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Dyes and Pigments 51 (2001) 71–77

DYES and PIGMENTS

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Intramolecular H-bonding in the bay region of hypericin: an AM1 study

Dao-Ping Wang[a], De-Xin Kong[a], De-Zhan Chen[a], Hong-Yu Zhang[b,c,*]

[a]*Department of Chemistry, Shandong Teachers' University, Jinan 250014, PR China*
[b]*College of Life Sciences, Shandong Teachers' University, Jinan 250014, PR China*
[c]*Laboratory for Computational Biology, Shandong Provincial Research Center for Bioinformatic Engineering and Technique, Zibo University, Zibo 255091, PR China*

Received 21 November 2000; received in revised form 4 January 2001; accepted 24 May 2001

Abstract

The semi-empirical quantum chemical method AM1 was employed to study intramolecular H-bonding in the bay region of hypericin (HYP). It was found that three isomers of HYP, which correspond to structures with 0, 1, and 2 H-bonds in the bay region, readily interconvert, and that the isomer with two H-bonds is the predominant species in polar or nonpolar solvents. It was also shown that the formation of intramolecular H-bonding in the bay region facilitates proton dissociation and intramolecular proton transfer, providing an alternative explanation for the experimentally observed transient species produced from HYP © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Hypericin; Intramolecular hydrogen bond; Intramolecular proton transfer; Perylenequinonoid pigments; p*K*a value

1. Introduction

Perylenequinonoid pigments, including hypocrellin A, hypocrellin B, cercosporin, hypericin (HYP) and isohypericin (iso-HYP) (Fig. 1) are photosensitizers that have attracted considerable attention in recent years [1–7]. Structure–activity relationship studies indicate that 4,9-dihydroxy-3, 10-perylenequinone is the active center of perylenequinonoid pigments [7–9], and that fast intramolecular proton transfer in the ground state of perylenequinonoid pigments facilitates intersystem crossing ($S_1 \rightarrow T_1$), enhancing the quantum yield of the T_1 state of such pigments [7]. In addition, the acidity of the hydroxyl group of perylenequinonoid pigments increases in the excited state, which is a beneficial feature in photodynamic therapy [7,10,11].

Although the various perylenequinonoid pigments are similar in structure, they exhibit distinct photosensitization characteristics. For example, the energy barrier to intramolecular proton transfer involving hypocrellin in the excited state is 2.12 ± 0.070 kcal/mol, while that for HYP is only 0.044 ± 0.008 kcal/mol [12]. The p*K*a for HYP is approximately 6 units lower than that of hypocrellin A, hypocrellin B, and cercosporin [13,14]. Since chemical and physical properties are normally related to molecular structures, it is likely

* Corresponding author. Tel.: +86-533-2780271; fax: +86-533-2780271.
E-mail address: zhysdtu@jn-public.sd.cninfo.net (H.-Y. Zhang).

0143-7208/01/$ - see front matter © 2001 Elsevier Science Ltd. All rights reserved.
PII: S0143-7208(01)00057-2

that the characteristics of HYP arise from its unique structure, in that HYP has two OH groups in the bay region (positions 1 and 2, Fig. 1). It has been found that the facile ionization of HYP is due to the relative proximity of the OH groups, one of which is H-bonded to the O atom of the second OH in a sterically crowded environment [13]. Results of calculations of theoretical heats of formation for intramolecular H-bond formation in the bay region suggest that HYP structures possessing intramolecular H-bonds in the bay region are especially stable [15].

Despite the aforementioned studies, several questions regarding the intramolecular proton transfer process remain unanswered. For instance: (1) What is the energy barrier for intramolecular H-bond formation? (2) Is it possible for HYP to form two intramolecular H-bonds in the bay region (cf. Fig. 2)? (3) How does intramolecular H-bonding influence the photophysics of HYP, including proton dissociation and intramolecular proton transfer? AM1 calculations have been used to address these questions, the results of which are summarized in this paper. Since HYP isomers

