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Solvent Effects on the Tautomerism of Apigeninidin

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Abstract: Solvent effects are found to be responsible for the predominance in water solution of a highly unstable tautomer of apigeninidin *in vacuo*. We present free energy perturbation in molecular dynamics simulations and self-consistent reaction field calculations of the relative solvation of the anionic tautomers of apigeninidin.

Anthocyanidins (derivatives of 2-phenylbenzopyrilium salts) are compounds widely spread in nature. They provide most of the pink, orange, red, violet and blue colours of flowers.[1] Moreover, they are known to exert pharmacological activities toward several enzymes.[2]

Apigeninidin (5,7-dihydroxy-2-(4'-hydroxyphenyl)benzopyrilium chloride) is one of the most common anthocyanidins. Owing to the presence of three acidic hydroxyls, it gives rise in solution to a variety of dissociated neutral and anionic tautomeric forms which are very difficult to characterize. These tautomers are likely to play different roles on both fronts of flower colouring[1] and pharmacological activity;[2,3] therefore, the knowledge of the tautomeric composition of apigeninidin in solution is important and timely for the understanding of the above-mentioned phenomena.

Preliminary answers to this question were obtained from the calculation of the relative stabilities of the tautomeric forms generated by anthocyanidins by means of molecular orbital (AM1) calculations.[4] Those calculations also provided the first description of the electronic structures of these tautomers appeared in the literature. Later on, we experimentally determined the tautomeric composition of apigeninidin in solution from UV-VIS spectroscopy of selectively methoxylated analogs.[5] While qualitative agreement between theory and experiment was generally obtained, prediction failed for a particular anionic tautomer of apigeninidin. The anionic tautomer A5$7^-$ (Figure), predicted to be 16 Kcal/mol less stable than A5$4^-$ and 14 Kcal/mol less stable than A7$4^-$ by AM1 *in vacuo*,[4] turned out to be the most abundant tautomer of apigeninidin in water solution.[5] Solvation model calculations on these tautomers based on a semiempirical formulation of the virtual charge model[4,6] predicted the A5$7^-$ tautomer to be better solvated than the other tautomers (-0.97 Kcal/mol with respect to A5$4^-$), but not sufficiently to compensate for the 16 Kcal/mol energy difference of this form *in vacuo* (Table).

On the other hand, the marked preference for A5$7^-$ in solution is likely attributable to solvent, because this tautomer has a considerably higher dipole moment compared to A5$4^-$ and A7$4^-$ (Table).

First of all, we tested the reliability of the AM1 hamiltonian in predicting the relative stabilities of these tautomers *in vacuo* by extending calculations to *ab-initio* wavefunctions. RHF calculations with STO-3G, 3-21G and 3-21G** basis sets[7] give relative stabilities that are closely comparable to AM1 (Table); these findings led us

to re-examine solvent effects on these anionic tautomers making use of two distinct models, one based on an explicit representation of water molecules, the other based on a dielectric continuum representation.

Figure: Structures of the three anionic tautomers of apigeninidin.

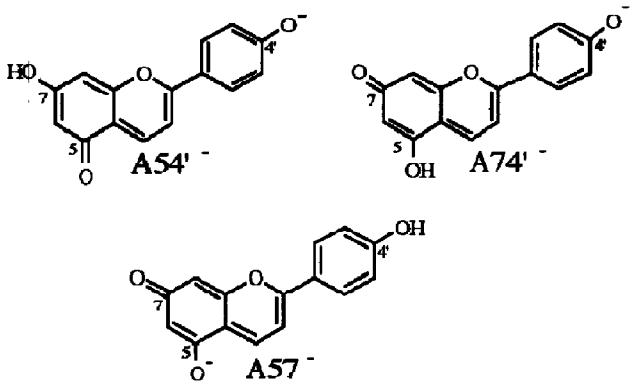

Table: Experimental and theoretical data of the three anionic tautomers of apigeninidin.

