Chemical Reactivity as a Tool To Study Carcinogenicity: Reaction between Estradiol and Estrone 3,4-Quinones Ultimate Carcinogens and Guanine†

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Received November 28, 2003

In this article we study the chemical reactions between guanine and two ultimate carcinogens, the 3,4-quinone forms of the estrogens estrone (E_1) and estradiol (E_2). DNA was truncated to guanine, i.e. no deoxyribose moiety was included. Due to a complex reaction that involves proton transfer via water molecules we applied linear free energy relationships rather than computation of the transition state and activation energies. The minima corresponding to reactants and products were obtained on the B3LYP/6-31G(d) level. The effects of hydration were considered using the solvent reaction field of Tomasi and co-workers and the Langevin dipoles model of Florian and Warshel. No significant difference in reaction free energy for the reaction involving estrone and estradiol metabolites was found, despite the fact that for the two substances different carcinogenic activities were reported. Differences in carcinogenicity may be therefore attributed to other types of interactions or reactions such as (i) specific interactions of the carbonyl or hydroxyl group with DNA giving rise to different activation free energies for the reactions, (ii) the reaction of depurination and subsequent effects on the DNA, (iii) enzymatic or nonenzymatic oxidation steps (P450, aromatase, peroxidases, O_2) and detoxification reactions (catechol-*O*-methyl transferase, S-transferase), or (iv) binding of the hormone to its nuclear receptors.

1. INTRODUCTION

Carcinomas are associated with chemical modifications of nucleic acids. Damages can be produced by synthetic or natural chemicals such as (poly)aromatic hydrocarbons or aflatoxins,[1] free radicals, or reactive oxygen species which can be issued from photochemical reactions or enzyme activity. Oncogenes are altered versions of genes implied in cellular growth and division, which can be of cellular or viral origin. A significant proportion of carcinoma is believed to originate from environmental factors.[2] Carcinogenicity of polyaromatic compounds has been the subject of many experimental and computational studies.[3−9] Polyaromatic compounds are not carcinogenic per se and are called procarcinogens, whereas their metabolites are carcinogenic and are called ultimate carcinogens.

Nevertheless a certain class of procarcinogens is inherent to the human body. This includes steroid hormones that have a partial aromatic structure. Carcinogenesis associated with this class of compounds is called endogeneous, and indeed hormonal carcinogenesis is believed to be responsible for a number of cancers, such as ovary, uterus, mammary gland, and prostate. In particular, a serious controversy is at its height with regard to the inherent risks of hormone replacement therapy and estrogens' link to breast cancer (Women's Health Initiative,[10,11] Million Women Study[12]). As is the case for polyaromatic hydrocarbons, hormones themselves are not carcinogenic, but, aside from an effect which could result from binding to their nuclear receptors, they have to be activated to reactive metabolites to be cancer initiators. Indeed, endogenous estrogen (E) metabolites, through catechol estrogens (CE) formation, have been shown to exhibit genotoxic properties which can lead to carcinogenic DNA mutations.[13] They can be oxidized to two types of o-quinones (Q) which bind to DNA giving either stable adducts, in the case of E-2,3-Q, or depurinating adducts in the case of E-3,4-Q.[14] In the latter, these adducts, formed at N7 of guanine[15] or at N3 of adenine,[16] are lost from DNA by presumable cleavage of the glycosidic bond, leaving apurinic sites which are tumor-initiating in a number of human cancers. However, the 2-hydroxylation of estrogens pathway might not be to neglect.[17] Some xenobiotics, such as dioxin, aromatic hydrocarbons, or pesticides, influence the expression level of cytochromes P450. Indeed dioxin, as well as xenoestrogens, lead to the diminution of the expression of CYP1A1 and not CYP1B1, which could unbalance production of catechols in favor of 4OH-E, associated with a higher genotoxicity.[18]

The level of carcinogenicity of E-3,4-Q seems to be highly dependent on the species and type of tissue (e.g. human breast,[19] hamster kidney,[20] rat mammary gland, or prostate[21]). In $B6C3F_1$ mice liver for instance, E_1-3,4-Q (estron derived quinone) was very carcinogenic and toxic, whereas E_2-3,4-Q (estradiol derived quinone) was not, which is not understood yet.[14] In SENCAR mice skin, E_2-3,4-Q could be at the origin

† Dedicated to George W. A. Milne, a long-term editor of JCICS, our friend and colleague.

