
# Asian Journal of Biochemistry

ISSN 1815-9923

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Biochemistry 2 (5): 354-358, 2007
ISSN 1815-9923
© 2007 Academic Journals Inc.

# Molecular Modelling Analysis of the Antioxidant Activity of Probucol

Fazlul Huq
Discipline of Biomedical Science, School of Medical Sciences,
Faculty of Medicine, Cumberland Campus, C42, The University of Sydney,
P.O. Box 170, Lidcombe, NSW 2141, Australia

**Abstract:** Probucol (PBC) is a phenolic antioxidant that was once used as a lipid lowering agent. However, in addition to having moderate low density lipoprotein lowering ability, the drug is also found to significantly lower high density lipoprotein levels and cause QTc (corrected heart rate) prolongation. Molecular modelling analyses based on molecular mechanics, semi-empirical (PM3) and DFT (at B3LYP/6-31G* level) calculations show that PBC has a much larger LUMO-HOMO energy difference than its major metabolite SPQ so that PBC would be more kinetically inert than SPQ. Whereas the molecular surface of PBC is found to possess significant amount of electron-rich (red and yellow) regions so that it can act as an antioxidant, that of SPQ is found to possess significant amount of electron-deficient (blue) regions so that it can react with cellular nucleophiles glutathione and nucleobases in DNA. Reaction with glutathione would induce cellular toxicity due to glutathione depletion whereas the oxidation of nucleobases would cause DNA damage.

**Key words:** Probucol, antioxidant, lipid lowering agent, spiroquinone, molecular modelling

## INTRODUCTION

Probucol (PBC; 4,4'-isopropylidenedithio)bis(2,6-die-*tert*-butylphenol]) is a phenolic antioxidant that was once used as a lipid lowering agent (Barnhart *et al*., 1977; Buckley *et al*., 1989). However, in addition to having moderate low density lipoprotein (LDL) lowering ability, the drug is also found to significantly lower High Density Lipoprotein (HDL) levels and cause QTc (corrected heart rate) prolongation. Because of these adverse effects, PBC was actually withdrawn from the market.

The strong antioxidant properties of PBC are believed to contribute to its anti-atherogenic effects. It may be noted that oxidative stress is a major risk factor of atherosclerosis (Jeon *et al*., 2005). Compelling evidence indicates that Reactive Oxygen Species (ROS) can induce epithelial dysfunction and macrophage activation, resulting in the release of cytokines and growth factors that stimulate matrix remodelling and proliferation of smooth muscle cells. Oxidation processes are also involved in the crosslinking of collagen fibres, resulting into long-term vascular constriction. In a clinical trial, PBC was found to reduce carotid artery intima-media thickness in patients with hypercholesterolemia to the same extent as pravastain and to show significantly lower incidence of cardiac events than placebo group (Sawayama *et al*., 2002).

PBC is metabolized to spiroquinone (SPQ). SPQ and its metabolites have been suspected to be responsible for causing QTc prolongation in some patients. Figure 1 gives the metabolic pathway for PBC in humans (Meng, 2006). In this study, molecular modelling analyses have been carried out using the program Spartan '02 (Spartan, 2002) to investigate the relative stability of PBC and its metabolite SPQ with the aim of providing a better understanding on their relative toxicity. Previous studies have shown that xenobiotics or their metabolites which are kinetically labile and abound in electron-deficient regions on the molecular surface tend to induce cellular toxicity due to glutathione depletion and cause DNA damage due to oxidation of nucleobases in DNA (Huq, 2006a, b).

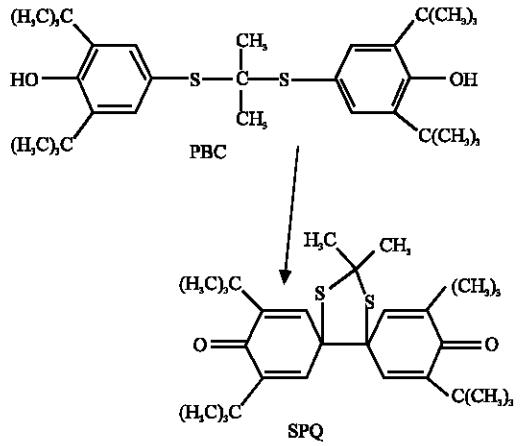



Fig. 1: Metabolic pathway for PBC based on Meng (2006)

## MATERIALS AND METHODS

This being entirely theoretical study, only molecular modelling calculations were carried out in the study.

