Pergamon

Tetrahedron Letters 43 (2002) 6181–6184

TETRAHEDRON LETTERS

Prevention of UV-light induced *E*,*Z*-isomerization of caffeoyl residues in the diacylated anthocyanin, gentiodelphin, by intramolecular stacking

Kumi Yoshida,[a,*] Reiko Okuno,[b] Kiyoshi Kameda[b] and Tadao Kondo[c]

[a]*Graduate School of Human Informatics, Nagoya University, Chikusa, Nagoya 464-8601, Japan*
[b]*School of Life Studies, Sugiyama Jogakuen University, Chikusa, Nagoya 464-8662, Japan*
[c]*Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8602, Japan*

Received 6 June 2002; revised 27 June 2002; accepted 28 June 2002

Abstract—Prevention of UV-B light induced *E*,*Z*-isomerization of caffeoyl residues in a diacylated anthocyanin, gentiodelphin, by molecular stacking was studied. We first isolated an isomer of gentiodelphin containing a *Z*-caffeoyl residue from an acidic methanol solution, irradiated with UV-B light. The isomerized pigment had only one *Z*-configurated caffeoyl residue, attached to the glucose of the 5-position. Under the irradiation conditions the other caffeoyl residue linking to the glucose on B-ring stacked to the anthocyanidin nucleus and did not isomerize. In acidic and neutral aqueous solutions the content of the *Z*-isomer was very low when the isomerization reaction was at equilibrium, intramolecular stacking of the caffeoyl residues being stronger than in acidic methanol. Therefore, intramolecular stacking may prevent light-isomerization of the α,β double bond of caffeoyl residues. Under physiological conditions the pigment was more tolerant of UV-irradiation, which may play an important role in quenching solar radiation energy in flower petals.

Although flowers bloom under exposure to strong sunlight, their petal color is stable for several days or more. Flower pigments, anthocyanins, are tolerant of UV-light and may confer some protection on DNA, like flavones.[1–7] Many anthocyanins contain cinnamoyl derivative residues, e.g. *p*-coumaroyl, caffeoyl, and feruloyl moieties. In general, their α,β double bonds readily isomerize on UV-B irradiation. However, only a few anthocyanins bearing the acyl moiety with *Z*-configuration have been reported,[8–19] and polyacylated anthocyanins containing two or more aromatic acyl moieties with *Z*-configurated cinnamoyl derivatives have not been described to our knowledge. The question why cinnamoyl derivative parts of polyacylated anthocyanins in living petal cells do not isomerize to *Z*-forms under strong solar radiation is attracting from the viewpoint of the biological functions of anthocyanins.

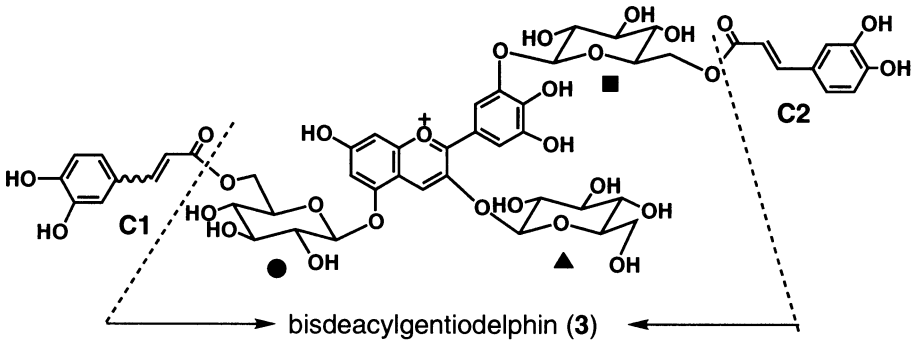
Keywords: gentiodelphin; polyacylated anthocyanin; intramolecular stacking; *E*,*Z*-isomerization; UV-B light; caffeoyl residue.

* Corresponding author. Tel.: +81-52-789-5638; fax: +81-52-789-4230; e-mail: yoshidak@info.human.nagoya-u.ac.jp

Polyacylated anthocyanins are remarkably stable in neutral or weakly acidic media,[20–24] their acyl residues being considered to stack to the anthocyanidin nucleus intramolecularly, which prevent hydration reactions and pigment decoloration.[20–24] The intramolecular stacking may play some important role in prevention of *E*,*Z*-isomerization. In this report we first describe in vitro *E*,*Z*-isomerization of a diacylated anthocyanin, gentiodelphin (**1**)[20], from blue petals of *Gentiana triflora*, on UV-B irradiation. We then discuss the prevention of isomerization under physiological conditions and the relationship of molecular stacking to protection against UV (Scheme 1).