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10.1021/ci030424n CCC: $27.50
Published on Web 02/18/2004

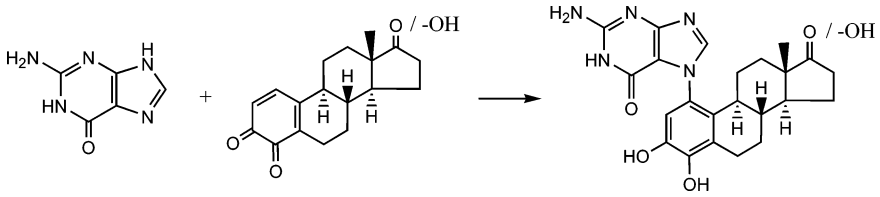

Figure 1. Reaction between guanine and E_1 or E_2-3,4-Q, following a Michael reaction mechanism.

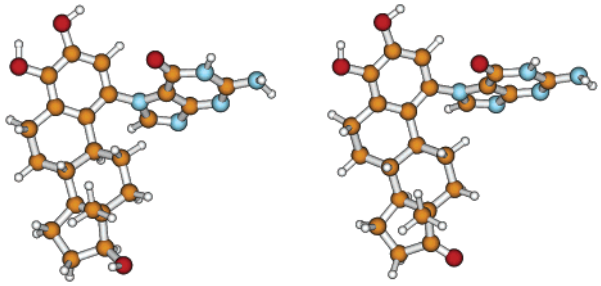

Figure 2. DFT optimized structures of 4-hydroxy-estradiol-1-N7 guanine (left) and 4-hydroxy-estrone-1-N7 guanine (right) adducts.

of oncogenic H-*ras* mutations due to DNA depurination by a predominant rapidly depurinating 4-hydroxy E_2-N3 adenine adduct.[22] In calf thymus with the inclusion of Cu(II) and NADPH, single strand breaks as well as aldehydic lesions were induced in the DNA for both E_2-3,4-Q and E_2-2,3-Q.[23]

Oxidation of estrogens to the quinone forms is catalyzed by cytochrome P450 and different peroxidases. P450 type is important for the specific enzyme activity, which may for instance favor the formation of 16α-hydroxylated estrogens, also chemically reactive and potentially mutagenic.[24] COMT (catechol-*O*-methyltransferase) plays a crucial role in lowering the potential for DNA damage, through methylation of catechol estrogens into inactive methoxyestrogens, which in return can exert feedback inhibition of P450.[25] The detoxifying S-transferase lowers the levels of CE-Q through formation of conjugates with glutathione.A common feature of ultimate carcinogens is their electrophilicity. As such they can easily attact DNA and in particular guanine at position N7 or adenine at position N3. To our best knowledge, the only computational study up to now dealing with carcinogenicity of estrogens was the contribution of Picazo and Salcedo,[26] who addressed the difference in carcinogenicity of the two procarcinogens estrone and estradiol using DFT calculations, with no DNA target included. They concluded that the difference in carcinogenicity can be attributed to the difference in electrostatic potential and to the fact that estrone has more aromatic character than estradiol.

In this work we addressed by using DFT calculations the chemical reaction between either estrone or estradiol 3,4-quinone ultimate carcinogens and guanine, taken as a model for DNA, forming the 4-hydroxy(E_1 or E_2)-1-N7 guanine adducts. The subsequent reaction involving depurination was not considered. The geometries of the reactants and products were optimized in vacuo first at the semiempirical PM3 level and refined at DFT B3LYP level. Hydration free energies were calculated using either the PCM solvent reaction field method of Tomasi and co-workers[27] or the Langevin dipoles method of Florian and Warshel.[28,29] Activation free energy for each reaction was estimated using the linear free energy relation.

2. COMPUTATIONAL METHODS

2.1. In Vacuo Calculations. In vacuo calculations were performed on a semiempirical MO level PM3 and a Density Functional Theory (DFT) level B3LYP. Both methods proved to be efficient for describing chemical processes in systems of biological interest. DFT calculations were performed using the basis set 6-31G(d). The double-zeta basis set augmented with polarization functions on the heavy atoms is flexible enough to faithfully describe chemical processes while being still computationally tractable. Since the systems studied are relatively large, the applied DFT level is a good compromise between quality of the results and CPU effort. Initial structures were obtained by model building using the program package Molden.[30]

The structures corresponding to estradiol in the 3,4-quinone form, estron in the 3,4-quinone form, guanine, and products of both ultimate carcinogens with guanine were built and the geometries were optimized on the PM3 level, followed by geometry optimization on the DFT level. Thus geometry optimizations were applied to all reactants and products. Vibrational analysis was performed in the harmonic approximation to prove that the minima are real minima rather than saddle points. In addition we also calculated the zero point energy corrections in the harmonic approximation.