**Computational Methods**

The geometries of PBC and its metabolite SPQ have been optimized based on molecular mechanics, semi-empirical and DFT (density functional theory) calculations, using the molecular modelling program Spartan '02. Molecular mechanics calculations were carried out using MMFF force field. Semi-empirical calculations were carried out using the routine PM3. DFT calculations were carried at B3LYP/6-31G* level. In optimization calculations, a RMS gradient of 0.001 was set as the terminating condition. For the optimized structures, single point calculations were carried out to give heat of formation, enthalpy, entropy, free energy, dipole moment, solvation energy, energies for HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital). The order of calculations: Molecular mechanics followed by semi-empirical followed by DFT ensured that the structure was not embedded in a local minimum. To further check whether the global minimum was reached, some calculations were carried out with improvable structures. It was found that when the stated order was followed, structure corresponding to the global minimum or close to that could ultimately be reached in all cases. Although RMS gradient of 0.001 may not be sufficiently low for vibrational analysis, it is believed to be sufficient for calculations associated with electronic energy levels.

## RESULTS AND DISCUSSION

Table 1 gives the total energy, heat of formation as per PM3 calculation, enthalpy, entropy, free energy, log P, surface area, volume, dipole moment and energies of HOMO and LUMO as per both PM3 and DFT calculations for PBC and its metabolite SPQ. Figure 2 and 3 give the regions of negative electrostatic potential (greyish-white envelopes) in (a), HOMOs (where red indicates HOMOs with high electron density) in (b), LUMOs in (c) and density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral) in (d) as applied to the optimized structures of PBC and its metabolite SPQ.

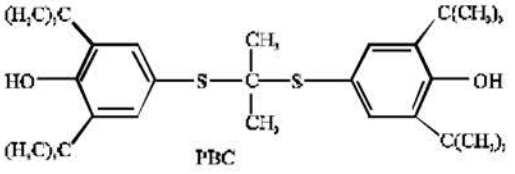
Table 1: Calculated thermodynamic and other parameters of PBC and its metabolite SPQ

| Molecule | Calculation type | Total energy (kcal mol$^{-1}$/ atomic unit*) | Heat of formation (kcal mol$^{-1}$) | Enthalpy (kcal mol$^{-1}$ K$^{-1}$) | Entropy (cal mol$^{-1}$ K$^{-1}$) | Free energy (kcal mol$^{-1}$) | Solvation energy (kcal mol$^{-1}$) |
|---|---|---|---|---|---|---|---|
| PBC | PM3 | -95.14 | -89.72 | 410.25 | 209.78 | 347.70 | -5.42 |
| | DFT | -1999.79 | | 411.86 | 208.43 | 349.75 | -5.07 |
| SPQ | PM3 | -24.22 | -21.89 | 467.74 | 214.56 | 403.77 | -2.33 |
| | DFT | -2155.71 | | 468.92 | 213.31 | 405.35 | -2.13 |

Table 1: Continued

| Molecule | Calculation type | Log P | Area (Å$^2$) | Volume (Å$^3$) | Dipole moment (debye) | HOMO (eV) | LUMO (eV) | LUMO-HOMO (eV) |
|---|---|---|---|---|---|---|---|---|
| PBC | PM3 | 9.92 | 505.60 | 500.34 | 4.1 | -8.52 | -0.52 | 8.00 |
| | DFT | 9.92 | 515.97 | 505.47 | 5.2 | -5.82 | -0.42 | 5.40 |
| SPQ | PM3 | 9.45 | 516.83 | 553.16 | 3.7 | -9.36 | -1.00 | 8.36 |
| | DFT | 9.45 | 532.17 | 561.34 | 4.9 | -6.21 | -1.99 | 4.22 |