Gentiodelphin (**1**) has two caffeoyl residues, C1 and C2. We have already revealed its conformation in acidic methanol solution, only C2 stacking to the anthocyanidin.[25] In order to clarify the relationship between intramolecular stacking and *E*,*Z*-isomerization reaction, an acidic methanol solution of **1** (5×10^{-5} M in 0.5% trifluoroacetic acid (TFA)–MeOH) was irradiated with a high-pressure mercury arc

0040-4039/02/$ - see front matter
PII: S0040-4039(02)01319-9



	C1	C2
gendiodelphin (**1**)	*E*	*E*
Z-isomer (**2**)	*Z*	*E*

Scheme 1.

lamp.[†] After a few minutes new peak (**2**) appeared with **1** on HPLC chromatography,[‡] gentiodelphin (**1**, 169 mg, 0.14 mmol) was dissolved in 300 ml of 0.5% TFA/MeOH, then the solution was stirred at 0°C and irradiated with the mercury arc for 30 minutes. Preparative HPLC (Develosil® ODS-HG 5, 20 mmϕ×250 mm) by stepwise elution from 0 to 15% aq. CH_3CN containing 0.5% TFA gave **2** as a dark red TFA salt (19 mg, 11%) with recovery of **1** (148 mg, 88%).

FABMS of **2** showed the same peak at $m/z=1113$ (M^+) as **1**. The ^1H NMR spectrum of **2** was similar to that of **1**, but the $J_{\alpha,\beta}$ of one caffeoyl residue was 13.0 Hz, indicating a *Z*-configuration. The linkage position of the *Z*-caffeoyl moiety was determined ambiguously by combination of the NOE difference spectra and the HMBC spectrum, then the structure of **2** was determined to be 3-*O*-(β-D-glucopyranosyl)-5-*O*-(6-*O*-(*Z*)-caffeoyl-β-D-glucopyranosyl)-3′-*O*-(6-*O*-(*E*)-caffeoyl-β-D-glucopyranosyl) delphinidin (Table 1). This is the first report of a polyacylated anthocyanin containing a *Z*-configurated caffeoyl residue.

Under these irradiation conditions other isomers such as C1 or both C1 and C2 could not be found *Z*-forms, suggesting that the acyl residue stacked to the anthocyanidin nucleus is resistant to *E*,*Z*-isomerization. To clarify the relationship between the manner of intramolecular stacking and prevention of *E*,*Z*-isomerization, *Z*-isomer contents were compared in acidic methanol, acidic water and phosphate buffer of pH 6.0. The solutions (5×10^{-4} M), each filled into 1.0 mm path-length quartz cuvettes were irradiated with the high-pressure mercury arc at room temperature. The radiation energy was controlled at 2000 μw/cm^2 with cut off of light wave shorter than 290 nm with a UV-cut filter.[§] The isomerization reaction reached equilibrium within a few minutes (Fig. 1). In acidic methanol solution the equilibrium ratio for **1** to **2** was 80:20, while in both acidic water (0.5% aq. TFA) and neutral aqueous solution (phosphate buffer, pH 6.0), ratio was 99:1. The equilibrium of *E*,*Z*-isomers in the diluted solutions (5×10^{-5} M in 10 mm path-length quartz cuvettes) was the same as that of 5×10^{-4} M solutions, indicating that prevention of isomerization reaction is caused by intramolecular association. The equilibrium ratio of *E*,*Z*-isomerization of methyl caffeate (1×10^{-3} M) with/without bis-deacyl gentiodelphin (**3**, 5×10^{-4} M) in acidic methanol was 60:40, and in acidic and neutral aqueous solutions was 95:5, suggesting that there is no intermolecular stacking effect between methyl caffeate and the chromophore under those conditions.

The three dimensional conformation of **1** in acidic aqueous solutions can well explain the suppression of formation of the *Z*-isomer. In acidic D_2O the chemical shift of α,β protons of C1 in **1** moved toward a higher field, nearly the same as these of C2 (Table 1), indicating both caffeoyl residues stacked to the anthocyanidin nucleus. A molecular modeling study with QUANTA97/CHARMm 23.2 software also showed the conformation of **1** in acidic methanol and in aqueous media to be different.[26] As shown in Fig. 2, both caffeoyl moieties, C1 and C2, stack to the nucleus in aqueous solutions.