	%(a)	ΔE AM1(b)	ΔE_{sol} VCM(c)	μ(d)	STO-3G	ΔE(e) 3-21G	3-21G**	ΔG_{sol} FEP(f)	ΔE_{sol} SCRF(g)
A54'-	4.2	0	0	2.8	0	0	0	0	0
A74'-	11.9	2.04	0.73	7.1	0.82	1.75	2.00	-0.2±0.7	-2.2
A57-	83.9	16.24	-0.97	19.3	19.37	13.85	13.90	-21.1±1.2	-28.9

(a) experimental abundance percentages of the anionic tautomers (ref. [5]); (b) relative stabilities (Kcal/mol) from AM1 hamiltonian (ref. [4]); (c) relative solvation energies (Kcal/mol) from virtual charge model (VCM) calculations (ref. [4]); (d) dipole moments from AM1 calcs. (Debyes); (e) relative stabilities (Kcal/mol) from *ab-initio* basis sets; geometries were completely optimized in STO-3G basis; 3-21G and 3-21G** are single-point calculations with STO-3G geometries; (f) solvation free energy differences (Kcal/mol) from free energy perturbation (FEP) calculations; error bars are the difference between forward and reverse perturbations. (g) relative solvation energies (Kcal/mol) from self-consistent reaction field (SCRF) calculations.

In the first model, the free energy perturbation method[8] in molecular dynamics simulations has been used to estimate the changes in free energy of solvation between the three anionic tautomers of apigeninidin. At this end, the A54'- tautomer was solvated in a periodic box of 480 TIP3P[9] water molecules, resulting in an initial box size of 32Å x 26Å x 20Å. The system was energy minimized and equilibrated at 300K for 10ps. Calculations were performed using the AMBER all atom force field and the AMBER 4.0 molecular dynamics program.[10] During simulations, all bond lenghts were constrained using the SHAKE[11] algorithm with a tolerance of 0.005Å, allowing a time step of 2fs. Solute and solvent were coupled to a constant temperature heat bath with a coupling constant of 0.2ps to maintain a temperature of 300K. A residue based cutoff of 8 Å was

employed. The atomic charges of the three tautomers were obtained from an electrostatic potential fit to a STO-3G wavefunction, using STO-3G optimized geometries.

In order to determine the free energy differences of solvation, the equilibrated $A54^{+}$ tautomer was gradually transformed into $A57^{-}$ (and into $A74^{+}$) in a series of 41 windows with 250 steps of equilibration followed by 250 steps of data collection at each window (41 ps of total simulation time), and the changes in free energy of solvation were calculated at each window.

In the second model, self-consistent reaction field (SCRF) calculations[12] were performed at the RHF/STO-3G level using Gaussian92 suite of programs;[7] a spherical cavity radius of 4.9 Å was adopted for the three tautomers and the dielectric constant of the medium was set to 80.

The results are reported in the Table (ΔG_{sol} from FEP and ΔE_{sol} from SCRF). Both SCRF and FEP calculations coherently assign an exceptionally high stabilization to the $A57^{-}$ tautomer. $A57^{-}$ is predicted to be better solvated than $A54^{+}$ by 21.1 Kcal/mol from FEP and by 28.9 Kcal/mol from SCRF; the more similar tautomers $A74^{+}$ and $A54^{+}$ are also found almost equally solvated (-0.2 Kcal/mol from FEP and -2.2 Kcal/mol from SCRF).

These results put in evidence a strong stabilization of $A57^{-}$ due to solvent. Moreover, they qualitatively reproduce the predominance of $A57^{-}$ in solution from the sum of the relative energies of solvation and the relative energies *in vacuo*, whatever method is chosen for the *in vacuo* (semiempirical AM1 or *ab-initio*) and solvent (FEP or SCRF) calculations. We conclude that the observed reversal of stability on going from *vacuo* to solvent is at the origin of the predominance of $A57^{-}$ in water solution.

This is one in a few examples where solvation effects are so strong to be able to favour such a highly unstable tautomer *in vacuo*. It is possible that a similar balance between intrinsic stabilities and environmental effects may also occur in nature to control the stabilization of anthocyanin pigments in the vacuole of plants. The properties of solvation of tautomers are also of the utmost importance in protein-ligand interactions, where solvation/desolvation and solvation substitution by protein binding residues effectively determine the strength of association.

On the whole, our results call attention to the need of considering solvent effects when phenomena in solution are to be investigated. On the front of methodology, free energy perturbation approaches within molecular dynamics simulations and *ab-initio* self-consistent reaction field calculations are both appropriate for reaching this goal, at least in our case where solvation effects are dominated by electrostatic energies.

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