*In atomic units from DFT calculations




PBC

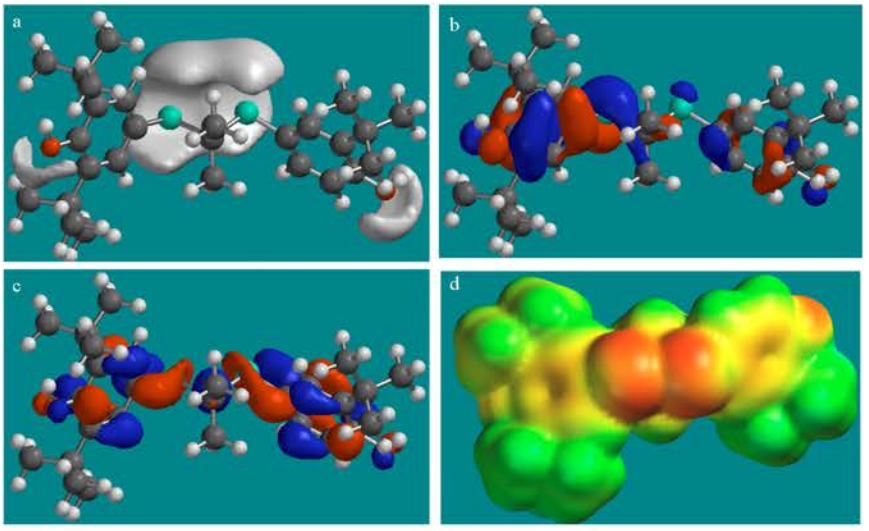



Fig. 2: Structure of PBC giving in (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral)

The calculated solvation energies from PM3 calculations of PBC and SPQ are respectively -5.42 and -2.33 kcal mol$^{-1}$ and corresponding log p-value are 9.92 and 9.45 (Table 1). The values suggest that both PBC and SPQ would have low solubility in water. This means both PBC an d SPQ would have higher solubiliy in lipid and therfore long biological half-life.

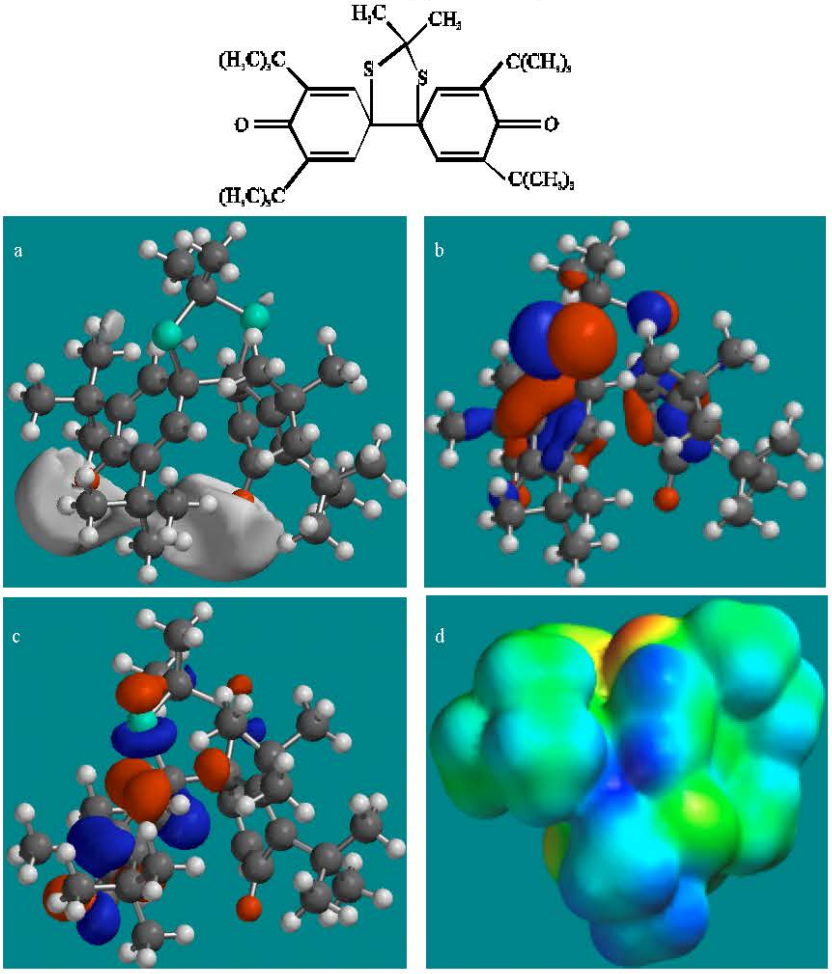



Fig. 3: Structure of SPQ giving in (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral)

The LUMO-HOMO energy differences for PBC and SPQ from DFT calculations are found to be 5.2 and 4.2 eV, respectively, indicating that the PBC would be more kinetically inert than its metabolite SPQ.

