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Molecular modelling of phthalates – PPARs interactions

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Abstract

Di(2-ethylhexyl) phthalate (DEHP) is the most widely plasticizer for polyvinyl chloride (PVC) that is used in plastic tubes, in medical and paramedical devices as well as in food storage packaging. The toxicological profile of DEHP has been evaluated in a number of experimental animal models and has been extensively documented. Its toxicity is in part linked to the activation of the peroxisome proliferator-activated receptor α ($PPAR_\alpha$). As a response, an intensive research for a new, biologically inert plasticizer has been initiated. Among the alternative studied, tri(2-ethylhexyl) trimellitate (TEHTM) or trioctyl trimellitate (TOTM) has attracted increasing interest. However, very little information is available on their biological effects. We proceeded to dock TOTM, DEHP and its metabolites in order to identify compounds that are likely to interact with $PPAR_\alpha$ and $PPAR_\gamma$ binding sites. The results obtained hint that TOTM is not able to bind to PPARs and should therefore be safer than DEHP.

Keywords: *DEHP, MEHP, TOTM, PPARs, docking*

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Introduction

Plastic materials require the addition of certain amount of plasticizer to obtain specific physico-chemical and mechanical properties required for practical applications. Di(2-ethylhexyl) phthalate (DEHP) is the predominant plasticizer used to make polyvinyl chloride (PVC) plastics more flexible and pliable. Mono-ethylhexyl phthalate (MEHP) is the active metabolite of DEHP. Its widespread usage in medical and paramedical appliances as well as in food storage packaging has led to DEHP being present as an ubiquitous environmental contaminant [1,2]. Given its high production volume and common use, humans are exposed through ingestion, inhalation, dermal and medical devices. For these reasons, plasticizers have been subjected to fairly extensive safety testing. So, toxic hazards associated with DEHP have extensively been investigated in a number of experimental animal models [3–6]. Phthalates adversely affect the male reproductive system in animals including hypospadias, cryptorchidism, reduced testosterone production and decreased sperm counts [7]. DEHP and the related compounds impair fertility of both sexes. Given the well-characterized testicular toxicity in the male, the ovary was considered a likely target for toxicity in the female [8].

MEHP is unique among the phthalates in that it suppresses aromatase in the granulosa cells, altering estradiol production in the ovary [8]. However, these effects are much more severe after in utero than adult

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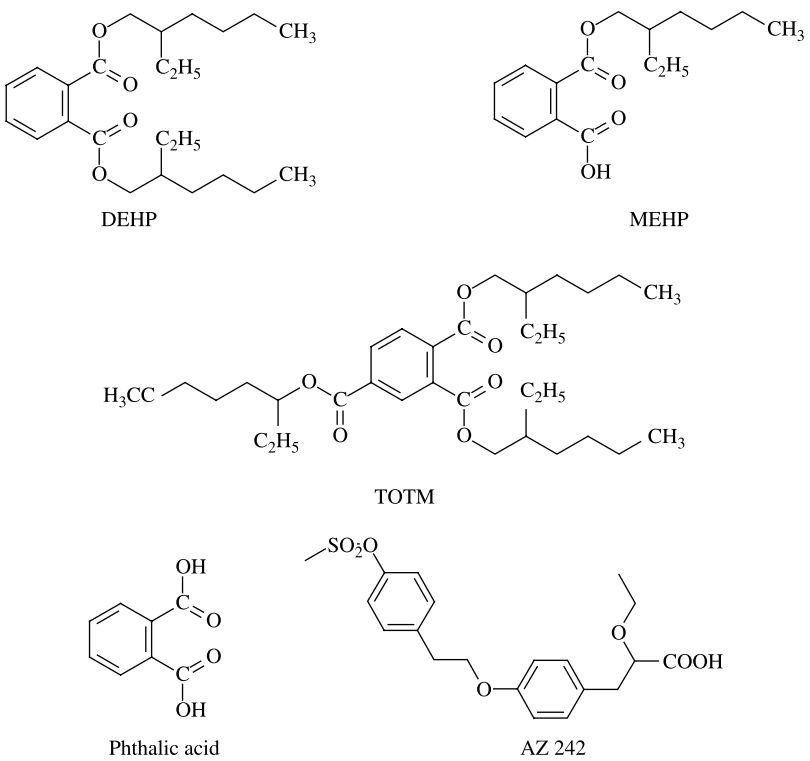

Figure 1. Structures of DEHP, MEHP, TOTM, phthalic acid and AZ 242.