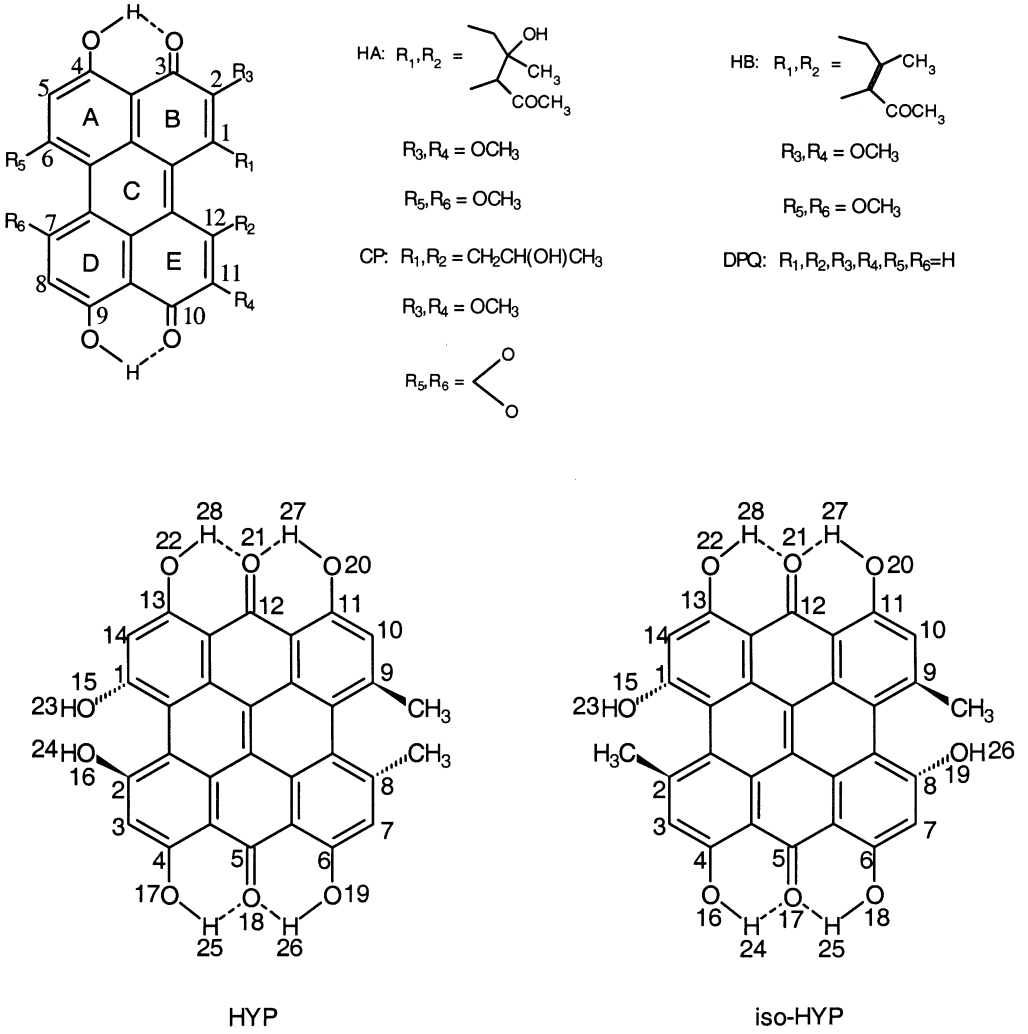

Fig. 1. The structures of perylenequinonoid pigments, including hypocrellin A (HA), hypocrellin B (HB), cercosporin (CP), dihydroxy-perylenequinone (DPQ), HYP, and iso-HYP.

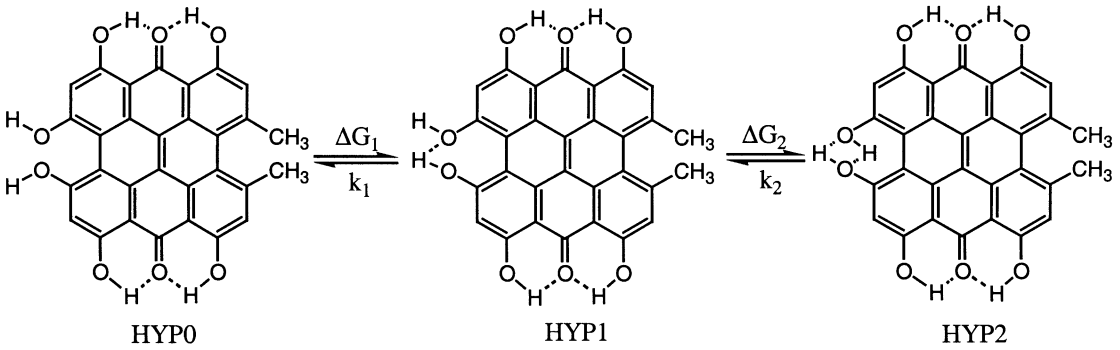

Fig. 2. Structures of HYP0, HYP1, HYP2.

arising from intramolecular proton transfer at positions 4, 6, 11 and 13 were not thermally stable [15], our work considered only the stable structures.

2. Methods

The MMX molecular mechanics method [16] operating under the PCMODEL program was used to optimize the molecular structures of HYP isomers. Then, quantum MO calculations were carried out to finalize geometry optimizations, using the AM1 semi-empirical method [17]. The keyword TS was employed for transition state calculations, and only one virtual frequency was generated for the transition state. All calculations were performed using GAUSSIAN 94 [18].