In the case of PBC and SPQ, the electrostatic potential is found to be more negative mainly around the two oxygen centres, indicating that the positions may be subject to electrophilic attack. It may be noted that in the case of both PBC and SPQ, oxygen and sulfur bear significant amount of negative charge of the order -0.46 unit for O and -0.36 for S in the case of PBS, -0.55 unit for O and -0.46 unit for S in the case of SPQ), supporting the idea that atoms can act as electron donor centres.

In the case of PBC and SPQ, both the HOMOs with high electron density and LUMOs are found to be centered mostly on the non-hydrogen atoms of the two phenyl rings.

The overlap of HOMO with high electron density and region of negative electrostatic potential at some positions, gives further support to the idea that the positions may be subject to electrophilic attack.

The molecular surface of PBC is found to abound in neutral (green) and electron-rich (yellow and red) regions, indicating that the drug may be subject to lyophilic and electrophilic attacks. The presence of electron-rich regions on the molecular surface can explain why PBC can act as an antioxidant. The molecular surface of SPQ is found to abound in neutral (green) and electron-deficient (blue) regions, indicating that the metabolite may be subject to lyophilic and nucleophilic attacks. Nucleophilic attacks can be due to glutathione and nucleobases in DNA. Reaction with glutathione will induce cellular toxicity by compromising the antioxidant status of the cell whereas that with nucleobases in DNA will cause DNA damage.

When surface area and volume of PBC are compared with those of SPQ, it is found that the values differ significantly (Table 1) so that PBC and SPQ may not bind to the same binding site of the receptors.

## CONCLUSION

Molecular modelling analyses based on semi-empirical and DFT calculations show that PBC has a much larger LUMO-HOMO energy difference than its metabolite SPQ, indicating that SPQ would be more kinetically labile than the parent drug. The molecular surface of PBC is found to possess significant amount of electron-rich (yellow and red) regions, thus providing an explanation as to why PBC can act as an antioxidant. The molecular surface of SPQ however is found to possess significant amount of electron-deficient regions so that it can react readily with glutathione and nucleobases in DNA. Reaction with glutathione would induce cellular toxicity due to glutathione depletion whereas oxidation of nucleobases would cause DNA damage.

## ACKNOWLEDGMENT

Fazlul Huq is grateful to the Discipline of Biomedical Science, The University of Sydney for the time release from teaching.

## REFERENCES

Barnhart, J.W., D.J. Rytter and J.D. Molello, 1977. An overview of the biochemical pharmacology of probucol. Lipids, 12: 29-33.

Buckley, M.M., K.L. Goa, A.H. Price and R.N. Brogden, 1989. Probucol. A reappraisal of its pharmacological properties and therapeutic use in hypercholesterolaemia. Drugs, 37: 761-800.

Huq, F., 2006a. Molecular modelling analysis of the metabolism of tamoxifen. Int. J. Pure Applied Chem., 1: 155-163.

Huq, F., 2006b. Molecular modelling analysis of the metabolism of zaleplon. J. Pharmacol. Toxicol., 1: 328-336.

Jeon, S.M., Y.B. Psrk, O.S. Kwon, T.L. Huh, W.H. Lee, K.M. Do, T. Park and M.S. Choi, 2005. Vitamin E supplementation alters HDL-cholesterol concentration and paraoxonase activity in rabbits fed high-cholesterol diet: Comparison with probucol. J. Biochem. Molecular Toxicol., 19: 336-346.

Meng, C.Q., 2006. Inhibitors of the Expression of Vascular Cell Adhesion Molecule-1. In: Wood, A. (Ed.), Ann. Rep. Med. Chem., 41: 197-210.

Sawayama, Y., C. Shimizu, N. Maeda, M. Tatsukawa, N. Kinukawa, S. Koyanagi, S. Kashiwagi and J. Hayashi, 2002. Effects of probucol and pravastatin on common carotid atherosclerosis in patients with asymptomatic hypercholesterolemia Fukuoka atherosclerosis trial (FAST). J. Am. Cardiol., 39: 610-616.

Spartan '02, 2002. Wavefunction, Inc. Irvine, CA, USA.