Photoabsorption spectra of a natural polyphenol compound for therapeutic applications: the protocatechuic acid in dilute water solution at room temperature†

Costantino Zazza* and Nico Sanna

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The UV-Vis absorption spectra of the protocatechuic acid, a potential new drug for the treatment of cancerous tumors and central nervous diseases, is for the first time fully reproduced in water solution at room temperature; these results open up the routes to integrated experimental/simulated studies in order to identify characteristic spectroscopic features of such a molecule and its derivatives within different hosting receptors in nature.

1. Introduction

Polyphenols constitute an intriguing source of natural compounds showing potentially relevant therapeutic applications;[1] in particular, it was recently found that they suppress cancer cell growth in human hepatocellular carcinoma.[2] This basic feature of polyphenols might explain the reduced risk of many cancers in the presence of a diet that includes regular consumption of fruits and vegetables which are rich in these natural antioxidants.[3] However, their ability to affect cancer cell growth is not only related with strong antioxidative properties, but, most importantly, with their effective interaction with basic cellular mechanisms. In this context, in a short communication Borbulevych *et al.*[4] have clearly revealed, by X-ray analysis, the formation of a complex between protocatechuic acid (3,4-dihydroxybenzoic acid, PCA, see Fig. 1) and lipoxygenase-3 (LOX-3)[5] metabolic enzyme. As the same authors report, this findings might be of fundamental importance in cancer theraphy; this is because such a chemical interaction offers us the opportunity to efficiently control the presence of free LOX enzyme which is known to have a key role in cancer progression.[4] Moreover, several studies have reported extensively that PCA may be a suitable candidate for the treatment of Parkinson's disease due to its well-known neuroprotective properties.[6] Also very recently, PCA isolated from the kernels of *Alpina oxyphylla* is found to promote neural stem cells (NSCs) proliferation preventing their apoptotic cell death;[7] this latest experimental evidence can pave the way to new frontiers in the clinical treatment of central nervous system diseases acting on cell proliferation and *anti*-apoptotic effects induced by PCA. As a conclusive remark, it is worth noting that much attention should be paid to administration dose and timing-dependent effects in clinical applications of phenolic antioxidants.[8]

CASPUR, Supercomputing Centre for University and Research, via dei Tizii 6/b, 00185 Rome, Italy. E-mail: costantino.zazza@caspur.it; Fax: +39 06 4957083; Tel: +39 06 44486720

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However, at present, the real cellular and mechanistic routes behind the observed action of protocatechuic acid in different systems are not yet completely understood. This is, unequivocally, due to the intrinsic complexity of the biological targets involved. In fact, to the best of our knowledge, the only integrated experimental and (static) computational studies concern the metal complexation of PCA with aluminium.[9,10]

Thus, as first step, a full characterization of the electronic properties of PCA in physiological conditions (*i.e.* dilute water solution, 298 K) is mandatory. This is the reason why we have decided, starting from a previous theoretical and experimental work,[9] to computationally evaluate the photoabsortion spectra of protocatechuic acid in water solution at room temperature. For this purpose, we combine time-dependent density functional theory (TD-DFT)[11] calculations with 10 ns of classical molecular dynamics (MD) simulations carried out on the PCA solute in explicit water solution. TD-DFT electronic degrees of freedom of PCA are coupled, in the framework of the perturbed matrix method (PMM) procedure,[14] with the classical sampling, in equilibrium conditions at 298 K, of the surrounding environment. The currently applied methodology allows us to obtain perturbed electronic wavefunctions (and related property) of a chromophore, *e.g.* PCA, during a perturbing field trajectory which changes according to a standard MD propagator. Finally, solvation effects at the solute/solvent interface on the valence excitation energies of PCA are also evaluated by means of the conductor-like polarizable continuum method (CPCM)[15] including explicit solvent water molecules; in this context, MD simulations were essential for establishing starting geometries for CPCM-based calculations.

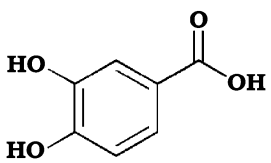

Fig. 1 Molecular structure of the protocatechuic (PCA) acid; here, we report the fully protonated stable form at low pH conditions in water dilute solution.