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exposure [7]. Based on these data, the link between MEHP activity and its toxic effect on the granulosa cell was hypothesized. Recent epidemiological evidence indicates that boys born to women exposed to phthalates during pregnancy have an increased incidence of genital malformations and spermatogenic dysfunction, signs of a testicular dysgenesis syndrome [9]. Data reported elsewhere indicated that phthalates toxicity may be mediated by the peroxisome proliferators-activated receptors (PPARs) [10–12]. These receptors compose a class of nuclear receptors involved in glucidic and lipidic metabolism. They are divided in three isoforms, of which α and γ are of particular interest. $PPAR_{\gamma}$ are highly expressed in human adipose tissue where many lipophilic compounds tend to accumulate. $PPAR_{\alpha}$ control the oxidation of the fatty acids in the liver.

Recent studies have been reported the activation of $PPAR_{\alpha}$ and $PPAR_{\gamma}$ by phthalate monoesters [13]. Studies in human populations suggest an association between phthalate exposure and adverse reproductive health outcomes. For example, a higher phthalate monoester levels in women urine living near a plastics manufacturer is correlated with pregnancy complications such as anemia, toxemia, and preeclampsia [8].

A limited number of animal studies suggest that exposure to phthalate esters may be associated with altered thyroid function, but human data showed that urinary MEHP concentrations were associated with altered free T4 and/or total T3 levels in adult men [14].

Because people at risk for reproductive toxicity of phthalates are likely to include those exposed occupationally as well as those exposed during medical treatments such as hemodialysis, blood transfusion, parenteral nutrition, an active research for an alternative plasticizer has been initiated. Tri(2-ethylhexyl) trimellitate (TEHTM) or trioctyl trimellitate (TOTM), an ester of trimellitic acid has been increasingly attractive because of its potential for lower leachability [15,16]. However, little information was available on TOTM biological effects. Before using TOTM as alternative to DEHP, some investigations are needed such as the molecular interaction between $PPAR_{\alpha}$ and $PPAR_{\gamma}$ binding sites. Therefore, we proceeded to dock TOTM, DEHP and its degradation products (MEHP and phthalic acid: PA) in order to compare and specify the potential interactions of these ligands with $PPAR_{\alpha}$ and/or $PPAR_{\gamma}$.

Materials and methods

Molecular modelling studies were performed using SYBYL software version 6.9.1 [17] running on Silicon Graphics Octane 2 workstations. Three-dimensional models of DEHP, MEHP, TOTM and phthalic acid (Figure 1) were built from a standard fragments library, and their geometry was subsequently optimized using the Tripos force field [18] including the electrostatic term calculated from Gasteiger and Hückel atomic charges. As the pK_a of ionizable compounds such as

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MEHP or phthalic acid are unknown, the SPARC (SPARC Performs Automated Reasoning in Chemistry) online calculator was used to determine the species occuring at physiological pH (7.4) (http://ibmlc2.chem.uga.edu/sparc/index.cfm) [19]. The method of Powell available in the Maximin2 procedure was used for energy minimization until the gradient value was smaller than 0.001 kcal/mol.Å. The structures of the human $PPAR_\alpha$ and $PPAR_\gamma$ ligand-binding domain were obtained from their complexed X-Ray crystal structures with the tesaglitazar (AZ 242) available in the RCSB Protein Data Bank (http://www.pdb.org) [20] (PDB ID: 1I7G and 1I7I, respectively) [21]. Flexible docking of the compounds into the receptor active site was performed using GOLD 3.0.1 software [22]. The most stable docking models were selected according to the best scored conformation predicted by the GoldScore [22] and X-Score scoring functions [23]. The complexes were energy-minimized using the Powell method available in Maximin2 procedure with the Tripos force field and a dielectric constant of 4.0 until the gradient value reached 0.01 kcal/mol.Å. The anneal function was used to define a 10Å hot region and a 15Å region of interest around the ligand.

Results and discussion

The first step of our docking study was to check the protonation state of the molecules under investigation in order to test the form putatively binding to the Ligand Binding Domain (LBD) of the PPARs. By doing so, we thus tried to approximate at best the real binding conditions to be able to put forward meaningful conclusions. Different ionic species of a molecule differ in physical, chemical and biological properties and so it is important to be able to predict which ionic form of the molecule is present at the site of action. The pH of the environment and the pK_a of its ionizable groups will determine the charge associated with a molecule. We therefore sought the most probable forms of the compounds prior to their docking. For this mean, the SPARC online calculator allows a prediction of the fraction of each species at physiological pH. This prediction was carried out for all the molecules bearing a moiety reasonnably chargeable at physiological pH. Among the compounds, MEHP and phthalic acid contain carboxylic acid groups (Figure 1). It appeared that both are negatively charged at physiological pH (7.4) (Figures 2 and 3). Interestingly, both acid functions of phthalic acid are under their carboxylate form at this pH with only a tiny fraction of protonated acid on one of the function. However, this fraction is so small that it will seldom interact with the receptor at all. For MEHP, the situation is much clearer as there is only one form under these conditions, with the acid deprotonated.