In conclusion, molecular associations of caffeoyl residues of gentiodelphin with the anthocyanidin nucleus become stronger in aqueous media than in methanol solution. This intramolecular stacking of acyl residues prevents light-isomerization of the *E*-form. The light energy absorbed with caffeoyl residues might be charge-transferred to the anthocyanidin nucleus to

[†] Eikosha, high pressure mercury arc (EHB-WU-100, 100 W), radiating from 250 to 600 nm, mainly at 365 nm.

[‡] HPLC analysis was carried out using a Develosil® ODS HG-5 column (4.6 mmϕ×250 mm) with 17.5% aq. CH_3CN containing 0.5% TFA as the eluent. Analysis was carried at 40°C with a flow rate of 1.0 mL/min and peaks were detected at 530 nm.

[§] UV-29 filter (Toshiba Glass). Light permabilities at 260, 290 and 340 nm were 10%, 50% and 90%, respectively. The radiation energy was measured using a UV radiometer UM-10 with a UM-360 receptor unit (MINOLTA).

Table 1. Assignment of ^{1}H NMR spectra of gentiodelphin (**1**) and its mono *Z*-isomer (**2**) (600 MHz)

		1[a]			**2**[a]		**1**[b]
H-4	8.74	s		8.76	s		8.32
H-6	6.96	d	2.0	6.80	d	2.0	6.84
H-8	6.81	d	2.0	6.70	brs		6.67
H-2′	7.73	brs		7.76	brs		7.28
H-6′	7.88	brs		7.93	brs		8.00
▲- 1	5.06	d	7.5	5.04	d	7.5	
2	3.75	dd	9.0, 7.5	3.73	dd	9.0, 7.5	
3	3.61	t	9.0	3.58	t	9.0	
4	3.48	t	9.0	3.46	t	9.0	
5	3.69	ddd	9.0, 7.0, 2.0	3.37	brdd	9.0, 5.0	
6a	4.09	dd	12.0, 2.0	4.04	brd	12.0	
6b	3.84	dd	12.0, 7.0	3.80	brdd	12.0, 5.0	
●- 1	5.23	d	7.5	5.18	d	8.0	
2	3.81	dd	9.0, 7.5	3.72	dd	9.5, 8.0	
3	3.68	t	9.0	3.62	t	9.5	
4	3.62	t	9.0	3.50	t	9.5	
5	3.88	ddd	9.0, 6.5, 2.0	3.81	m		
6a	4.66	dd	12.0, 2.0	4.50	brd	12.0	
6b	4.41	dd	12.0, 6.5	4.54	dd	12.0, 7.0	
■- 1	5.21	d	7.0	5.22	d	8.0	
2	3.68	m		3.65	m		
3	3.68	m		3.65	m		
4	3.41	t	9.0	3.40	m		
5	3.85	ddd	9.5, 9.0, 2.0	3.86	m		
6a	4.71	dd	12.0, 2.0	4.68	brd	11.0	
6b	4.35	dd	12.0, 9.5	4.37	dd	11.0, 9.5	
C1 α	6.23	d	16.0	5.78	d	13.0	5.75
β	7.47	d	16.0	6.88	d	13.0	6.91
2	6.98	d	2.0	7.18	d	2.0	6.74
5	6.74	d	8.0	6.53	d	8.0	6.57
6	6.86	dd	8.0, 2.0	6.94	dd	8.0, 2.0	6.57
C2 α	5.91	d	16.0	5.91	d	16.0	5.71
β	7.05	d	16.0	7.06	d	16.0	6.87
2	6.37	d	2.0	6.35	d	2.0	6.22
5	6.51	d	8.0	6.51	d	8.0	6.53
6	6.34	dd	8.0, 2.0	6.33	dd	8.0, 2.0	6.22

The coupling constant of **1** in 10% TFA*d*-D_2O was the same as that in 10% TFA*d*-CD_3OD. The difference of chemical shifts of **1** between rt and 40°C in 10% TFA*d*-CD_3OD is within 0.05 ppm.
[a] In 10% TFA*d*-CD_3OD at rt.
[b] In 10% TFA*d*-D_2O, at 40°C.