2. Theoretical and computational details

2.1 Classical molecular dynamics simulations of PCA compound in water

We have performed two MD simulations of 10 ns for the protocatechuic acid (the first one considering the optimized structure of the solute at B3LYP/CPCM/6-31G(d,p) level and the second one at VSXC/CPCM/6-31G(d,p)) at 298 K in NVT ensemble in water solution. Both simulations were initiated with the PCA molecule at the centre of a rectangular box of 21 nm^3 filled with SPC water molecules[16] at its typical liquid density of 997.1 kg m^{-3}. Furthermore, for PCA solute we used the standard Gromos96 force field.[17] In this context, the atomic point charges over PCA were recalculated, taking into account the optimized geometry (at B3LYP/CPCM/6-31G(d,p) and VSXC/CPCM/6-31G(d,p) level) in conductor-like polarizable continuum model (CPCM)[15] in conjunction with B3LYP,[12] VXSC[13] density functionals and 6-311++G(d,p) basis set by adopting a Merz-Kollman (MK) scheme and ESP fitting procedure.[18] In this respect, before doing any classical/statistical sampling in dilute solution, CPCM represents a reasonable and well-known computational tool for evaluating the polarization of the surrounding environment on a solute molecule in its own electronic ground state.[19] Further, as far as the relative position of the OH moieties in the solute is concerned, we selected the most stable rotamer as suggested in ref. 9. Note that a relatively high rotation barrier value of almost 10 kcal mol^{-1} around cycle-C bond was predicted in that case.

The investigated system (*i.e.* PCA solute in dilute water solution) was gradually heated from 50 to 298 K using short (50 ps) MD simulations upon solvent relaxation. Afterwards, the MD trajectories were propagated for 10 ns using an integration step of 2.0 fs and the temperature was kept constant by the isokinetic temperature coupling.[20] In this respect, having stored a molecular frame every 0.5 ps, we have therefore applied PMM computational procedure[14] (see next subsection) over 20.000 MD conformations in equilibrium conditions. Long range electrostatics was computed by the particle mesh Ewald (PME) method,[21] with 34 wave vectors in each dimension and a 4th order cubic interpolation. A cut-off of 1.1 nm was used and the pair list was updated every 5 integration steps. All simulations were carried out using the Gromacs software.[22]

2.2 Mixed TD-DFT/MD calculations

In order to address the electronic properties of the protocatechuic acid (PCA) in dilute water solution we combine time-dependent density functional theory (TD-DFT) calculations with classical MD sampling in physiological conditions (see subsection 2.1). We then selected PCA molecule as quantum centre (QC), with the surrounding SPC solvent molecules acting as an electrostatic perturbation according to the standard PMM procedure.[14] More precisely, by using PMM computational procedure, we model the solvent perturbation as an external and homogeneous electric field changing according to the (classical) mechanical behavior of the surrounding media and acting on the solute (that is the quantum centre, QC) centre of mass. Moreover, for a comparison with static calculations reported in literature by E. Andrè *et al.*,[9] we accomplished PMM simulations using exactly the same unperturbed description, *e.g.* B3LYP/6-31G(d,p) and VSXC/6-31G(d,p). Indeed, the molecular structure of the QC was optimized at B3LYP/6-31G(d,p) and VXSC/6-31G(d,p) level of theory in CPCM model,[15] and an unperturbed Hamiltonian matrix of dimension [13 × 13], was constructed using time dependent density functional theory (TD-DFT) calculations in gas-phase conditions (*i.e.* TD-B3LYP/6-31G(d,p) and TD-VSXC/6-31G(d,p)). It is worth noting that the unperturbed permanent electric dipole moments of the electronic eigenstates considered in our PMM simulation (the ground and the first twelve electronic excited states), as well as the transition moments above them were also calculated. In so doing, by applying PMM we can address, at a relatively low computational cost, the relationship between a conformationally flexible environment, like the present one, and the excitation wavelengths of the PCA molecule during a nanosecond time-scale molecular dynamics simulation in water. Quantum chemical calculations within time-dependent formalism were performed using both linear response theory as currently implemented in the Dalton 2.0 code[23] and random-phase approximation in Gaussian03 package.[24] Finally, the semi-classical theoretical approach was also extended performing PMM simulations at TD-B3LYP/6-311++G(d,p) level of theory and compared with PMM/TD-B3LYP/6-31G(d,p) and CPCM-based calculations also in the presence of explicit water molecules surrounding PCA chemical group. In this respect, CPCM calculations including up to five solvent molecules interacting with the solute were accomplished, using as starting coordinates the most sampled local conformations (at the solute–solvent interface) spanned by means of classical MD sampling at 298 K.