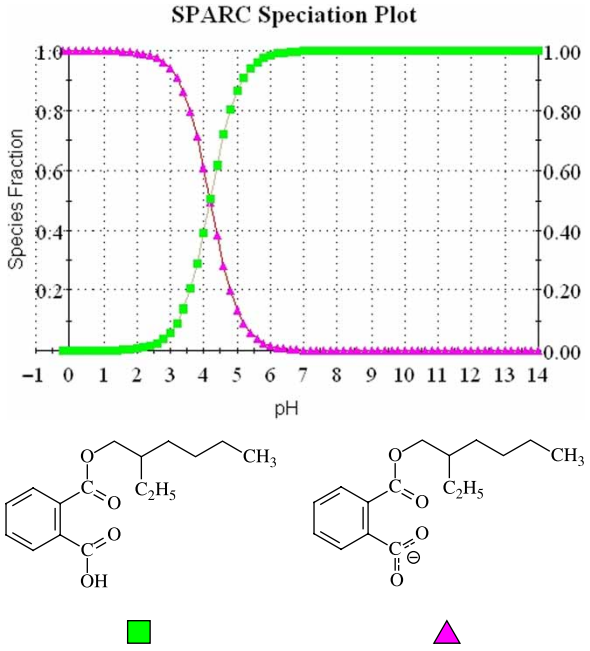

Figure 2. Fraction of each species of MEHP *versus* pH.

Docking simulations were carried out in order to predict the binding mode of these compounds into the active sites of $PPAR_\alpha$ and $PPAR_\gamma$ formerly occupied by tesaglitazar (AZ 242). Automated docking of the ligands into the $PPAR_\gamma$ active site provides multiple docking solutions. They were ranked by the consensus scoring GoldScore/X-Score. The consistency of the binding mode was verified by superimposing all the 30 solutions and a visual inspection of the top ranked was performed to retain the conformations forming the

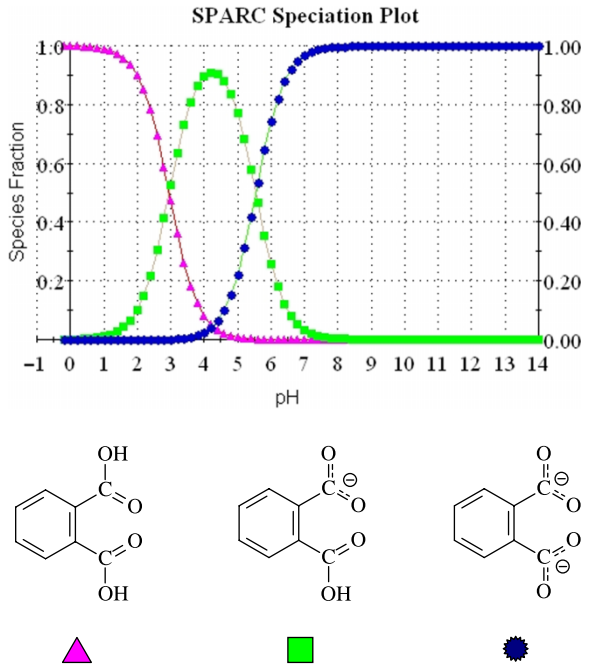

Figure 3. Fraction of each species of phthalic acid *versus* pH.

