K01 A Study on Conformational Similarity of the (R)- and (S)-Enantiomers of Rivastigmine and the Binding Features with AChE by MO Calculations

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Introduction. Rivastigmine is a carbamate inhibitor of acetylcholinesterase(AChE) in use for treatment of Alzheimer's disease(AD) under the trade name of Exelon and has an asymmetric center (see Figure 1). Its (S)-enantiomer has a 10-fold affinity for brain G_1 AChE, its chemical stability, its longer duration of action in vivo, and its good tolerability[1]. The protein crystallographic structure of the complex of NAP, (-)-S-3-[1-dimethylamino)ethyl]phenol, as a leaving group (Figure 1) and Torpedo californica AChE (*Tc*AChE) has been reported[1].

In this work, the lowest-energy conformations of the (R)- and (S)-enantiomers of rivastigmine are to be analyzed and the interaction energy in the complex will be decomposed.

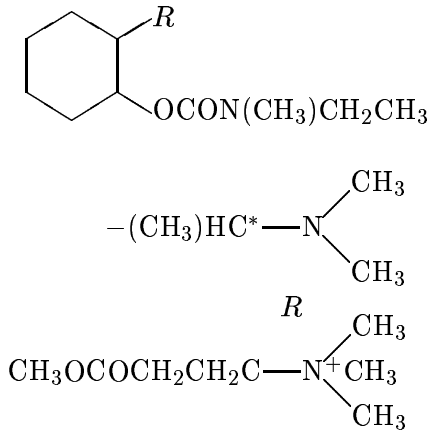
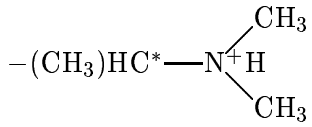


Figure 1: Structures of Rivastigmine, ACh and NAP

Methods. *Ab initio* MO calculations were employed throughout this study. The conformation analyses of (R)- and (S)-enantiomers of Rivastigmine were performed at B3LYP/6-31G(d,p), rotating two dihedral angles, PHI (Φ) and PSI (Ψ), at 8.78° increments (the dihedral angles defined here shown in Figures 2 and 3). The coordinates obtained here were to be fully optimized at B3LYP/6-31G** by Gaussian 98. The zero-point corrected energies (ZPE) at 298° K, using the PCM solvent model, were calculated by Gaussian 03. The interaction energies were decomposed by Kitaura-Morokuma (KM) method[2] by GAMESS.

Results and Discussion. First of all, the proton affinities of the trimethylamine of rivastigmine have been estimated at a B3LYP/6-31G(d,p) level (Table 1 and 2). The nitrogen atom is protonated in the physiological pH range, 6.5 - 8.0, which corresponds to the quarternary amine of acetylcholine (Figure 1).



Trimethylammonium

On the contrary, the nitrogen atom in the carbamate group of rivastigmine was assumed to be hardly protonated, since the proton affinity had shown a negative value, -221.7 kcal/mol at a B3LYP/6-31G** level (Table 1). The zero-point corrected energies using the solvent PCM model were calculated in order to predict the protonation affinities in such an enzyme active site pocket, where the dielectric constant ϵ is in the range 2-10 (Table 2).

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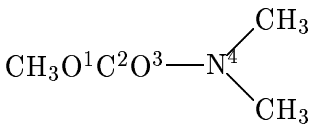
[1] P. Bar-On, C. B. Millard, M. Harel, H. Dvir, A. Enz, J. L. Sussman, and I. Silman, *Biochemistry*, **41**, 3555-3564 (2002) and therein.

[2] K. Kitaura and K. Morokuma, *Int. J. Quantum. Chem.*, 325–340 (1976).

Second, the conformation analyses of (R)- and (S)-rivastigmine were performed, by the calculation of the potential energy surfaces (PES) (Figure 2).



