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Stereochemistry of Asymmetric "(Net) Hydride Transfer" in an Intercoenzyme Model Reaction System

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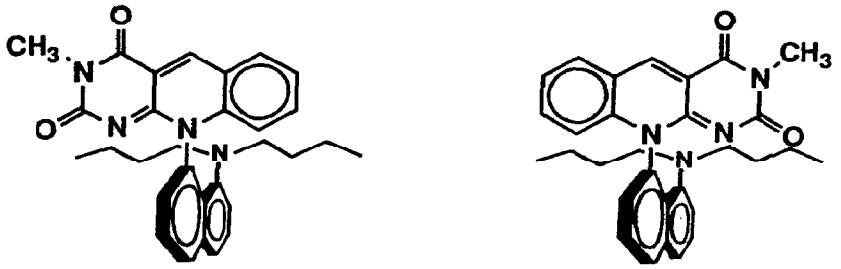
Abstract: X-ray crystal structure analysis revealed the absolute configuration of compound 2 and thus proved the absolute configuration of 5-deazaflavin (+)-1 possessing both axial and planar chirality to be (S). Model reactions of "(net) hydride transfer" between 1 and Me2PNPH enanatiomers revealed that (S)-(+)-1 oxidizes (4S)-Me2PNPH more rapidly than its (4R) isomer and that (R)-(−)-1 oxidizes (4R)-Me2PNPH more rapidly than its (4S) isomer. These results strongly suggest that an enatioselectivity on Me2PNPH by chiral 1 is not solely determined by the availability of C(4) hydrogen but by the "(net) hydride donor-acceptor" interactions involving the interaction between the pyrimidine site of 5-deazaflavin molecule and the carbamoyl group of the NAD(P)H model as the important contributor.

Flavin and 5-deazaflavin[1] are the main coenzymes which play important roles in redox reactions in biological systems. Recently, "flavoenzyme models"[2], flavin derivatives embodied in a chiral environment, have been synthesized to investigate the mechanism of asymmetric "(net) hydride transfer" reactions with substrates. Shinkai[2a] synthesized chiral (5-deaza)flavins included in cyclophane structure and we synthesized 5-deazaflavin 1[2b,c] possessing both axial and planar chirality. These models revealed that the chiral flavin faces significantly affect asymmetric "(net) hydride transfer" reactions with NAD(P)H (models). Recently, we synthesized a series of 5-deazaflavins with axial chirality at pyrimidine ring moiety[2d,e,f] and observed the successful diastereotopic face activation of 5-deazaflavin molecule and chiral recognition with and without metal assistance[2f] in the intercoenzyme model reactions. These results suggested that a stereoselective interaction between the pyrimidine site of 5-deazaflavin molecule and the carbamoyl group of NAD(P)H model significantly facilitates the asymmetric "(net) hydride transfer" reactions.

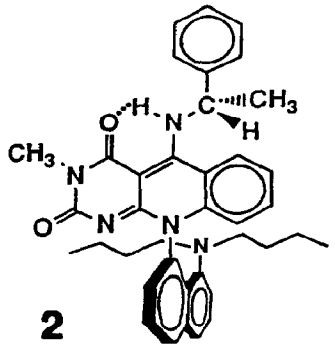
In the present paper, we wish to report the absolute configuration of a flavoenzyme model 1 and identify the stereochemistry of reactive face of each enantiomer of this model compound. The consideration of



(S)-(+)-1 Scheme 1. **(R)-(-)-1**

transition state stereochemistry of this asymmetric "(net) hydride transfer" reaction strongly suggests interaction(s) within the "(net) hydride donor-accepter" pair involving the interaction between the pyrimidine site of 5-deazaflavin molecule and the carbamoyl group of an NAD(P)H model as one of the crucial factors. The intervention of ternary complex, "dFl•Mg^{2+}•NADH model"[2e,f] was shown in kinetics studies.

Compound **2** was synthesized from (+)-**1** and (S)-(−)-α-phenylethylamine as described in a previous paper[2b] without concomitant racemization of (+)-**1** and α-phenylethyl moiety. Recrystallization of **2** from ethanol gave a pale yellow crystal which was subjected to an X-ray analysis. The structure and crystal data of **2** were shown in Figure 1. The analysis assigned (S) configuration[3] to the (+)-**1** moiety in **2** in the light of (S) configuration of α-phenylethyl moiety in **2**. In other words, for (+)-**1**, the only face open to the interaction with attacking chemical species should be assigned as *si* face with respect to C(5) position (Scheme 1.). Similarly, (R) configuration was assigned to (−)-**1** and the *re* face of 5-deazaflavin (R)-(−)-**1** opens to interact with substrates (Scheme 1.).