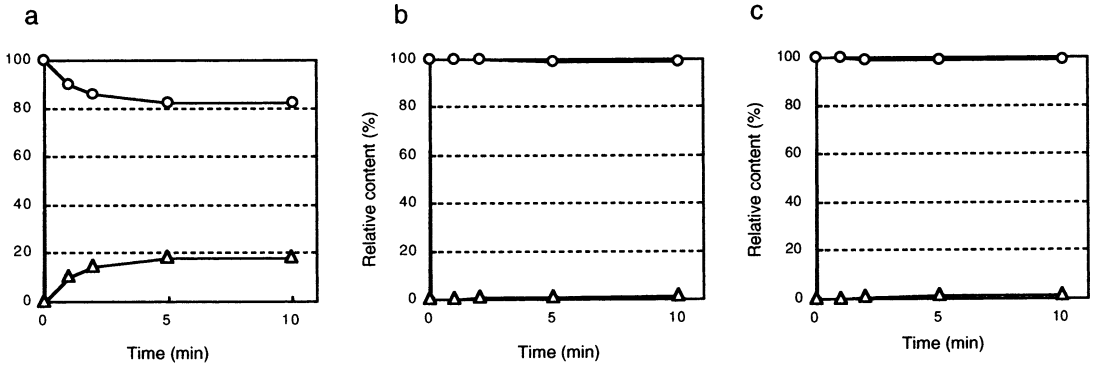


Figure 1. Isomerization of **1** (5×10^{-4} M) to **2** by UV-light. ○: **1** and △: **2**. (a) In 0.5% TFA–methanol; (b) in 0.5% TFA–H_2O; (c) in phosphate buffer, pH 6.0.

be released without any bond cleavage or isomerization reaction. These mechanisms may work more effectively in flower petal cells in which the concentration of anthocyanins must be higher than 10^{-3} M. This could be the reason why polyacylated anthocyanins with *Z*-form cinnamoyl derivatives are not found in nature.

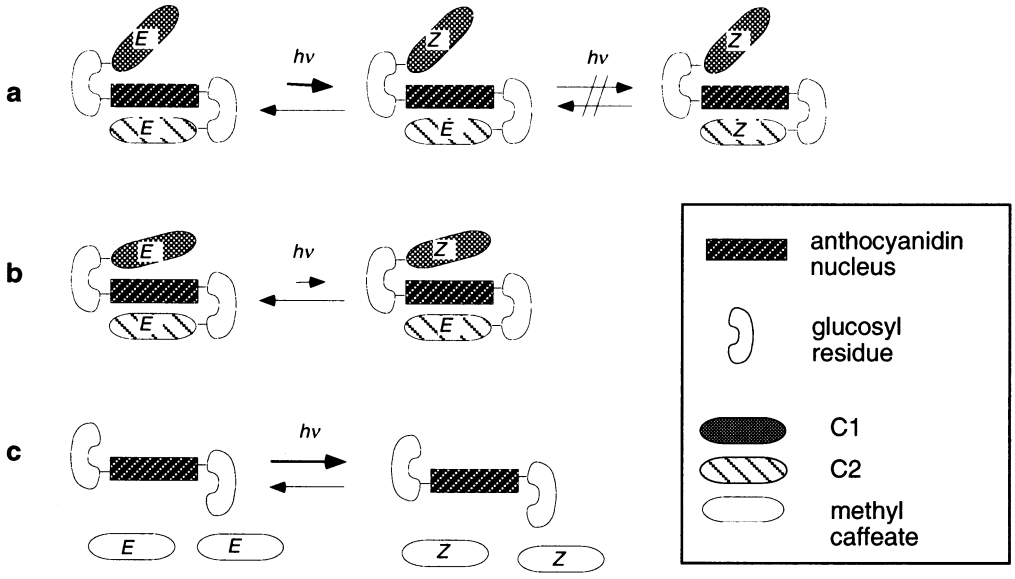


Figure 2. Schematic stacking conformation of **1** in different solutions and suppression of isomerization to the *Z*-form. (a) In acidic methanol solution. Only C2 is stacked to the anthocyanidin nucleus; (b) in acidic or weakly acidic aqueous solutions. Both C1 and C2 are stacked to the anthocyanidin nucleus; (c) methyl caffeate with **3** in acidic methanol, acidic water and neutral water solutions. No intermolecular stacking is apparent.

The fact that anthocyanins are stable under physiological conditions is rational in terms of plant survival. Some special mechanism for quenching light energy, e.g. emitted fluorescence, might be expected. This UV-protection (including screening effect of DNA) may be one of the most important biological functions of anthocyanins in living petal cells.