2.2. Hydration Free Energies. To calculate free energies of hydration for the studied species we applied two methods. The first is the PCM solvent reaction field method of Tomasi and co-workers applying a realistic cavity shape. The solute cavity is composed of interlocking spheres. For a review see ref 27. The applied PCM method is closely related to the solvation model developed by Baldridge and co-workers.[31] The Langevin dipoles method calculates the free energy of hydration as the reversible work necessary for embedding the solute described by a set of point charges to the grid of the Langevin dipoles, together with a proper parametrization. By displacing the solute (50 times in our calculations), thermal averaging is performed and the main lack of the solvent reaction field is in this way overcome. DFT and semiempirical MO calculations were run with a Gaussian-03[32] suite of programs. Langevin dipole calculations were performed using CHEMSOL versions 1.1 and 2.1 packages kindly provided by Jan Florián.[33,34] We followed the authors' recommendation to use Merz−Kollman charges calculated at the HF/6-31G(d) level for CHEMSOL 1.1, while for version 2.1 charges were calculated at the B3LYP/6-31G(d) level with an included solvent reaction field. The HF/6-31G-(d) wave function exaggerates with predicted dipole moments, what corresponds to the situation of polarized wave function in solution. All calculations were performed on a cluster of dual-CPU PC/Linux processors (AMD Athlon XP 1600+ model; 512 MB RAM each).

2.3. Linear Free Energy Relation. The studied reactions are electrophilic substitutions and are associated with a

Table 1. Free Energy and Free Energy Components for Reactions between Estron and Estradiol in Their 3,4-Quinone Form (E_1-3,4-Q and E_2-3,4-Q, Respectively, i.e. Ultimate Carcinogens) and Guanine[g]

	ΔE[a]	ΔZPE[b]	ΔG_{hydr}[c]	ΔG_{hydr}[d]	ΔG_{hydr}[e]	ΔG_{react}[f]
E_1-3,4-Q	−19.19	2.06	10.96	10.97	15.64	−1.49
E_2-3,4-Q	−18.96	2.17	11.40	10.93	15.17	−1.62

[a] B3LYP/6-31G(d) calculated gas-phase energies. [b] B3LYP/6-31G(d) calculated zero point energy (ZPE) corrections. The ZPE was calculated as ZPE(product) − ZPE(reactants). [c] Free energy of hydration differences was obtained using Langevin dipoles (LD) method with ChemSol 1.1 parametrization. Merz−Kollman charges were calculated using HF/6-31G(d) wave function (gas phase) applied to the B3LYP/6-31G(d) optimized geometry. [d] Free energy of hydration differences was obtained using PCM solvent reaction field of Tomasi in conjunction with HF/6-31G(d) wave function. [e] LD free energy of hydration differences using ChemSol 2.1 parametrization, where Merz−Kollman charges were calculated at B3LYP/6-31G(d) level using Tomasi's PCM SCRF. [f] Reaction free energy $\Delta G_{react} = \Delta E + \Delta ZPE + \Delta G_{hydr}$[e]. We feel that the LD method with ChemSol 2.1 parametrization is the most reliable. [g] (Free) energy of reaction was calculated as (free) energy of the product (adduct with guanine) minus (free) energy of reactants. All (free) energies are in kcal/mol.

complex mechanism involving proton transfer via several solvent molecules. Location of a transition state and calculation of activation free energy for such a complex reaction is not practical. In the present case we are dealing with two closely related reactants since the two estrogen ultimate carcinogens only differ in a carbonyl or hydroxyl group being at a large topological distance from the reactive carbon atom. The linear free energy relation seems to be the method of choice to estimate the activation free energy. The method is empirical and states that in a series of chemical reactions involving similar reactants and having the same mechanism, the reaction with the most favorable reaction free energy will have the lowest free energy of activation. The rationale behind this is that if one approximates reactant and product free energy hypersurface wells with parabolas, they are expected to have about the same curvatures since we are dealing with similar species. Clearly, the point of their intersection will be lower if the product parabola is lower, giving rise to lower activation free energy for the reaction. Application of linear free energy relationships in enzyme catalysis is well established and is described in ref 35.

