \pm 2$ kJ / mol is suggested for the title reaction. The computed reaction enthalpy $\Delta_{r}H^{\circ} = -124.7 \pm 2$ kJ / mol is in good agreement with experimental value.

This quasi barrierless hydrogen abstraction is quite fast and does not have a single step reaction mechanism. The putative pathway between the transition state and the early π-complex state was characterized. The stability of the initial π-complexes was found to be $\Delta H^{\circ}_{\pi\text{-complex}}$ = -7.1 kJ / mol. The product complex was found between the transition state and the product with the stability of -127.2 kJ / mol.

All possible reactions of the propene-OH system was studied using the QCISD(T)/cc-pVTZ//BH&HLYP/6-31G(d) level of theory. All important transition state structures were determined in this potential energy surface (PES). The formation of the π–complex is the initial step and there are three possible reactions of the propene-OH system: H-abstraction, terminal addition, and non-terminal addition. All of them have quasi negative activation enthalpy related to infinity separated propene and OH (-1.2, -5.1 and -6.5 $\mathrm{kJmol^{-1}}$ respectively). The highest barrier belongs to the H-abstraction channel; however, the products of other two channels can go back to π–complex or go towards low-lying transition states. These low-lying transition states are: the conversion 2-β-hydroxy-propyl radicals to 1-methyl-ethoxy radicals with 7.1 $\mathrm{kJmol^{-1}}$ and dissociation 2-β-hydroxy-propyl of to ethenol and methyl radical with 9.7 $\mathrm{kJmol^{-1}}$ quasi activation enthalpy. Note that their relative enthalpies are higher by at least 8.3 $\mathrm{kJmol^{-1}}$ than those of the transition state of H-abstraction from propene and after the TS of H-abstraction, the products can revert back in contrast to the addition channels. In addition, not only the negative activation enthalpy ($\Delta^{\ddagger}H^{\circ}$ = -1.2 $\mathrm{kJmol^{-1}}$) and strongly exothermic ($\Delta_r H^{\circ}$ = 120.2 $\mathrm{kJmol^{-1}}$) behaviour predict that H-abstraction must be important in a propene and OH system, but the positive reaction entropy (ΔS° = 9.5 $\mathrm{Jmol^{-1}K^{-1}}$) also show similar properties. Theoretical $\Delta_r H^{\circ}$ of H-abstraction is in good agreement with the experimental $\Delta_r H^{\circ}_{\mathrm{exp}}$ = -126.2 $\mathrm{kJmol^{-1}}$. The deviation is mainly due to high spin-contamination (0.95) of allyl radical. All in all, allylic H-abstraction reaction is important at any temperature in (poly)alkenes and OH systems. The overestimation of the barrier of H-abstraction reaction can come from its sensitivity to the reasonable level of theory and sufficiently large basis set because of the formation of the conjugated allylic system.

Another important point: the kinetics of the $CH_2CHO + O_2$ reaction was experimentally studied in 2 quasi-static and a discharge flow-reactor at temperatures ranging from 298 to 660 K and pressures between 1 mbar and 46 bar with helium as bath gas. The CH_2CHO radicals were produced by laser-flash photolysis of ethyl vinyl ether at 193 nm and by the reaction F + CH_3CHO, respectively. Laser-induced fluorescence excited at 337 or 347.4 nm was used to monitor the CH_2CHO concentration. The reaction proceeds via reversible complex formation with subsequent isomerization and fast decomposition: $CH_2CHO + O_2 \Leftrightarrow O_2CH_2CHO \rightarrow HO_2CH_2CO \rightarrow$ products. Rate coefficients for the first and second step were determined (k_1, k_{-1}, k_2) and analyzed by a master equation with specific rate coefficients from RRKM theory. Molecular and transition-state parameters were obtained from quantum chemical calculations. A third-law analysis lead to the following thermodynamic parameters for the first step: $\Delta_R S°_{300K(1)} = -144$ J K^{-1} mol^{-1} (1 bar) and $\Delta_R H°_{300K(1)} = (-101 \pm 4)$ kJ mol^{-1}. From the falloff analysis the following temperature dependences for the low and high-pressure limiting rate coefficients were obtained: $k_{1(0)} = 5.14 \times 10^{-14} \exp(210\ K/T)$ cm^{-3} s^{-1}, $k_{1(\infty)} = 1.7 \times 10^{-12} \exp(-520\ K/T)$ cm^{-3} s^{-1}, and $k_{2(\infty)} = 1.3 \times 10^{12} \exp[-(82 \pm 4)\ kJ\ mol^{-1}/RT]$ s^{-1}. Readily applicable analytical representations for the pressure and temperature dependence of k_1 were derived to be used in kinetic modeling.

The reaction of 1-Methylvinoxy radicals, CH_3COCH_2, with molecular oxygen been investigated by experimental and theoretical methods as a function of temperature (291 – 520 K) and pressure (0.042 – 10 bar He). Experiments have been performed by laser photolysis coupled to a detection of 1-Methylvinoxy radicals by laser induced fluorescence LIF. The potential energy surface calculations were performed using ab inito molecular orbital theory at the G3MP2B3 and CBSQB3 level of theory based on the density function theory optimized geometries. Derived molecular properties of the characteristic points of the potential energy surface were used to describe the mechanism and kinetics of the reaction under investigation. At 295K, no pressure dependence of the rate constant for the association reaction has been observed: $k_{1,298K} = (1.18 \pm 0.04) \times 10^{-12}$ $cm^3 s^{-1}$. Bi-exponential decays have been observed in the temperature range 459 – 520 K and have been interpreted as equilibrium reaction. The temperature dependent equilibrium constants have been extracted from these decays and a standard reaction enthalpy of $\Delta H_{r,298K} = (-105.0 \pm 2.0)$ kJ mol^{-1} and entropy of $\Delta S_{r,298K} = (-143.0 \pm 4.0)$ J $mol^{-1} K^{-1}$ were derived, in excellent agreement with the theoretical results. Consistent heats of formation for the vinoxy and the 1-Methylvinoxy radical as well as their O_2 adducts are recommended based on our complimentary experimental and theoretical study $\Delta H_{f,298K} = 13.0 \pm 2.0 / -32.9 \pm 2.0 / -85.9 \pm 4.0 / -140.6 \pm 4.0$ kJ mol^{-1} for CH_2CHO, CH_3COCH_2 radicals and their adducts, respectively.