Anthocyanins and related compounds. Detecting the change of regime between rate control by hydration or by tautomerization

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Fernando Pina

REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Monte de Caparica, Portugal

ABSTRACT

The network of chemical reactions involving anthocyanins and related compounds has been historically considered as the chemical transformations taking place when the flavylium cation (which is generally stable only in very acidic solutions) disappears upon a pH jump to higher pH values (direct pH jump). In this work profit is taken from the appearance of flavylium cation from equilibrated solutions at moderately acid pH values, (reverse pH jumps) as well as upon irradiation of *trans*-chalcone in the cases where photochemistry is available. Reverse pH jumps, flash photolysis and reaction quantum yields unequivocally show that at sufficiently acidic pH values the tautomerization becomes the rate determining step of flavylium cation formation. This is a lucky situation since gives kinetic information about tautomerization, the most difficult process to access in the anthocyanins and related compounds network of chemical reactions.

The thermodynamic and kinetic details of flavylium base photochromic systems are described. Mathematical expressions for direct and reverse pH jumps, stopped flow and flash photolysis are presented as well as the procedure to obtain all of the rate and equilibrium constants of the system.

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1. The network of chemical reactions involving flavylium compounds

Flavylium compounds are derived from the 2-phenylbenzopyrylium, Scheme 1. The systematization of the kinetic steps regarding the photochromism of flavylium derivatives is closely related to the knowledge of the pH dependent network of chemical reactions involving these compounds, Scheme 2. Historically, only after understanding this pH dependent network of reactions was it possible to account for the mechanism of the photochromism of flavylium compounds [1–8].

The flavylium cation is stable only at very acidic pH values. At moderately acidic solutions a series of reactions involving at least five species occur. Nowadays the sequence of pH dependent chemical reactions involving flavylium derivatives is firmly established, a research that took almost one century after the description of the first flavylium compound by Büllow [9]. A convenient way to study the network of chemical reactions involving flavylium compounds is to carry out pH jumps: (i) direct pH jumps defined as the increasing of pH of an equilibrated solution of a flavylium cation generally at pH $\leq$ 1; (ii) reverse pH jumps from solutions equilibrated at higher pH values back to flavylium cation [10].

In the case of a direct pH jump, the flavylium cation can deprotonate to give the quinoidal base, as an example the compound 4′,7-dihydroxyflavylium, eq. (1)


$$K_a = k_a/k_{-a} \qquad \text{(deprotonation)}$$

(1)
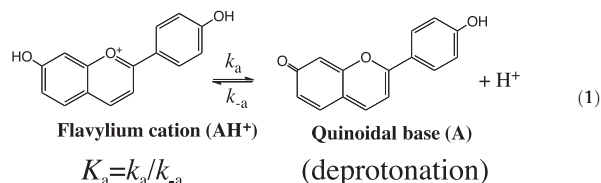
In competition with this reaction the hydration of the flavylium cation by addition of water at position 2 takes place, eq. (2)

E-mail address: fp@fct.unl.pt.

Flavylium cation (AH⁺)   **Hemiketal (B)**

$K_h = k_h / k_{-h}$ **(hydration)**   (2)
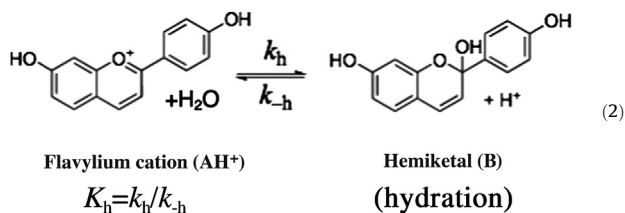
On the other hand, the hemiketal (chromene) is involved in a tautomeric process that leads to the ring opening with formation of a *cis*-chalcone. This reaction usually occurs in the sub-second timescale.

Hemiketal (B)   ***cis*-Chalcone (Cc)**

$K_t = k_t / k_{-t}$ **(tautomerization)**   (3)
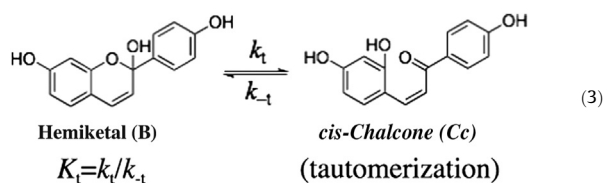
Finally the *cis*-chalcone isomerizes and gives the *trans*-chalcone, in the timescale of seconds or many days depending on the substitution pattern of the flavylium salt.


***cis*-Chalcone (Cc)**   ***trans*-Chalcone (Ct)**

$K_i = k_i / k_{-i}$ **(isomerization)**   (4)
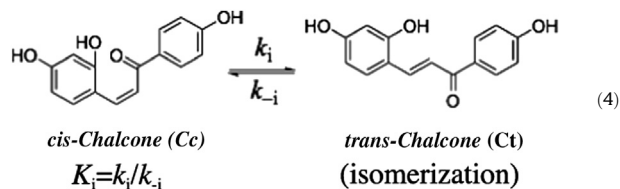
The sequence of reactions is represented in Scheme 2.