3. RESULTS AND DISCUSSION

The free energies for both reactions as well as their components are collected in Table 1. It is clear that there is no significant difference between reaction free energies for the reactions of both carcinogens with guanine. Neither in vacuo values of energies differ from each other nor the contributions from hydration free energies within each method of calculation. Interestingly, we noticed that use of the semiempirical method PM3 yielded the same in vacuo results, providing reaction enthalpies of −17.36 and −17.13 kcal/mol for E_1-3,4-Q and E_2-3,4-Q, respectively. This gives additional proof that the in vacuo contribution to the reaction free energy is basically identical for both reactions. We believe that the applied DFT level is reliable, and we checked the obtained stationary points to be minima rather than saddle points by performing vibrational analysis in the harmonic approximation for all the species. The calculated zero point contributions to reaction free energies for both reactions are basically identical.

Solvation free energies were modeled on three levels. All three methods predict no substantial difference between hydration free energy contributions for the reactions. Our calculations give strong evidence that in the guanine alkylation step there is no difference in E_1 and E_2 quinones reactivity. Linear free energy relation is an established and widely used method in physical organic chemistry, and we see no reason it would not work in our case. We believe that inclusion of an explicit or even a chemically reactive solvent, for example on Car-Parrinello level, while keeping truncation of DNA to guanine would not change the results. We can conclude that both chemical reactions leading to guanine alkylation have not significantly different free energies of reaction and that the corresponding rate constants are basically the same.

How can then observed possible differences in carcinogenicity of both estrogens be addressed? We offer more possible answers. One reason could be that DNA modeled by guanine is truncated too much, and specific interactions between DNA and the carbonyl or hydroxyl group in E_1 or E_2-3,4-Q, respectively, might affect the chemical reactivity. From the computational point of view this limitation could be overcome by extending the system and/or using QM/MM methods that are developed and ready to be used. Another possible explanation is linked to the fact that E_1 and E_2 undergo other metabolic transformations at different rates. In particular reactions catalyzed by enzymes such as catechol-*O*-methyl transferase (COMT), glutathione-S-transferase, P450 (CYP families), aromatase, or peroxidases play a key role uphill from the reaction we considered, and the synthesis of the estrogens genotoxic metabolites depends on the expression and activity levels of these enzymes. For instance, evidence is given that the genotype of COMT is linked to breast carcinogenesis.[36] (See also ref 37 for a review of genetic polymorphisms and breast cancer risk.) Endogenous estrogens themselves are able to modify the activity of the enzymes producing their metabolites. In fact, the most frequently evoked mechanism in the development of some cancers due to estrogens prolonged exposure is the stimulation of cellular growth by chronic activation of estrogens receptors. Thus, the biochemistry of these receptors is important to consider for a difference in E_1 and E_2 carcinogenic effects. In addition to all these possibilities, one has to remember that not only DNA but proteins are also targets for reactions with quinones,[38] as well as cellular lipids and some metallic ions (iron and copper).[39] Finally, we would like to draw attention to another candidate reaction where the reaction rates may differ, i.e. depurination of the adducts through cleavage of the chemical bond between deoxyribose and the purine base.

All in all, steroid hormone induced carcinogenesis is associated with an extremely complex set of biochemical transformations. The fact that 31 different metabolites of estrogens were identified in the mammary gland carcinoma tissue[40] tells enough about the complexity of the reactions. We believe that those processes must be better understood at the molecular level and more particularly under the physicochemical point of view. The methods for modeling chemical reactions in solution are developed and ready to be used. We are sure that molecular modeling of chemical reactivity will play an important role in cancer research and

will finally contribute to improve the prevention and the treatment of cancer.

ACKNOWLEDGMENT

P.H. is grateful to the Ligue du Doubs contre le Cancer of France for financial support of this work. J.M. gratefully acknowledges the Ministry of Science and Technology of the Republic of Slovenia for financial support. J.M. would like to thank University of Sains Malaysia for hospitality during his stay in Penang where this work was initiated and the Laboratoire de Physique Moléculaire of the University of Franche-Comté in Besançon (France) for visiting professorship.

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CI030424N