Acknowledgements

This study was financially supported by Ministry of Education, Science, Sports, Culture and Technology, Japan with a Grant-in-Aid for Scientific Research on Priority Areas No.08219237, for Scientific Research (C) NO. 09660147, and for COE Research No 07CE2004, and by the Sugiyama Jogakuen University Fund.

References

1. Tevini, M. *Stratospheric Ozone Depletion. UV-B Radiation in the Biosphere*; Springer-Verlag: Berlin, Heidelberg, 1994; pp. 37–62.
2. Beggs, C. J.; Wellmann, E. *Photomorphogenesis in Plants*; Kluwer Academic Publishers: Dordrecht, 1994; pp. 733–751.
3. Markham, K.; Gregory, J.; Tanner, J.; Merdelyn, C. L.; Whitecross, M. I.; Nayudu, M.; Mitchell, A. *Phytochemistry* **1998**, *49*, 1913–1919.
4. Chalker-Scott, L. *Photochem. Photobiol.* **1999**, *70*, 1–9.
5. Harborne, J. B.; Williams, C. A. *Phytochemistry* **2000**, *55*, 481–504.
6. Burchard, P.; Bilger, W.; Weissenböck, G. *Plant Cell Environ.* **2000**, *23*, 1373–1380.
7. van de Staaji, J.; de Bakker, N. V. J.; Oosthoek, A.; Broekman, R.; van Beem, A.; Stroetenga, M.; Aerts, R.; Rozema, J. *J. Photochem. Photobiol. B* **2002**, *66*, 21–29.
8. Yoshida, K.; Kondo, T.; Kameda, K.; Goto, T. *Agric. Biol. Chem.* **1990**, *54*, 1745–1751.
9. Kondo, T.; Yoshida, K.; Yoshikane, M.; Goto, T. *Agric. Biol. Chem.* **1991**, *55*, 2919–2921.
10. Markham, K. R.; Ofman, D. J. *Phytochemistry* **1993**, *34*, 379–385.
11. Nakatani, N.; Kikuzaki, H.; Hikida, J.; Ohba, M.; Inami, O.; Tamura, I. *Phytochemistry* **1995**, *38*, 755–757.
12. Hosokawa, K.; Fukunaga, Y.; Fukushi, E.; Kawabata, J. *Phytochemistry* **1995**, *38*, 1437–1441.
13. Hosokawa, K.; Fukunaga, Y.; Fukushi, E.; Kawabata, J. *Phytochemistry* **1995**, *39*, 1293–1298.
14. Markham, K. R. *Phytochemistry* **1996**, *42*, 1035–1038.
15. Inami, O.; Tamura, I.; Kikuzaki, H.; Nakatani, N. *J. Agric. Food Chem.* **1996**, *44*, 3090–3096.
16. Hayashi, K.; Shinagawa, H.; Suzuki, A.; Tsukui, A. *Food. Sci. Int. Tokyo* **1998**, *4*, 25–28.
17. Toki, K.; Saito, N.; Honda, T. *Phytochemistry* **1998**, *48*, 729–732.
18. Slimestad, R.; Aaberg, A.; Andersen, O. M. *Phytochemistry* **1999**, *50*, 1081–1086.
19. Ando, T.; Saito, N.; Tatsuzawa, F.; Kakefuda, T.; Yamaakge, K.; Ohtani, E.; Koshi-ishi, M.; Matsusake, Y.; Kokubun, H.; Watanabe, H.; Tsukamoto, T.; Ueda, Y.; Hashimoto, G.; Marchesi, E.; Asakura, K.; Hara, R.; Seki, H. *Biochem. Systematics Ecol.* **1999**, *27*, 623–650.
20. Goto, T.; Kondo, T.; Tamura, H.; Imagawa, H.; Iino, A.; Takeda, K. *Tetrahedron Lett.* **1982**, *23*, 3695–3698.
21. Goto, T. *Prog. Chem. Org. Nat. Prod.* **1987**, *52*, 113–158.
22. Brouillard, R. *The Flavonoids Advances in Research Since* 1980; Chapman and Hall: London, 1988; pp. 525–538.
23. Goto, T.; Kondo, T. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 17–33.
24. Kondo, T.; Kawai, T.; Tamura, H.; Goto, T. *Tetrahedron Lett.* **1987**, *28*, 2273–2276.
25. Yoshida, K.; Kondo, T.; Goto, T. *Tetrahedron* **1992**, *48*, 4313–4326.
26. Yoshida, K.; Toyama, Y.; Kameda, K.; Kondo, T. *Phytochemistry* **2000**, *54*, 85–92.