3. Results and discussion

3.1. The thermodynamics of intramolecular proton transfer

The hydroxyl groups in the bay region of HYP may exist as indicated in Fig. 2, where HYP0, HYP1 and HYP2 arise from 0, 1, and 2 H-bonds in the bay region, respectively. For the interconversion of these isomers, ΔG_1 and ΔG_2 are the changes in Gibbs free energy and k_1 and k_2 are the corresponding equilibrium coefficients. The results summarized in Table 1 indicate that enthalpy (H) and entropy (S) decrease as the number of H-bonds increases. Using the data in Table 1 and the well-known equation $\Delta G = -RT \ln k$, we found that $k_1 = 0.86\ s^{-1}$ and $k_2 = 2.81\ s^{-1}$, which indicate that the proportions of HYP0, HYP1 and HYP2, are 23.36, 20.09 and 56.64%, respectively.

Table 1
Thermodynamic properties for three isomers of HYP ($T = 298.15$ K)

	H (kJ/mol)	S (J/mol)	G (kJ/mol)
HYP0	264.78	747.07	42.04
HYP1	263.63	741.94	42.42
HYP2	260.58	740.31	39.86

To determine the ease of the interconversion for the three isomers, we calculated the rotation barrier associated with the O–H group. The dihedral angle for H(24)–O(16)–C(2)–C(3) was set at 90°, and the keyword TS was used to optimize the resultant structure. Only one virtual frequency was obtained in each transition state. The energy barriers and zero-point energy (ZPE) corrections are listed in Table 2. The energy barriers for HYP0→HYP1 and HYP1→HYP2 are 14.91 and 7.50 kJ/mol, respectively. When one considers that the energy barrier associated with a typical chemical reaction is 80–250 kJ/mol, it is evident that interconversions amongst the three isomers are fairly easy.

Table 3 contains the dipole moments for three HYP isomers. The data show that increasing the number of H-bonds enhances the dipole moment. Since polar solvents stabilize structures that have

large dipole moments, the relative proportions of the 3 isomers would change with solvent polarity.

It is believed that the Gibbs free energy of HYP in a homogeneous medium of dielectric constant ε_r can be compared with the Gibbs free energy in a similar medium ($\varepsilon_r = 1$) using Eq. (1) [19]:

$$\Delta G_{solv} = -(N_0/4\pi\varepsilon_0) \times \left(\mu^2/r^3\right) \times [(\varepsilon_r - 1)/(2\varepsilon_r + 1)] \quad (1)$$

where ΔG_{solv} is the Gibbs free energy change in a polar solvent, μ is the dipole moment, r is the molecular radius, ε_0 is the vacuum permitivity, and N_0 is Avogadro's number. In this study, methanol was used as the solvent, $\varepsilon_r = 32.66$, and $r = 6$ Å [20]. The results of these calculations are shown in Table 3, where G, ΔG_{solv} and G_{solv} correspond to the Gibbs free energy without solvent effects, the change in Gibbs free energy, and the Gibbs free energy with the solvent effects, respectively. We found that G_{solv} decreases as the number of H-bonds increases. In this case, $\Delta G_1 = 0.28$ kJ/mol, $\Delta G_2 = -2.65$ kJ/mol, $k_1 = 0.89$ s^{-1}, $k_2 = 2.91$ s^{-1} and, the proportions of HYP0, HYP1 and HYP2 are 22.32, 19.87 and 57.81%, respectively, which is a little different from the proportion of these isomers in the absence of solvent effects.

Table 2
Energy barriers for intramolecular H-bond formation in the bay region of HYP (kJ/mol)

HYP	HYP0→HYP1	HYP1→HYP2
Ground state[a]	189.11	188.81
Transition state[a]	204.02	196.31
Energy barrier	14.91	7.50

[a] Includes ZPE correction.

Table 3
Gibbs free energy for the three isomers of HYP in methanol

	Dipole moment (debye)	G (kJ/mol)[a]	ΔG_{solv} (kJ/mol)[b]	G_{solv} (kJ/mol)[c]
HYP0	0.32	42.04	−0.014	42.03
HYP1	0.89	42.42	−0.11	42.31
HYP2	1.23	39.86	−0.20	39.66

[a] Without solvent effects.
[b] With solvent effects.
[c] Total free energy in solution.

3.2. Influence of intramolecular H-bonding on proton dissociation

It is interesting to note that the pKa value for HYP (~1.7) is much lower than that of iso-HYP (7.5) [13], which possesses only one OH group in the bay region, and hypocrellin A (8.22), which has no OH group in the bay region [14]. Thus, it is evident that intramolecular H-bonding in the bay region plays an important role in lowering the pKa of HYP.