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interactions considered to be essential for the activity. These interactions are mainly a net of hydrogen bonds with Tyr314, His440, Tyr464 for $PPAR_{\alpha}$, and the corresponding His323, His449, Tyr473 for $PPAR_{\gamma}$. Only phthalic acid and MEHP can bind into $PPAR_{\alpha}$ and $PPAR_{\gamma}$ active sites. In $PPAR_{\alpha}$, the aromatic group of all 30 solutions for phthalic acid are superimposed, with two sets of, respectively, 20 and 10 conformers differing by the placement of the carboxylates. The less numerous family points both of them toward Tyr464, while the most numerous has one pointing toward this residue and the other pointing toward Phe273. Moreover, the scoring of the two sets gives a slightly better consensus in favour of the larger set. In this conformation, phthalic acid interacts into $PPAR_{\alpha}$ binding site via hydrogen bonding with Tyr314, His440 and Tyr464. It interacts also via hydrogen bonding with Ser280 and Gln277. Another hydrophobic interaction was shown between its aromatic group and the phenyl group of Phe273. Into $PPAR_{\gamma}$ binding site, one conformation only has been found. One of its two carboxylate group forms hydrogen bonds with His323, Tyr473, His449 and Ser289 while the other is not involved in hydrogen bond but can be engaged in an electrostatic interaction with Phe282 (Figure 4). It is noteworthy that Gln286 of PPARγ, that corresponds to Gln277 of PPARα, is oriented toward the outside of the binding site and is therefore unable to bind to phthalic acid as its counterpart does in PPARα. This slight difference results from the different confomation of this residue in the crystallographic data. However, it is not sterically constrained and would most surely form a hydrogen bond with the ligand if allowed to move during the docking. Into $PPAR_{\alpha}$ LBD, the carboxylate group of MEHP forms hydrogen bonds with Tyr314, Tyr464 and Ser280. Another hydrogen bond occurs between His440 and the ester carbonyl group. Its aromatic ring is involved in a hydrophobic interaction with Phe273 side chain. Interestingly, of the 30 solutions found for MEHP, all are superimposed, with the exception of 8 low ranked solutions positionned at the entrance of the pocket. Into $PPAR_{\gamma}$ binding site, the carboxylate group of MEHP interacts via hydrogen bonds with His323, His449, Tyr473 and Ser289 (Figure 5). Two distinct conformations are found for the aliphatic chain of the ester, occupying either the upper or the lower part of the Y-shaped pocket of the PPAR. However, although the two families are roughly as numerous and there is no difference in ranking, the phthalic head and its carboxylate group are exactly superimposed throughout the 30 conformations. As only hydrophobic interactions can be formed by the aliphatic chain, this capacity to occupy either part of the pocket makes sense. It is noteworthy that Phe273(282) of $PPAR_{\alpha(\gamma)}$ was recently reported to play an important role in binding affinity through solvent effect [24].

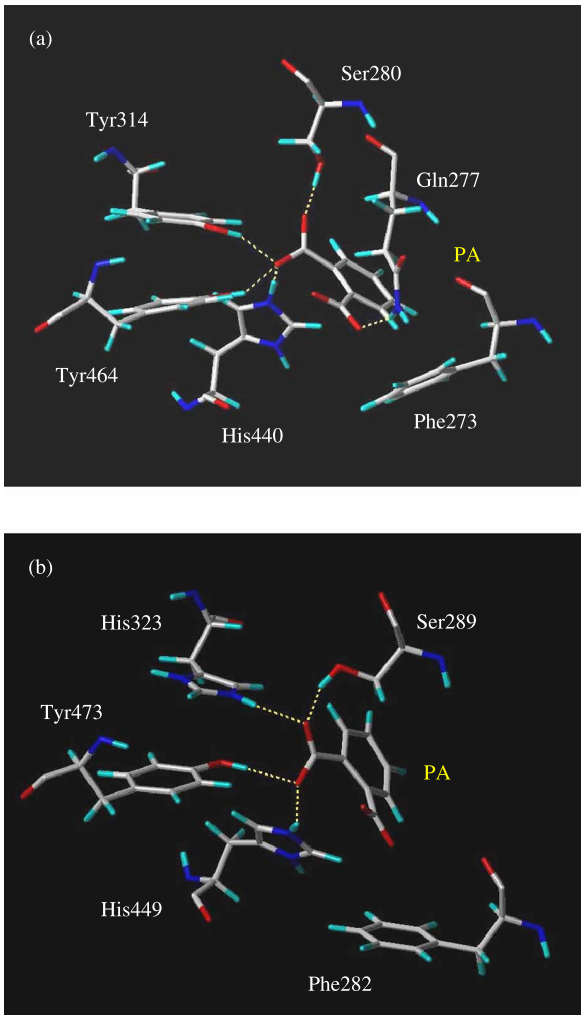

Figure 4. Docking of phthalic acid (PA) into $PPAR_{\alpha}$ (a) and $PPAR_{\gamma}$ (b) (hydrogen bonds are rendered as dashed yellow lines).