3. Results and discussion

The UV-Vis absorption spectra of PCA system in aqueous solution, calculated at PMM/TD-DFT/MD (10 ns, 20.000 conformations) level of theory and arising from $^1\pi \rightarrow \pi^*$ electronic excitations,[9] have been reported in Fig. 2 while our numerical results are summarized in Table 1, where experimental assignments in acid conditions are also reported.[9] Looking at our data, it is interesting to note that at PMM/B3LYP/6-31G(d,p) level: (i) the lowest electronic excitation having a λ_{max} = 293 nm, is fully reproduced. (ii) The second PMM/B3LYP/6-31G(d,p) transition, with a maximum at 262 nm, is slightly overestimated with respect to experiments (λ_{max} = 257 nm); (iii) the same trend has been observed for the third signal, a shoulder experimentally detected at almost λ_{max} = 216 nm, which shows, within our simulation conditions, a maximum at 222 nm, see Table 1. On the other hand, CPCM model at the same unperturbed level, *i.e.* B3LYP/6-31G(d,p), is found to: (i) underestimate the first lowest singlet peak; (ii) the second excitation agrees quite well with experimental findings having a maximum at lightly shorter wavelength, 251 nm; (iii) the shoulder at 216 nm is accurately reproduced. However, changing the functional as suggested by a previous investigation, PMM/VSXC/6-31G(d,p)† data

provides a theoretical picture worse than B3LYP one; in fact, all the lowest lying electronic transitions were found systematically at longer excitation wavelengths (see Table 1). The same trend, even though to a lesser extent, was observed at CPCM/VSXC/6-31G(d,p) level of computation. In turn, CPCM and IEF-PCM reports similar results.[9] Finally, as a conclusive remark, it should be noted that both PMM and CPCM results have not been able to correctly reproduce the different intensity between the first two electronic excitations which are predicted as having a comparable oscillator strength (see Table 1 and solid line in Fig. 2). In order to further refine the theoretical modelling we extended our level of computation including a triple-zeta atomic basis set with diffuse functions, *e.g.* 6-311++G(d,p). The emerged spectra (dashed line depicted in Fig. 2) agree well with the experimental assignments;[9] as a matter of fact, as can be seen in Table 1, the first two computed absorption bands fall at 293 and 259 nm, respectively. As expected, also the CPCM model is found to increase its performance predicting a first maximum at 288 nm and a second at 258 nm. However, it is worth to note that only with the inclusion of the atomistic fluctuations (driven by thermal effects) the intensity pattern was successfully reproduced by our PMM simulations (see Fig. 2). The observed change in the intensity, when passing to 6-311++G(d,p) basis set, is strongly connected with an increase in density of the perturbed electronic excited states, in the range 250–270 nm, which reinforces the absorption band mainly arising from the strongest $^1\pi\rightarrow\pi^*$ signal in such an interval.[9] The only discrepancy still regards the theoretical prediction of the third signal, the shoulder with a maximum at about 216 nm, which is found lying at slightly longer excitation wavelengths (225 nm using PMM procedure and 224 nm with CPCM model, respectively). At last, we would like to clarify that for a quantitative comparison between experiments[9] and computations, in Fig. 2 the statistical distributions of the lowest $^1\pi\rightarrow\pi^*$ excitation wavelengths have been weighted according to the corresponding Einstein coefficients (B_{ij}) combined with the probability densities [$\rho(\lambda)$] of excitation in the sampled wavelength (λ) space. $\rho(\lambda)$ are approximated by the occurrence frequencies to find the chromophore within a given excitation energy interval divided by the corresponding λ interval itself.[14]

As a final step, since the CPCM model is largely used in literature with explicit water molecules in interaction with the solute,[25] we extracted from our MD simulation the structure of the first hydration shell around PCA which is shown in Fig. 3. In so doing, inspired by MD sampling, we identify three regions (termed as #1, #2 and #3 in Fig. 3) around PCA chemical group where water molecules were explicitly added to CPCM-based calculations. Subsequently, a geometry optimization was carried out providing a local minimum in which the water molecules are found basically confined within the first shell sampled by MD simulation (see Fig. 3 and Fig. 4). In turn, the cluster composed by PCA and $3H_2O$ in CPCM model, at B3LYP/6-311++G(d,p) level of theory, provides vertical excitation wavelengths in line with those previously predicted by means of PMM (see the row labelled CPCM($3H_2O$)/B2 in Table 1); notwithstanding, it should be also noted that although the introduction of water molecules in CPCM cavity affects the excitation energies (in particular