Methyl Carbamate

For simplicity, N-dimethyl derivative of methyl carbamate was used for the proton affinity calculation. The nitrogen lone pair electrones within the carbamate were delocalized, $n_{N4} \rightarrow \sigma^*_{C2-O3}$ = 51.52 kcal/mol at B3LYP/6-31G(d,p) (Table 3). The proton affinities were calculated in an aqueous solution and organic solvents as well as *in vacuo*. Thus, protonation occurs on the nitrogen atom within trimethylamine in a most hydrophobic site. The delocalization of the lone pair electrons on the oxygen and nitrogen atoms in the carbamate system were analyzed by the deletion method of NBO analysis[3] at B3LYP/6-31G** and HF/6-31G** levels (Table 3).

The lowest-energy conformations of (R)- and (S)-rivastigmine were analyzed by geometry optimizations using the initial dihedral angles obtained in the potential energy surfaces. The C-H group of the methylamine and the carbamate O=C group forms a weak hydrogen bond (C-H $\cdots$ O=C = 2.37 Å) and this attractive interaction is seen to make the conformations unflexible (Figure 3).

The dihedral angles (Φ,ψ) of the lowest-energy conformations were (71.94, -81.88) and (66.24, -75.71) in degrees for the (R)- and (S)-rivastigmine, respectively. Each conformation was folded with the weak H-bond between the methyl group and the carbonyl oxygen (Figure 3). On the different pharmacological activities revealed by the two enantiomers, the methyl group attached on the asymmetric carbon plays an important role.

The pharmacophoric similarity was found in the conformations of both rivastigmine and acetylcholine and the distances between the quarternaly methylamine and the carbonyl oxygen were estimated to be near, 5.69 and 5.11 Å, respectively (Figures 3 and 4).

A method has been proposed for the analysis of components of molecular interaction energy within the Hartree-Fock approximation by Kitaura and Morokuma[2]. Nevertheless, few analyses of the interaction for the enzyme-ligand binding have been reported, although important at a pharmacological point of view. The following separation of the energy stabilization ($\Delta E = E_{complex} - E_{monomers}$) is calculated.

$$\Delta E = ES + PL + EX + CT + MIX \qquad (1)$$

ES is the electrostatic interaction. PL is the polarization interaction. EX is the exchange repulsion. CT is the charge transfer or electron delocalization interaction. MIX, the coupling term, is actually the difference between the total SCF interaction energy ΔE_{SCF} and the sum of the above four components. The KM method has been employed to examine the interaction for the complex between NAP and the carbamyl moiety bound to the Oγ atom of SER 200 of AChE (Figure 4). The initial coordinates obtained in Protein Data Bank (pdb1gqr), adding hydrogen atoms by a standard method, were optimized at HF/6-31G for the energy analysis. The decomposed energies of the complex in this work have been compared with those of $NH_3 \cdots H_2O$ H-bond complex previously reported (Table 4). In this work, the intermolecular weak H-bonds between the C-H of the methyl group and the carbonyl oxygen of the carbamyl moiety were observed, C-H $\cdots$ O=C = 2.16 and 2.82 Å (Figure 4).

It is concluded that the conformations of the (R)- and (S)-enantiomers of rivastigmine are similar and also to acetylcholine (Figures 3 and 4). Both of these compounds are bound and then, carbamylated or acylated by AChE. The methyl group attached on the asymmetric carbon might influence on the biochemical reactivities, as described above in introduction. Other important interactions, for example hydrophobic, and $\pi - \pi^*$ interactions, are hardly estimated in *ab initio* MO calculations.

[3]Alan E. Reed, Larry A. Curtiss and Frank Weinhold, *Chem. Rev.*, **88**, 899-926 (1988).

Table 1: Calculated Proton Affinities of the Two Nitrogen Atoms of Rivastigmine in kcal/mol.

Nitrogen Atoms	B3LYP/6-31G**	ZPE at B3LYP/6-31G**	ZPE(PCM) at B3LYP/6-31G**
Trimethylamine[a)]	251.7	276.9	283.8[b)] in water
Methyl Carbamate	-221.7	-213.0	-258.4 in water

a) The proton affinities on the nitrogen within trimethylamine were calculated for the whole rivastigmine coordinates, already optimized at B3LYP/6-31G**; b) See Table 2.

Table 2: Calculated Proton Affinities of Trimethylamine using the PCM Model in kcal/mol.