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Crystal Data
Formula : $C_{38}H_{41}N_5O_2$
M.W. 599.78
Orthorhombic
Space Group $P2_12_12_1$
a = 20.903(3) Å
b = 19.561(2) Å
c = 7.927(1) Å
Z = 4
D_{obs} = 1.200 g•cm^{-3}
D_{calc} = 1.229 g•cm^{-3}
V = 3241.2(1) Å^3
R = 0.042

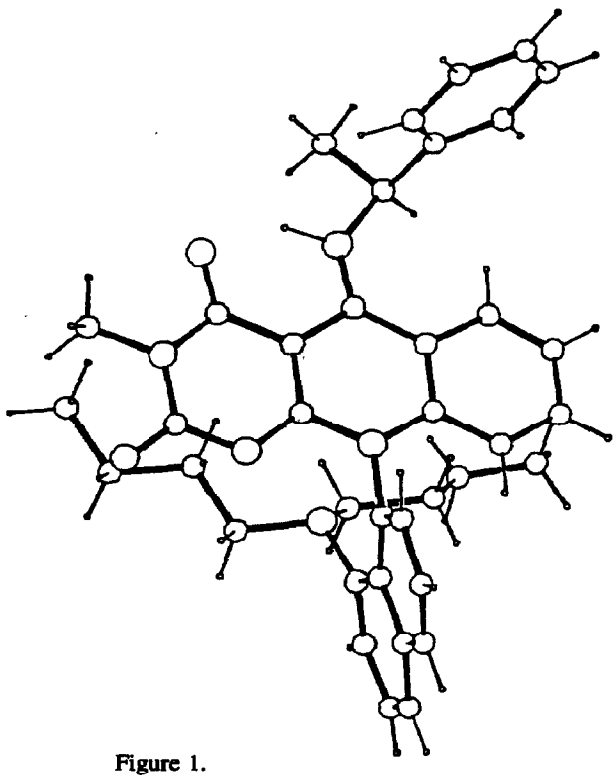
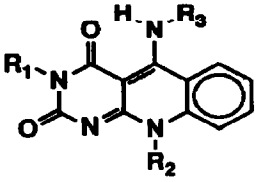


Figure 1.

There are two tautomeric isomers proposed for the 5-deazaflavin derivatives substituted by amino groups at C(5) position[4]; one is 5-amino form **3**, and the other is 5-imino form **4** (Scheme 2) and there have been controversies of this problem. The X-ray analysis showed that N(Amine)-C(5), C(5)-C(4a), C(4a)-C(10a), and C(10a)-N(1) distances are between that of single and double carbon-nitrogen bond or carbon-carbon bond[5], implying that the compound **2** takes a structure between **3** and **4**. However, the refinement showed that a hydrogen atom resides on the nitrogen atom attached at C(5) position (Figure 1), suggesting that the compound **2** takes amino form **3**, rather than imino form **4** that depicted in our previous paper[2b]. In **2** as well as **3**, the hydrogen atom on nitrogen atom attached at C(5) position forms a hydrogen bonding with

oxygen atom of the carbonyl group at C(4) position and the hydrogen bonding will fix the conformation of the chiral α-phenylethyl group and may give a significant effect on the chromatographic separation of **2** from the other diastereomer composed of (R)-(–)-**1** and (S)-(–)-α-phenylethylamine as was reported previously [2b].



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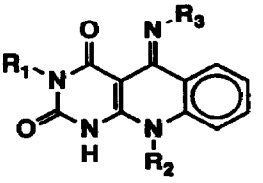

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Scheme 2.

To investigate the unequivocal stereochemistry of the asymmetric "(net) hydride transfer" reactions between flavoenzyme model **1** and an NAD(P)H model, and to have a clue on the flavoenzyme reactions, oxidation reactions by **1** of Me2PNPH[6] enantiomers were carried out. Me2PNPH enantiomers were oxidized by racemic **1** in acetonitrile in the dark under argon in the presence or in the absence of magnesium perchlorate and the ratios of the reduction products from **1** were measured by an HPLC method on a chiral stationary phase (CHIRALCEL OD) (Table 1.). Table 1 shows that magnesium ion enhances both the rate and chiral recognition of the asymmetric "(net) hydride transfer" reactions[7]. There is a tendency that (4S,9S)-Me2PNPH is oxidized by (S)-(+)-**1** more rapidly than (R)-(–)-**1**, vindicating that the 5-deazaflavin molecule (S)-(+)-**1** reacts on its *si* face with the NAD(P)H face possessing *Pro-R* hydrogen at C(4) position[8].

Table 1. Oxidation reaction of Me2PNPH by racemic **1**

Run	Me2PNPH	$Mg(ClO_4)_2$*	Reaction Time (hr)**	Reduced Product of (S)-(+)-**1** (%)	Reduced Product of (R)-(–)-**1** (%)	$k_{\psi(S)}/k_{\psi(R)}$[9]
1	(4S,9S)	0	5	65	35	1.9
2	(4S,9S)	25	1	76	24	3.2
3	(4S,9S)	100	0.5	>98	<2	>50[7]
4	(4R,9R)	0	5	33	67	1/ 2.0
5	(4R,9R)	25	1	28	72	1/ 2.6
6	(4R,9R)	100	0.5	<2	>98	<1/50[7]

* equivalent to [Me2PNPH], [(±)-**1**] = 1.5×10^{-2} (M), [Me2PNPH] = 7.5×10^{-4} (M)
** at 298K, Complete disappearance of Me2PNPH was observed by TLC.

