Quantum chemical calculations on the metabolic activation of paracetamol

Quantum chemical *ab initio* calculations were used to investigate a new hypothetical mechanism for the oxidation of paracetamol by cytochrome P450. The calculations reveal that an initial hydrogen abstraction from the phenolic oxygen atom is energetically most favorable and can explain the formation of all known metabolites of paracetamol.

Cytochromes P450 are enzymes involved in the oxidative metabolism of a wide variety of endogenous and exogenous compounds. The main biological function of this enzyme system is the incorporation of an oxygen atom from molecular oxygen into the substrates leading to hydroxylation, epoxidation, hetero-atom oxygenation, or dehydrogenation. The hepatic cytochrome P450 enzyme system is responsible for the biotransformation of the well-known mild analgesic and antipyretic drug paracetamol (PAR) to the toxic electrophilic metabolite N-acetyl-para-benzoquinonimine (NAPQI) (Figure 1). Upon an overdosage of PAR, the reactive metabolite NAPQI can cause severe liver damage in man and experimental animals by depleting the hepatic glutathione levels [1]. Besides NAPQI, two other minor metabolites are formed, notably 3-hydroxy-PAR and para-benzoquinone (Figure 1).

The mechanism of metabolic activation of PAR to its three metabolites by cytochrome P450 has been the subject of many toxicological studies during the last 20 years [2, 3]. However, until now, it has not been possible to detect experimentally elusive intermediates that, in principle, might be formed during the biotransformation of PAR within the active site pocket of cytochrome P450. In such cases a theoretical study might be useful. In the present paper use was made of *ab initio* energy and spin distribution calculations to support a new hypothetical mechanism of activation of PAR to its metabolites.

Considering the size and complexity of the molecules involved, as well as the computer-intensive nature of the *ab initio* methods employed, this work could hardly have been done without supercomputer(s).

Strategy and experimental procedures

Cytochrome P450 enzyme reaction is supposed to consist of a transfer of an iron-bound activated oxygen atom from the enzyme to the substrate via one-electron steps [4]. As initial one-electron step in the metabolic activation of PAR, a hydrogen abstraction either from the phenolic hydroxyl group or from the nitrogen atom in the acetylamo side chain is taken (Figure 2). The abstracted hydrogen atom is proposed to react with the activated oxygen atom of cytochrome P450 to yield a hydroxy radical and a phenoxy- or nitrogen-centered PAR radical. Subsequent reactions of the PAR radicals with this hydroxyl radical, involving second hydrogen abstraction, radical recombination and/or rearrangement reactions, are supposed to account for the observed metabolites of PAR. Which of the PAR radicals (phenoxy radical or nitrogen radical) is formed after the initial hydrogen abstraction from PAR, and the exact nature of the non-radical intermediates formed during subsequent reactions of these radicals, is unknown due to the fact that the reactions occurring in between the binding of PAR to the enzyme and the release of the metabolites by the enzyme are experimentally inaccessible. However, with the aid of the supercomputer and quantum chemical methods it is possible to calculate the relative stabilities of the two possible PAR radicals (phenoxy radical and nitrogen radical) formed after initial hydrogen abstraction. In the same way, the stabilities of the non-radical intermediates formed after subsequent reactions of the PAR radicals with the hydroxyl radical
can be calculated and compared. Thus one might get an idea about the reaction path of the metabolic activation of PAR by cytochrome P450. Before energy calculations were carried out the geometries of all radical and non-radical intermediates were optimized. The size and complexity of these intermediates and especially the radical species requires the use of \textit{ab initio} quantum chemical techniques and therefore implicitly the use of an advanced computer system. Pilot studies with semi-empirical methods (see next section) underline the need for the time-consuming \textit{ab initio} calculations. Use was made of the \textit{ab initio} program package GAMESS (General Atomic and Molecular Electronic Structure System) [5–7], containing over 250,000 lines Fortran and implemented, amongst others, on the computers of the Academic Computer Services Amsterdam (SARA), at the time the Cybers 205 and 995. In the \textit{ab initio} calculations a simplified enzyme system was used by substituting the cytochrome P450 enzyme complex for an oxygen atom. In order to comply with the law of conservation of spin momentum a singlet oxygen species had to be used in the calculations. The \textit{ab initio} calculations were performed at the linear combination of atomic orbitals/molecular orbitals/self-consistent field level using the STO3G [8] minimal basis set for geometry optimizations and a SV 6-31G [9] basis set for subsequent self-consistent field (SCF) calculations. The geometries of the parent compound PAR, intermediates and metabolites were fully optimized, implying variation of all bond distances, bond angles, and torsion angles. Radical species were optimized with the unrestricted Hartree-Fock (UHF)-formalism, which assumes different orbitals for different spins. However, it is known that in UHF-calculations admixture of higher spin states may distort the results, leading to too negative energies and unrealistic spin densities [10]. Therefore, SV 6-31G restricted Hartree-Fock spin distributions were calculated for the UHF geometries of the radicals. The \textit{ab initio} SV 6-31G energies were used to support the hypothetical mechanism of oxidation of PAR by cytochrome P450.