Molecule	ZPE (298° K) at HF/6-31G** using the PCM model			
	Dielectric Constant (ε)			
	78.5 (water)	10.36	4.9	2.023
trimethylamine	284.0	278.6	272.3	253.4

Table 3: Charge Transfer Energies Calculated by the Deletion Method of NBO Analysis for Methyl Carbamate in kcal/mol.

Charge Transfer	Energies in kcal/mol	
	Methods/Basis Set	
	B3LYP/6-31G**	HF/6-31G**
$n_{N4} \rightarrow \sigma^*_{C2-O3}$	51.52	44.88
$n_{O1} \rightarrow \sigma^*_{C2-N4}$	10.68	10.44
$n_{O1} \rightarrow \sigma^*_{C2-O3}$	33.07	29.24
$n_{O3} \rightarrow \sigma^*_{C2-N4}$	21.89	21.27
$n_{O3} \rightarrow \sigma^*_{O1-C2}$	30.90	28.50
Total	154.3	138.8

Table 4: Energy Decomposition of the Interaction in the Complex of NAP and the Carbamyl Moiety by Kitaura-Morokuma Method at HF/6-31G in kcal/mol.

Components	Interaction Energies		
	Intermolecular Complexes		
	NAP and Carbamyl Moiety Complex	$NH_3 \cdots H_2O$[a] (this work)	
ES	-8.61	-14.0	(-13.0)
EX	8.65	9.0	(10.0)
PL	-2.14(-1.70, -0.31)[b]	-1.1	(-1.14)
CT	-2.39(-2.00, -0.39)[b]	-2.4	(-2.46)
MIX	0.14	0.4	(0.01)
Total	-4.36	-9.0	(-6.50)

a) H. Umeyama and K. Morokuma, *J. Amer. Chem. Soc.*, **99**, 1316 (1977). The data in this reference were calculated at HF/4-31G and the values in this work at HF/6-31G**.
b) Decomposition energies for monomers of PL and CT are listed, (carbamyl moiety, NAP).