Comparing MEHP and phthalic acid docking into both PPAR subtypes, it is clear that the larger size of MEHP is far from being a disadvantage, as it increases the hydrophobic contact in the binding site and somewhat orient the free carboxylate toward direct interactions with the essential residues. It is fairly evident that phthalic acid and MEHP have a capacity to bind strongly to $PPAR_{\alpha}$ and $PPAR_{\gamma}$ and to activate them. On the other hand, more voluminous phthalic esters are not able to bind to either PPARs by the mean of hydrogen bonds. Without surprise, when both acids are esterised, there are only hydrophobic interactions left, even when the compound is placed in the binding site. This is the case for DEHP that is positionned in the pocket in two conformations, with the benzen ring at the middle of the Y- shaped binding site for both. One conformation is characterised by the occupation of the upper end part of the pocket and the Tyr464/473 access corridor, while the other is reminiscent of the placement of 2-BenzoylAminoBenzoic Acid (2-BABA), a partial agonist occupying only the part of the binding site at the opposite of

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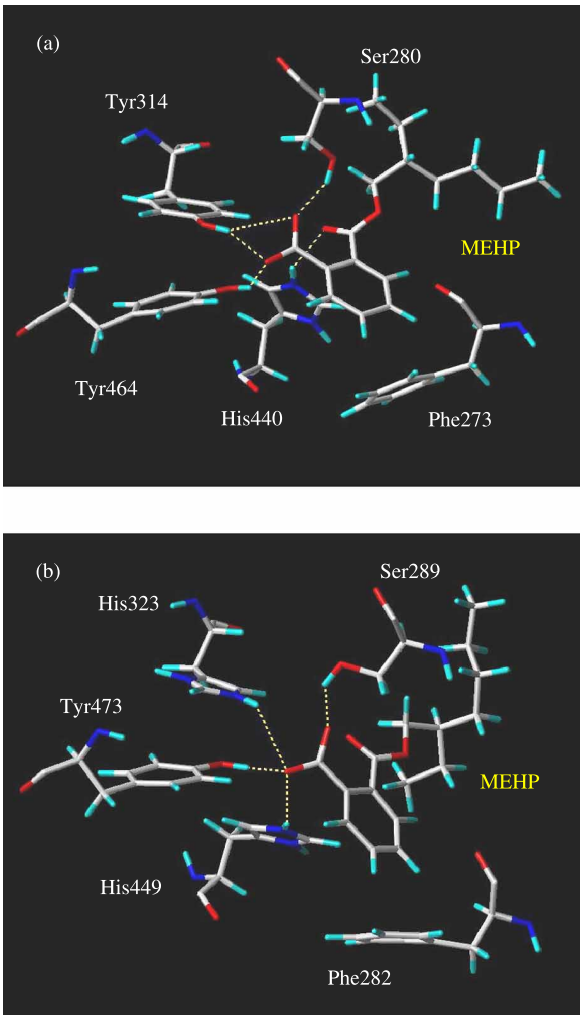

Figure 5. Docking of MEHP into $PPAR_{\alpha}$ (a) and $PPAR_{\gamma}$ (b) (hydrogen bonds are rendered as dashed yellow lines).

Tyr464/473 [25]. This result could hint to a less potent activation of PPARs by DEHP, when compared to its active metabolite MEHP. On the contrary, TOTM was not able at all to fit into the binding sites due to its vastly larger size. This could explain why it appeared to be devoided of PPARs dependent effects *in vitro*. Moreover, the large steric hindrance of its three esters should greatly reduce its *in vivo* degradation to an acid form of smaller size which could interact with PPARs, therefore greatly reducing its toxicity in respect of DEHP and its metabolite MEHP. Overall, the results of the docking study conduced on this limited set of compounds are in excellent agreement with the still sparse experimental data. If MEHP is well documented as a PPAR α and γ agonist [26], no such study has been published for TOTM up to now.

Conclusion

The docking study of phthalic acid, DEHP, its metabolite MEHP and a new plasticizer, TOTM, has been realised in order to assess the differences in PPAR α and γ binding modes between these compounds. In order to be as close as possible to biological conditions, the protonation state of the phthalic derivatives has been taken into account. Already known PPARs activators (phthalic acid itself and MEHP more prominently, DEHP to a lesser extent) have been found to bind to both PPAR subtypes. This binding can be described as fairly strong for phthalic acid and MEHP, and relatively weaker for DEHP, in correspondance with their placement in the binding site. TOTM was not able to fit in the binding site of either receptor due to its larger volume. This can shed a new light on earlier *in vitro* testing of its PPAR activation capacities. Taken together, the docking results are in excellent agreement with the biological data available and tend to further prove the interest of TOTM as a new plasticizer. Theoretical calculations therefore appear to be a significant tool in investigating the toxicity of plasticizers and could be employed to propose further improvments to innocuous compounds.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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