Considering these results and that the (4R) isomer of Me2PNPH reacts more sluggishly with (S)-(+)-**1**, even when the (4R)-H was disposed potentially available for the reaction, the major transition state of this "(net) hydride transfer" reaction could be postulated as depicted in Figure 2. Inspection of the Table 1 and the Figure 2 strongly suggests that all the following 3 factors are crucial in the"(net) hydride transfer" reactions between (5-deaza)flavin models and NAD(P)H models. 1) The interaction between the pyrimidine site of (5-deaza)flavin model and the carbamoyl group of NAD(P)H model, the interaction that is operating to bring reacting partners in close proximity and to secure the subsequent interactions. 2) Availability of the C(4)-H of NAD(P)H model in the proximity of the C(5) position of (5-deaza)flavin. 3) Interaction between the rings of two coenzyme models that secure face to face spacial arrangement of the reacting partners.

Only for the combination of (S)-(+)-**1** and (4S)-Me2PNPH and that of (R)-(–)-**1** and (4R)-Me2PNPH, these 3 factors are substantiated concomitantly. For the combination of (S)-(+)-**1** and (4R)-Me2PNPH and that of (R)-(–)-**1** and (4S)-Me2PNPH, only single or two factors can be sufficed simulta-

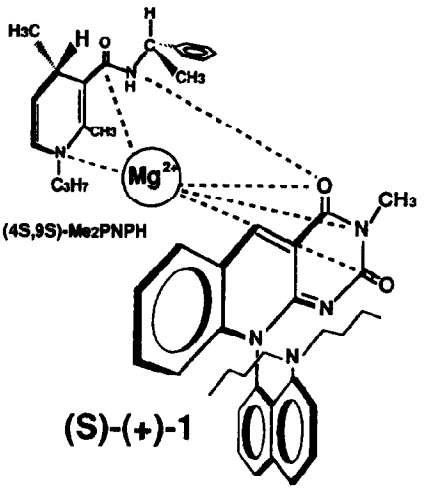

Figure 2.
Postulated Molecular Arrangement of (S)-(+)-1 and (4S,9S)-Me2PNPH in the Transition State of Asymmetric"(Net) Hydride Transfer" Reaction[10)]

neously. These mismatching conditions seem to afford much diminished reactivities as are examplified in the run 3 and the run 6 in Table 1.

Interestingly enough, the spacial arrangement of the two coenzyme models in the transition state proposed (Figure 2) is similar to that between NADPH and FAD observed at the active site of glutathione reductase reported by Pai[11)]. This similarity strongly implies that similar stereoselective interactions are operative between the two coenzyme models as well as those in enzymatic systems. The enhanced enantioselectivity observed in the present study suggests that the interactions would be strengthened by an intervention of magnesium ion in the model systems[10)] and magnesium ion plays a crucial role in an asymmetric "(net) hydride transfer" reaction in flavoenzyme models.

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5. The X-ray analysis shows the bond distances; N(Amine)-C(5), 1.337(3)Å; C(5)-C(4a), 1.426(4)Å; C(4a)-C(10a), 1.409(5)Å; C(10a)-N(1), 1.336(4)Å; C(4)-C(4a), 1.454(6)Å; and C(4)-O(4), 1.239(5)Å. These data exclude the Δ^{4-4a} enol structure for the compound 2. Final crystallographic coordinates, bond distances, bond angles, structure factors, and thermal parameters have been deposited with and can be ordered from the Cambridge Crystallographic Data Centre.
6. Ohno, A.; Ikeguchi, M.; Kimura, T.; Oka, S. *J. Am. Chem. Soc.*, **1979**, *101*, 7036-7040.
7. The similar experiments using higher concentration of (±)-1, Me2PNPH, and Mg^{2+} resulted in lower selectivities. This findings is in good agreement with the idea of competitive low selective reaction parallel to the ternary complex formation mechanism. Parallel progress of less selective reaction without magnesium ion implies that intrinsic selectivity of the reaction through the ternary complex formation mechanism is higher than that shown in Table 1 (<1/50 or >50).
8. As is the case with the report by Ohno in ref. 6, it has been found that the chirality on the ring (at C(4) position) of Me2PNPH gave far more significant effcts on the asymmetric "(net) hydride transfer" reactions with 1 than that on the side chain (at C(9) position). In separate experiments, the reactions with (4S,9R)-Me2PNPH gave similar results to those in the reactions with its (4S,9S) isomer.
9. In pseudo-first-order conditions, the products ratio is approximately equal to the ratio of pseudo-first-order rate constants.
10. Interaction between NAD(P)H model and magnesium ion is well discussed by Ohno. Ohno, A.; Kimura, T.; Yamamoto, H.; Kim, S. G.; Oka, S. Ohnishi, Y. *Bull. Chem. Soc. Jpn.*, 1977, *50*, 1535-1538. On addition of $Mg(ClO_4)_2$ in an acetonitrile solution of **1**, a blue-shift of UV-VIS spectrum was observed, suggesting an interaction between Mg^{2+} and the pyrimidine moiety of **1**. Magnesium ion most plausibly assists all the interactions shown in Figure 2.
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