Table 1 Excitation wavelengths (in nm) and related oscillator strengths (dimensionless) calculated using PMM procedure and CPCM calculations for the lowest valence electronic transitions of PCA molecule in water solution†. Note that experimental assignments at low pH are also shown[9]

Method	First band	Second band	Third band
PMM/B1	293(0.17)	262(0.15)	222(0.42)
CPCM/B1	279(0.18)	251(0.15)	216(0.32)
PMM/B2	293(0.12)	259(0.23)	225(0.46)
CPCM/B2	288(0.19)	258(0.16)	224(0.32)
CPCM($3H_2O$)/B2	292(0.20)	260(0.16)	226(0.33)
CPCM($5H_2O$)/B2	293(0.18)	258(0.12)	228(0.28)
PMM/V1	319(0.14)	273(0.16)	234(0.46)
CPCM/V1	300(0.16)	262(0.12)	228(0.30)
CPCM/V2	307(0.18)	269(0.14)	236(0.28)
CPCM($3H_2O$)/V2	315(0.19)	272(0.14)	237(0.14)
IEF-PCM/V1[9]	296(0.15)	261(0.11)	227(0.29)
Experimental data[9]	293(0.10)	257(0.16)	216(0.33)

Abbreviation: B1 stands for B3LYP/6-31G(d,p), B2 for B3LYP/6-311++G(d,p), V1 for VSXC/6-31G(d,p) and V2 for VSXC/6-311++G(d,p).

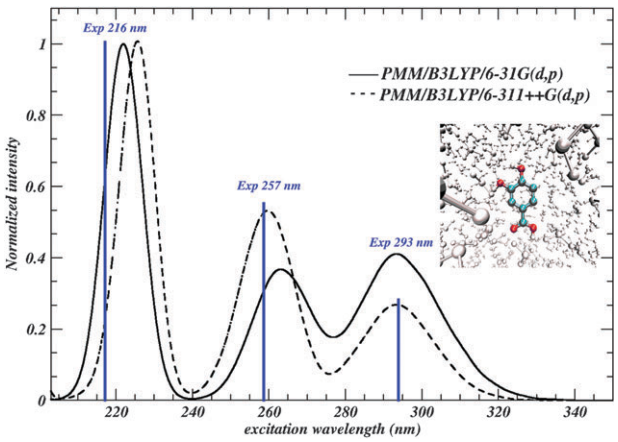

Fig. 2 Calculated, at PMM/B3LYP/6-31G(d,p) [solid line] and PMM/B3LYP/6-311++G(d,p) [dashed line] level of computation from 10 ns (20.000 configurations, 685 SPC[16] water molecules) MD classical simulation in water dilute solution in equilibrium conditions at 298 K, UV-Vis spectrum of PCA molecular system in the range between 200 and 350 nm. Note that the experimental data [blue vertical lines] and a pictorial view of the simulation box are also shown.

the position of the first absorption is red shifted toward the experimental value by 4 nm, as compared to the standard CPCM result carried out without explicit solvent molecules, see Table 1), the oscillator strengths remain basically unchanged. At last, the UV-Vis absorption spectra, as obtained by applying the VSXC functional in conjunction with 6-311++G(d,p) basis set and CPCM model, did not provide a satisfactory result. In fact, if compared with experiments, CPCM/VSXC/6-311++G(d,p) absorptions are strongly underestimated in energy. The same picture is observed taking into account the cluster model, *i.e.* PCA:$3H_2O$ (see Table 1). Therefore, at least at the level of theory applied, our results suggest that, contrary to previous observations,[9] the B3LYP functional performs fairly well on such a system in water, in particular when solvation effects are explicitly included in the