\section*{Results and discussion}

In a previous theoretical study on the metabolic activation of PAR [11] the semi-empirical all-valence electron molecular orbital method modified neglect of differential overlap (MNDO) [12] was applied to the geometry optimization of PAR and related compounds. The MNDO calculations revealed a minimum energy conformation of PAR in which the acetylamino side chain was rotated nearly perpendicularly relative to the phenyl ring. When repeating these MNDO calculations, we found in addition to this "perpendicular" conformation a second "flat" minimum energy conformation with the side chain in the plane of the phenyl ring. The "perpendicular" conformation was calculated to be 3.3 kcal/mol more stable than the "flat" conformation. However, it is known that the semi-empirical MNDO method underestimates conjugation effects [10]. Therefore, in the present study, \textit{ab initio} calculations were used.

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure2.png}
\caption{Paraacetamol is proposed to be oxidized by cytochrome P450 either via initial hydrogen abstraction from nitrogen atom in the acetylamino side chain or from the phenolic oxygen atom.}
\end{figure}

Figure 2 depicts the \textit{ab initio} STO3G energy of PAR as a function of torsion angle. The results clearly indicate that the acetylamino side chain is rather flexible and can be rotated out of plane up to 40° without a substantial loss of energy. Further results are published in [13].

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure3.png}
\caption{The \textit{ab initio} STO3G energy of paraacetamol as a function of torsion angle $\tau$. The energy is given in atomic units (1 a.u. $= 627.5$ kcal/mol).}
\end{figure}

The mechanism of oxidation of PAR as depicted in Figure 4 explains the formation of all known metabolites of PAR by one mechanism and is in accordance with a more and more generally accepted mechanism of oxidation of substrates by cytochrome P450 involving sequential one-electron steps. However, it must be kept in mind that the presented quan-
chemical approach is limited in the sense that only electronic aspects of the mechanism of oxidation are accounted for. Neither an influence of the environment of the active site on the metabolic profile nor a possible regio-selective metabolism of the substrate due to a specific orientation within the active site are accounted for in the approach used. Furthermore, due to the time-consuming ab initio calculations, also other simplifica-

tions had to be made. For example, the P450 enzyme complex had to be substituted by a singlet oxygen atom. A porphyrine ring with a thiolate and an activated oxygen species as axial ligands of the iron atom would be a more realistic representation of the activated oxygen complex of cytochrome P450. However, with the fast growth in supercomputer power this might become possible in the nearby future.

Acknowledgement
The authors thank Dr M.F. Guest (Daresbury Laboratory, UK) for the use of the program package GAMESS. Thanks are also due to the Academic Computing Services Amsterdam (SARA) for the use of their computers.

References
