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THE INHIBITION OF THE PHOTOCHEMICAL DIMERIZATION OF 1,3-DIMETHYLTHYMINE BY FLAVIN AND 5-DEAZAFLAVIN DERIVATIVES

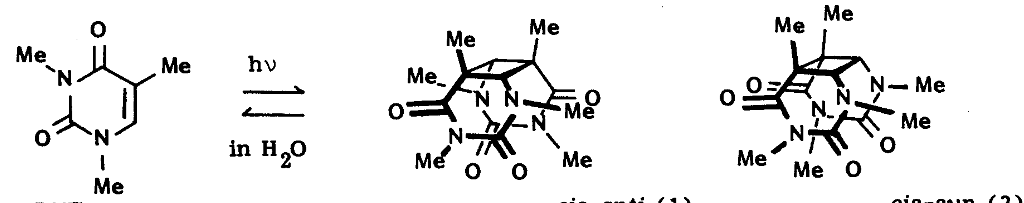
Kiyoshi Tanaka, Masahiro Kawase, Masako Okuno, Makiko Senda, Tetsutaro Kimachi
and Fumio Yoneda*
Faculty of Pharmaceutical Sciences, Kyoto University,
Sakyo-ku, Kyoto 606, Japan

We studied the effect of several flavin and 5-deazaflavin derivatives (flavin dyes) on the photochemical dimerization of 1,3-dimethylthymine as a model compound of DNA. These flavin dyes, especially those with the D-ribityl moiety such as riboflavin, 5-deazariboflavin and F_{420} chromophore, had a great inhibitory effect on 1,3-dimethylthymine photodimerization. But they did not detectably catalyze dimer cleavage at physiological pH levels.

KEYWORDS —— photochemical dimerization; 1,3-dimethylthymine; flavin dye; 5-deazaflavin; 1,3-dimethylthymine dimer; inhibitory effect; dimer cleavage; proflavine

During the past two decades there has been a large amount of interest in the photochemical formation and cleavage reaction of the thymine dimer in DNA which produces important effects in many biological systems.1) Therefore a number of model studies have been carried out on the reversible photochemical thymine dimerization in order to clarify possible mechanisms.2) On the other hand, it was also found that during irradiation acridine dyes such as proflavine inhibit the thymine dimerization in DNA.3,4)

Recently, Rokita and Walsh described a model repair system forthe DNA lesion in which some flavin and 5-deazaflavin derivatives catalyzed the photochemical cleavage of thymine dimers.5) This prompted us to report our observation on the the effect of flavin and 5-deazaflavin derivatives (flavin dyes) on the photochemical dimerization of 1,3-dimethylthymine (1,3-DMT) as a model of DNA (Chart 1).



1,3-DMT cis-anti (1) cis-syn (2)

Chart 1. Photochemical Dimerization of 1,3-Dimethylthymine and the Cleavage of Its Dimers

Cis-anti-(1) and *cis-syn*-1,3-dimethylthymine dimer (2) were prepared according to a known procedure.6) The flavin dyes used were riboflavin (3), 5-deazariboflavin (4),7) F_{420} chromophore (5),8) and three 5-deazaflavin derivatives (6-8).9) All of these are soluble in water. Proflavine (9) and acridine (10) were used as reference compounds for inhibitory experiments (Chart 2).

The experimental procedure for the photochemical dimerization of 1,3-dimethylthymine in the presence of a flavin dye is as follows. A 0.1 M aqueous solution of 1,3-dimethylthymine (50 ml), which includes 0.005 M flavin dye, was frozen in a Petri dish (160 mm in diameter) with dry ice-acetone. The frozen solution was irradiated for 10 h with a 10 W low pressure mercury lamp (Fuji Glass) at a distance of 20 mm. The progress of the reaction was monitored by HPLC (μ-Bondapak C_{18}, CH_3CN/H_2O (1/6), UV 236nm). After the reaction, the reaction mixture was concentrated *in vacuo* and the residue was placed on a column of neutral alumina (20x250 mm) in benzene. The column was eluted successively with benzene, ether, 1% EtOH/ether, and chloroform to yield the monomer and the dimers. The results are summarized in Table I.