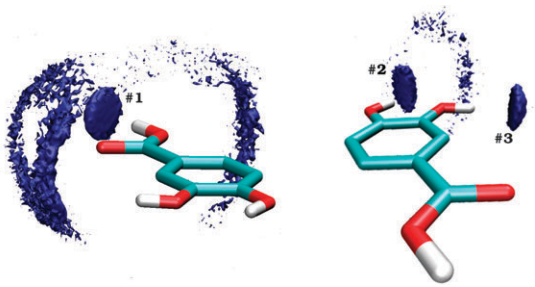

Fig. 3 Spatial distribution function (in blue color) of the water molecule oxygen atoms around the carboxylic (right panel) and catechol (left panel) moieties of the PCA molecule in solution as extracted from MD sampling at 298 K. The labels #1, #2 and #3 indicate the closest wet regions, at the solvent/solute interface, to PCA chemical group.

model system; as a consequence, the emerged necessity of changing Becke's parameters should be most likely ascribed to the incompleteness of the previous model[9] rather than to the intrinsic quality of B3LYP hybrid functional.[26] In this context, at the highest level of theory, that is CPCM($5H_2O$)/B3LYP/6-311++G(d,p) and characterized by two additional explicit water molecules interacting with the lone pair of the carboxylic oxygen atom of PCA group (see ESI†), the computed excitation wavelengths (293, 258 and 228 nm) being consistent with PMM estimation (293, 259 and 225 nm) at the same QM level of theory unequivocally confirms the reliability of the overall computational setup applied in this work.

As a final step we also checked the artificial solute dipole moment given by the explicit treatment of solute–solvent intermolecular interactions in our hydrated cluster models. In this context, ranging from the drug interacting with a continuum media to the largest complex (*i.e.* PCA:5H2O† in C-PCM) we observe a change in the permanent electric dipole moment of the drug molecule which remains confined within 0.23 Debye at B3LYP/6-311++G(d,p) level of theory (from 1.45 to 1.68 Debye). Furthermore, before concluding our work we would like to underline that, in the current investigation, we are disregarding (non-equilibrium/inertial) solvation dynamics effects following the lowest electronic transitions

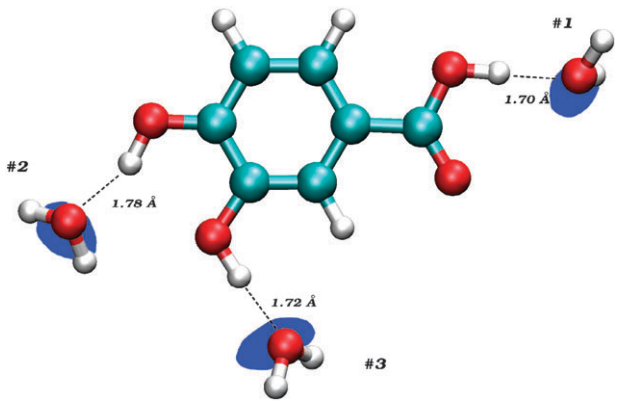

Fig. 4 Optimized geometry, at CPCM/B3LYP/6-311++G(d,p) level of computation, of the 1:3 complex of the PCA compound with explicit water molecules; the projection of the #1, #2 and #3 regions onto the plane defined by PCA molecule is also reported (blue areas).

of PCA solute; therefore, our results have to be considered as purely "vertical" with the solvation patterns at the interface in equilibrium at 298 K with the electronic ground state of the solute molecule.[27]

Conclusions

Summarizing, the UV-Vis spectrum of protocatechuic acid has been, for the first time, theoretically addressed in water solution at 298 K. To this end, PMM and CPCM calculations (the latter only in presence of explicit water molecules at the solute/solvent interface) show the accuracy of combining density functional theory and nanoseconds time-scale molecular dynamics simulations in reproducing the basic UV-Vis spectroscopic features of PCA in physiological conditions. Furthermore, the results obtained clearly reveal that accurate unperturbed electronic degrees of freedom and thermal effects are both essential in reducing the gap between simulations and experiments. In conclusion, this contribution represents a starting point for future experimental/theoretical investigations concerning the electronic properties (and related spectroscopic responses) of such a natural drug in configurationally complex hosting receptors which are supposed to be the biological targets for clinically relevant therapeutic applications. Having such an issue in mind, the biological complex between PCA and LOX-3 enzyme discovered by Borbulevych *et al.*[4] could represent a fascinating investigation system. As a matter of fact, the experimental measurement of the UV-Vis spectrum of PCA in LOX-3 coupled with the application of an integrated TD-DFT/MD approach might allow to identify characteristic (and diagnostic) spectroscopic signatures of such a natural drug *in silico*.

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