The AM1 calculated dissociation free energies for the three isomers of HYP and iso-HYP are given in Table 4. It is clear that the dissociation free energies of HYP1 and HYP2 are much lower than those associated with HYP0 and iso-HYP. This is consistent with the experimental results, which show that intramolecular H-bonding is beneficial to proton dissociation. The dissociation free energy for HYP1 is a little lower than that for HYP2 because the dissociation of the HYP2 proton is inhibited by intramolecular H-bonding. The dissociation free energy for HYP0 is even higher than that for iso-HYP, suggesting that the acidity of HYP0 is weaker than that for iso-HYP.

Having established that the three isomers of HYP coexist in solution, we turned our attention to a study of their monoanions. The free energies for the monoanions ($HYP0^-$ and $HYP1^-$) are listed in Table 4. These values indicate that $HYP1^-$ is more stable than $HYP0^-$ by 26.08 kJ/mol. Thus, the equilibrium constant for converting $HYP0^-$ to

Table 4
Dissociation free energy for protons in the bay region of HYP and iso-HYP (kJ/mol)[a]

	Parent molecule	Anion	Dissociation free energy	pKa [13]
HYP0	42.04	−195.92	1053.39	
HYP1	42.42	−222.00	1026.93	
HYP2	39.86	−222.00	1029.49	1.7
iso-HYP	46.72	−204.04	1041.59	7.5

[a] Gibbs free energy for H^+ is 1291.35kJ/mol, at $T = 298.15$ K.

$HYP1^-$ is 3.55×10^4 s^{-1}, making $HYP1^-$ the predominant monoanion in solution.

We also calculated the dissociation free energies for the top protons (H(25) and H(28)) of HYP. In the case of H(28) (Table 5), the proton dissociation free energy involving HYP2 is lower than that of the corresponding protons in HYP0, HYP1 and iso-HYP. However, in each case, the free energy is much higher than that involving the protons in the bay region, suggesting that the top protons are more difficult to dissociate than protons in the bay region. In fact, hypocrellin A has no hydroxyl group in the bay region, and its p*K*a is much higher than that of HYP and iso-HYP.

3.3. The influence of intramolecular H-bonding on proton transfer

Intramolecular proton transfer involving dihydroxy-perylenequinones is a key factor in the photosensitivity of perylenequinonoid pigments [15]. Until now, only the proton transfer involving the top protons (H(28)) has been studied [12,21–37]. Among the unresolved questions is whether the experimentally observed transient species arises from intramolecular proton transfer [38]. From the structure of $HYP1^-$, it can be assumed that intramolecular proton transfer involving a proton in the bay region of $HYP1^-$ can also occur. Therefore, we compared the intramolecular proton transfer processes involving the H(28) and bay region protons.

The AM1 calculated energy barriers for intramolecular proton transfer involving the H28 protons are 94.13 and 81.69 kJ/mol for HYP2 and $HYP1^-$, respectively (Table 6). However, the energy barrier for the proton in bay region of $HYP1^-$ is only 28.56 kJ/mol (Table 6), making it much lower than that for the H28 protons. The rather low energy barrier for $HYP1^-$ arises from the fact that the O(15)−O(16) distance (2.362 Å) is much shorter than that between oxygens O(16) and O(15) (>2.494 Å) [13]. Although the results of AM1 calculations have only qualitative significance, they suggest intramolecular proton transfer in the bay region is an alternative explanation for the observed transient species produced from HYP. In addition, the X-ray structure of $HYP1^-$ indicates that the O(15)–H(24) and H(24)–O(16) distances are 1.18 and 1.21 Å, respectively. These values are very similar to those from the AM1 calculated intramolecular proton transfer transition state involving $HYP1^-$, which are 1.21 and 1.23 Å. This supports the hypothesis that the proton in the bay region of $HYP1^-$ can undergo intramolecular proton transfer.

Table 5
Dissociation free energy for H(28) of HYP and iso-HYP (kJ/mol)[a]

	Parent molecule	Anion	Dissociation free energy
HYP0	42.04	−162.96	1086.35
HYP1	42.42	−159.10	1089.83
HYP2	39.86	−178.91	1072.58
iso-HYP	46.72	−158.48	1086.15

[a] The Gibbs free energy of H^+ is 1291.35 kJ/mol, $T=298.15$ K.

Table 6
Energy barriers (kJ/mol) to intramolecular proton transfer in HYP2 and $HYP1^-$

	HYP2[a]	$HYP1^-$[b]	$HYP1^-$[c]
Ground state	−899.25	−75.32	−75.32
Transition state	−805.12	6.37	−46.76
Energy barrier	94.13	81.69	28.56

[a] H(28) transfer from O(22) to O(21) in HYP2, including ZPE correction.
[b] H(28) transfer from O(22) to O(21) in HYP1, including ZPE correction.
[c] H(24) transfer from O(16) to O(15) in $HYP1^-$, including ZPE correction.

Acknowledgements

This research was supported by the Natural Science Foundation of the Shandong Province through Grant Nos. Q98D01133 and Q99B06.

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