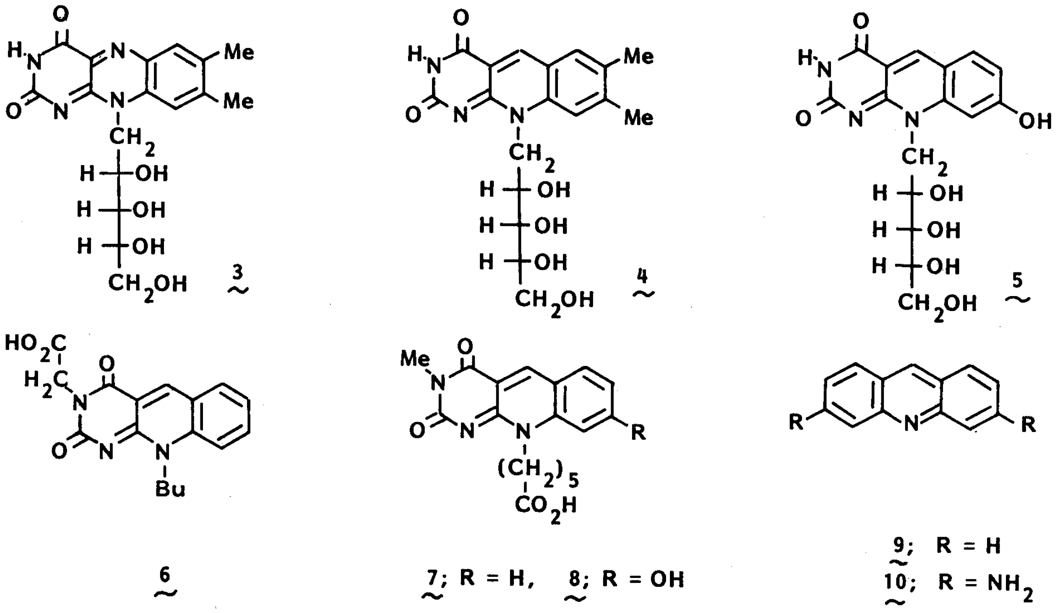


Chart 2. Flavin Dyes and Acridine Dyes

Table I. The Inhibition of 1,3- DMT Photodimerization by Flavin Dyes

Compound	Recovered 1,3-DMT (%)a)	Dimer (%)a)	
		cis-anti (1)	*cis-syn* (2)
None	73	8	14
3	93	1	1.5
4	95	0.6	1
5	96	0.7	2
6	83	3.5	8
7	89	3	4.5
8	84	3	3.5
9	84	2	5.5
10	88	5	6

a) Isolated yield.

The experimental procedure for the photochemical cleavage of 1,3-dimethylthymine dimer in the presence of a flavin dye was as follows. An aqueous solution (20 ml) containing 0.1 M 1,3-dimethylthymine dimer and 0.005 M flavin dye was frozen and then irradiated for 3 h or 10 h. The reaction mixture was worked up as described above to give the monomer (1,3-DMT) and the recovered dimer. The results are summarized in Table II.

Rokita and Walsh reported that the compound (4) catalyzed detectable thymine dimer cleavage nonenzymatically under highly basic conditions. Also Eker and coworkers10) demonstrated that 10-alkyl-8-hydroxy-5-deazaflavin, a model of F_{420} chromophore (5), could photosensitize a small amount of thymine dimer to thymine.

Table II. The Effects of Flavin Dyes on the Photochemical Cleavage of 1,3-DMT Dimers

Flavin dye	irradiation time (h)	Cleavage yield (%)[a]			
		cis-anti Dimer (1)		*cis-syn* Dimer (2)	
		Recovery of dimer	Monomer (1,3-DMT)	Recovery of dimer	Monomer (1,3-DMT)
None	3	81	19	83	17
5	3	80	20	86	14
4	3	80	20	83	17
3	3	81	19	86	14
None	10	38	62	47	53
5	10	37	63	49	51
4	10	31	69	46	54
3	10	38	62	47	53

a) Isolated yield.

Our experimental results indicate that the presence of a catalytic amount of flavin dyes significantly affects the rate of formation of 1,3-dimethylthymine dimer but does not affect its cleavage to the monomer at physiological pH levels. As can be seen in Table I, the flavin dyes that inhibit dimerization of 1,3-dimethylthymine most are riboflavin (3), 5-deazariboflavin (4) and F_{420} chromophore (5). These have the *D*-ribityl substituent on the 10 positon of the isoalloxazine skeleton. Furthermore, 5-deazaflavin derivatives (6-8), as well as the reference compounds acridine (9) and proflavine (10), are also inhibitory to some degree.

The inhibition phenomenon might be attributed to the dye-sensitized cleavage of the initially formed dimer. In order to exclude this possibility, the dimer cleavage experiments were carried out under conditions similar to those in the dimer formation. As can be seen from the results (Table II), the flavin dyes did not promote the dimer cleavage. It is also interesting that under our experimental condition, the *cis-anti*-dimer (1) splits into the monomer faster than the *cis-syn*-dimer (2) .

In conclusion, the flavin dyes used in the present study did not catalyze the cleavage of 1,3-dimethylthymine dimer at physiological pH levels. However, it is significant that the flavin dyes which possess the *D*-ribityl moiety at the 10 position on the isoalloxazine framework greatly inhibited the 1,3-dimethylthymine dimer formation. This suggests that naturally occurring flavins may prevent DNA damage by photochemical formation of thymine dimer in biological systems.

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