Thermodynamic and Kinetic Stability of Anthocyanidin: DFT and TD-DFT Calculation

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ABSTRACT

In this study, the thermodynamic and kinetic stability of cyanidin were investigated using density functional theory (DFT) and time-dependent DFT (TD-DFT) methods. The stability of cyanidin as a simple anthocyanidin has been described and the effect of keto-enol tautomerization on the color instability of this pigment relative to other counterparts with 3-*O*-substitution has been analyzed. The results reveal that the keto form at 3-position is thermodynamically preferable and the keto structure of chalcone (a secondary product of cyanidin hydration) is thermodynamically and kinetically preferable compared to the keto-structure of cyanidin flavylium cation. Therefore, the 3-hydroxyl group is responsible for cyanidin color instability and plays an important role in decomposition of anthocyanidin.

Keywords: Cyanidin, Stability, TD (TD-DFT), keto-enol tautomerization, anthocyanidin.

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INTRODUCTION

Anthocyanin compounds are considerable to be highly useful plant pigments that can be used in many applications. It can be used as food colorants [1–3], pigments in dye sensitized solar cell [4,5] antioxidants [6–9], anti-tumor, anti-inflammatory, anticarcinogenic [2,10–14] and also as indicators [15]. They are responsible for red, pink, and blue colors of flowers, vegetables and fruits [16–20]. Anthocyanin structures differ in the number of hydroxy and methoxy groups of the 2-phenylbenzopyrylium moiety (flavylium cation) [21]. Naturally occurring anthocyanins are always glycosylated at the C-3 position [22]. Anthocyanidins are the sugar-free anthocyanin chromophore that can be easily prepared by the acidic hydrolysis of glycoside residue. The main drawback reported for the anthocyanidins is their color instability; they are highly reactive and degrade under normal conditions and their color change to colorless compounds.

It was known that anthocyanidins exist in equilibrium (Scheme 1) in an aqueous solution depending on the pH. The red flavylium cation (AH^+) of the strong acidic medium is transformed to purple quinoidal base (A) at neutral solutions and further into blue quinoidal base anion (A^-) at the

basic medium (by proton transfer). Hydration of AH^+ to colorless hemiketal (pseudobase, **B**) in weakly acidic medium takes place followed by formation of chalcone (**C**) via ring opening of **B** [23–25]. It is supposed to inhibit the hydration and proton transfer reactions to preserve the red color of flavylum cation. The color stability of natural anthocyanins [1,26–28] have been ascribed to chemical structure and position of the substituents of anthocyanin [29,30] especially sugar [29,31] as well as other factors such as binding with metal ions [32] or other pigments [25]. Even though, variety of reports about anthocyanin stability have been intensively investigated and most of them are based on nature of anthocyanin structure [33,29,34,35,27,28,36,37], The high stability of 3-*O*-glucoside moieties than 3-hydroxyl anthocyanin haven't yet been understood [29,31]. Quantum chemical calculations provide a reliable rapid screening of the possibility of another suggested mechanism that can interpret color change in anthocyanidins under different conditions (Scheme 2) with saving time and resources. Therefore, we carried out such computational work to investigate possible conversion of cyanidin to keto structure using density functional theory.

Scheme 1: Transformation equilibria of cyanidin in aqueous solution.

Scheme 2: Keto-enol tautomerization of cyanidin in aqueous solution

COMPUTATIONAL DETAILS.

The most stable cyanidin conformer has been optimized using hybrid density functional theory with the dispersion corrected [38,39]. The B3P86–D2 method accurately described anthocyanin conformers [38,40], their thermodynamic [41] and absorption properties. Therefore, geometry optimizations of the keto and enol forms were carried out with the B3P86–D2 functional along with the cc-pVDZ basis set. The electronic properties were calculated using time-dependent DFT (TD-DFT) at the same level of optimization. The effect of presence of solvent such as water was examined using solvation model based on density (SMD) [42]. All calculations were carried out using Gaussian 09 program [43].

RESULTS AND DISCUSSION

Conformer of cyanidin

Cyanidin has a numbers of hydroxyl groups with different orientation. It is anticipated, from the small differences in the calculated energy (Figure 1), that most of conformers are possible in solution. Figure 1 shows relative zero-point corrected energies and free energies of all possible conformers at B3P86-D2/cc-pvdz in water. Figure 2 displays the structures of the most stable conformers of cyanidin with a relative energy difference of about 2 kcal/mol. Energy difference between the two most stable **A** and **I** structures is modest; **A** is more stable than **I** by 0.14 kcal/mol. The stability of them might be ascribed to strong hydrogen bond formed by such pattern of the hydroxyl group orientation. From hereinafter we will consider conformer **A** of cyanidin for further discussion (**Cy**).