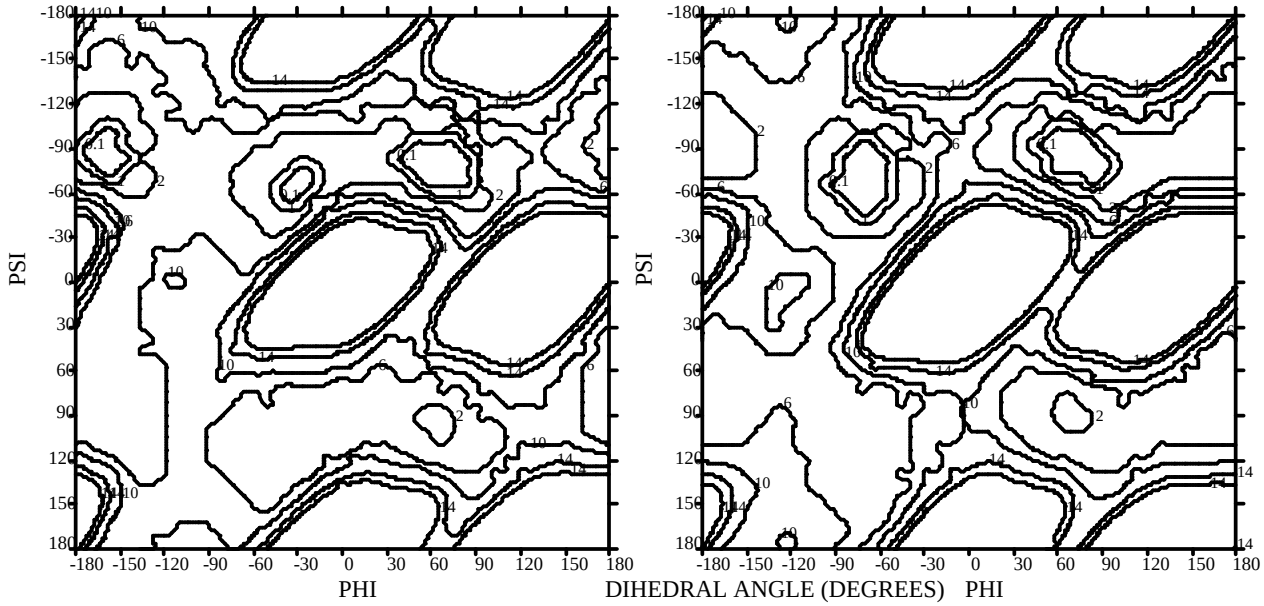


Figure 2: PES's of (S)- (left) and (R)-Rivastigmine (right) at B3LYP/6-31G**

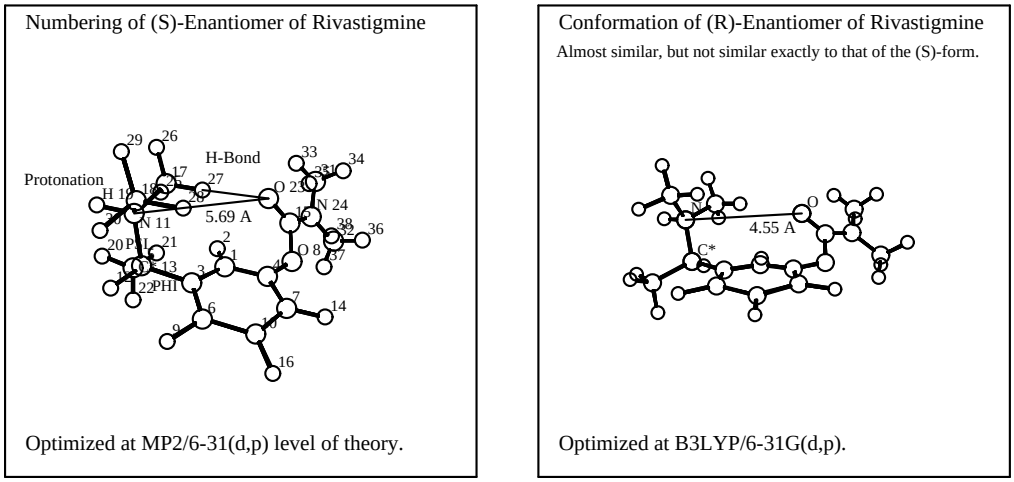


Figure 3: Conformations of (S)- (left) and (R)-Rivastigmine (right)

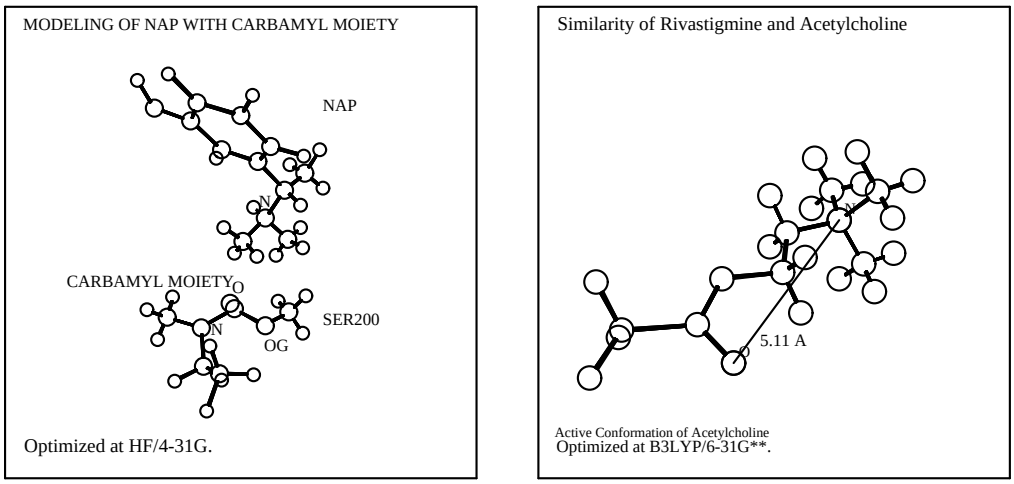


Figure 4: NAP and Carbamyl Complex (left) and Acetylcholine (right)