The coloured species of the network are the flavylium cation and the quinoidal base, the latter has its absorption maximum red shifted in comparison with the former.
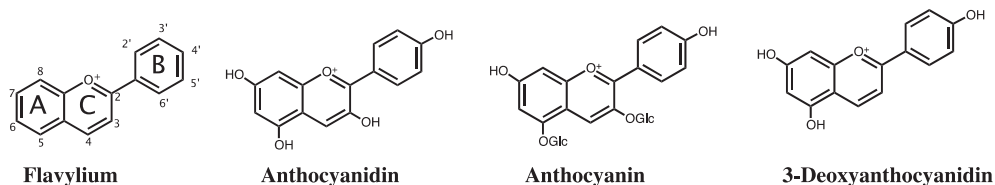
All these equilibrium and rate constants can in principle be calculated if the appropriate experiments are carried out (see below). Once the equilibrium constants are calculated, a very useful energy level diagram where all the network species are positioned [11] is easily obtained from the relationship, $\Delta G^0 = -RT \ln K$ where ΔG^0 is the standard Gibbs energy, R the gas constant, T the absolute temperature (in Kelvin) and K the equilibrium constant for each process of the network, see Scheme 2. It is worthy of note that the relative position of the several species is very dependent on the substituents of the flavylium core. For example in the case of anthocyanins the hemiketal **B** (resulting from the water attack in position 2) is the most stable species at higher pH values, while in 4′,7-dihydroxyflavylium it is **Ct**.

As shown in Scheme 3 the flavylium cation is stable at very acidic pH values. Raising the pH (direct pH jump) there is thermodynamic access to the species **A** and **B**. However, the rate of the deprotonation reaction to give **A** is by far faster than hydration to give **B**, and by consequence **A** is formed immediately after the direct pH jump. An important detail of the kinetic mechanism was discovered by Brouillard and Dubois [6–8]. In acid to moderately acid solutions the quinoidal base, **A**, is not reactive [12]. This implies that **A** is a kinetic product and the system evolves to the more stable species from the hydration of **AH⁺**. As a consequence the hydration becomes a function of the mole fraction of **AH⁺** ($[H^+]/([H^+] + K_a)$) available to react. Because **AH⁺** and **A** are in fast equilibrium when compared with the other reactions, they behave as a single species in the timescale of the other reactions [13].
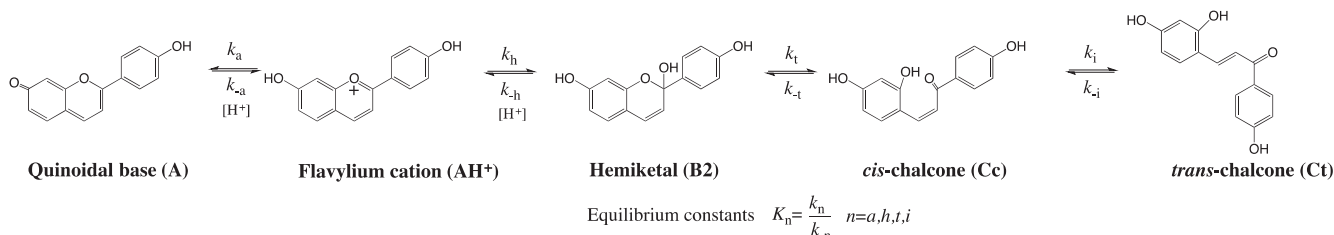
The analysis of the thermodynamic equilibria of the flavylium compounds becomes easier if eq. (5) is considered [6,7,14]. Eq. (5) is equivalent to a single acid–base equilibrium between flavylium cation and a conjugate base, **CB**, defined as the sum of the concentrations of the other species in the network, $[CB] = [A] + [B] + [Cc] + [Ct]$

$$AH^+ + H_2O \rightleftharpoons CB + H_3O^+ \quad K'_a = K_a + K_h + K_h K_t + K_h K_t K_i \quad (5)$$
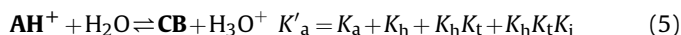
When a relatively high *cis*–*trans* isomerization barrier is present and the isomerization is much slower than the other reactions, it is very useful to define a pseudo-equilibrium attained before formation of significant amounts of **Ct**.

Flavylium   **Anthocyanidin**   **Anthocyanin**   **3-Deoxyanthocyanidin**

Scheme 1. Flavylium cation derivatives.

Quinoidal base (A)   **Flavylium cation (AH⁺)**   **Hemiketal (B2)**   ***cis*-chalcone (Cc)**   ***trans*-chalcone (Ct)**

Equilibrium constants $K_n = \dfrac{k_n}{k_{-n}}$ $n = a, h, t, i$

Scheme 2. Representation of the network of chemical reactions of the compound 4′,7-dihydroxyflavylium.