Keto-enol tautomerization

The thermodynamic and kinetic for keto-equilibrium structures of cyanidin starting from flavylum cation to the quinonoidal base passing through hemiketal and chalcone structures were investigated and compared with the keto-structures. Figure 3 depicts the potential energy diagram for the equilibrium from keto and enol flavylum cation. Figure 4 clearly shows the optimized equilibrium structures of cyanidin. Throughout this study three possible quinonoidal bases (**A**₄, **A**₅, **A**₇) have been considered. It will be easier to theoretically determine the equilibrium constant for H transfer reaction (acid-base equilibrium between **AH**⁺ and the possible **A**). The equilibrium constant can be estimated from the relation $\Delta G^0 = -RT \ln K_a$ where ΔG^0 is the standard Gibbs energy, R gas constant, T the absolute temperature in kelvin and K_a is the equilibrium constant. As reported previously [44], the proton transfer products (**A**) are kinetic products and disappear rapidly due to their instability, the higher free energy (22.8-24.9 kcal/mole) was found to **A**₄, **A**₅, **A**₇ formation. Other process like hydration, tautomerization and isomerization have their corresponding equilibrium constants K_h , K_t and K_i . The overall equilibrium is equivalent to combination of them. Hydration reaction and isomerization to keto form are thermodynamically in competition but the keto form is more thermodynamically favored. For the kinetic process taking place in the network of **AH**⁺ chemical reactions, the barriers for the keto formation from **AH**⁺ and from *cis*-chalcone (diketones) have been compared. The barrier height is lower in case of chalcone isomerization to 3-keto structure (49.1 kcal/mole) than flavylum cation isomerization to the 3-keto form (69.4 kcal/mole).

Both the hemiketal (**B**) from the keto and the enol forms of **AH**⁺ are involved in a tautomeric process that leads to the ring opening with formation of a *cis*-chalcone form. This reaction with keto form is again thermodynamically favored than the one occurs from enol-form. The diketones (**DK**) resulted from hemiketal ring opening is stable by 8.6 kcal/mole than *cis*-chalcone resulted from enol form. Even though the lower energy of tautomerization (1.7 kcal/mole) for keto form, the enol form needs 2.5 kcal/mole for tautomerization. Finally, depending on the flavylum substitution pattern the *cis*-chalcone thermally isomerizes and gives the *trans*-chalcone.

Figure 1: Relative stabilities of cyanidin conformers relative to **A** at B3P86-D2/cc-pVDZ in water. a) zero-point corrected energies and b) free energies.

Figure 2: Optimized structures of most stable cyanidin conformers at B3P86-D2/cc-pVDZ in water through SMD.

The pK_a calculation

For determining the pK_a the solvation free energies use thermodynamic cycle has been utilized. Table 1 collects the calculated K_a , K_h , K_t and K_i and available experimental data. The calculated value of pK_a are in the range of 9.50 and 11.0, while the experimental data is 4.8 indicating a double overestimated of the calculated pK_a of cyanidin.

Figure 3. Potential energy diagram for the decomposition of cyanidin (ΔG° , kcal/mol) at B3P86-D2/cc-pVDZ in water.

Figure 4: Optimized structures of cyanidin decomposition products at B3P86-D2/cc-pVDZ in water.

Table 1: B3P86-D2/cc-pVDZ calculated equilibrium constants of cyanidin at 298 K.

$K_{a4'}$	$pK_{a4'}$	K_{a5}	pK_{a5}	K_{a7}	pK_{a7}	K_h	K_t	K_i
9.66E-12	11.01	3.18E-10	9.50	1.91E-10	9.72 (4.8) ^a	5.88E-16	1.38E-02 (0.56) ^a	1.41E+04

^a ref. [44].

Electronic properties

The calculated longest wavelength of absorption ($\lambda_{\max}$) of the studied equilibrium structures of cyanidin are presented in Table 2. As seen from Table 2, the maximum absorption bands for cyanidin flavylum cation, 487 nm in water is slightly underestimated relative to the experimental value of 515 nm [31]. For chalcone, the calculated $\lambda_{\max}$ is in agreement with previous experimental reports. The $\lambda_{\max}$ of chalcone was in the ranges of (300-400, 200-270 nm) [45].

Table 2: calculated oscillator strength (f) and maximum wavelength ($\lambda_{\max}$) for the cyanidin equilibrium structures at TD-B3P86-D2/cc-pVDZ in water.

Enol-structure	$\lambda_{\max}$ (nm)	f	Keto-structure	$\lambda_{\max}$ (nm)	f
AH⁺	487	0.6139	AH⁺_k	364	0.3739
A_{C4'}	485	0.9709			
A_{C7}	515	0.5353			
B	188	0.6171	B_k	226	0.1245
Cc	201	0.2657	DK_c	343	0.1342
C_t	383	0.5181	DK_t	337	0.1723

CONCLUSIONS

In this study, thermochemistry and kinetics of cyanidin stability were calculated at B3P86-D2/cc-pVDZ. The results obtained can be summarized as follows:

- 1- Keto structures at 3-position is thermodynamically preferable reaction than hydration reaction.
- 2-Chalcone isomerization to 3-keto structure is thermodynamically and kinetically preferable than flavylum cation isomerization to 3-keto form.
- 3-The hydroxyl group at position 3 of anthocyanidin is responsible for cyaniding instability and plays an important role in decomposition of anthocyanidin.

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