Atropisomeric Flavoenzyme Models with a Modified Pyrimidine Ring

Atsuyoshi Ohno, Jun Kunitomo, and Yasushi Kawai

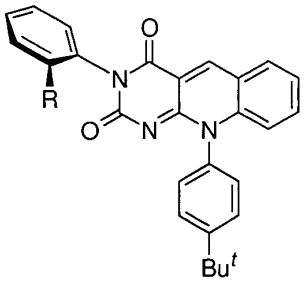
Abstract: Optically active 5-deazaflavin derivatives (3-aryl-10-(4-*tert*-butylphenyl)pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione) with an axial chirality at the pyrimidine ring have been synthesized, and the physical properties of these compounds have been investigated. In addition, (net) hydride-transfer reactions with NAD(P)H analogs have been carried out to elucidate the stereochemistry at the transition state of the reactions.

Keywords: flavin/ axial chirality/ NAD(P)H analog/ (net) hydride transfer/ stereochemistry

Flavoenzymes are the enzymes that require flavin coenzymes such as flavin adenine dinucleotide (FAD) or flavin mononucleotide (FMN) and catalyze redox reactions in biological systems. At the active site of flavoenzymes, flavin coenzymes are covalently bound or tightly held to apoproteins to form chiral environments and polar functional groups of apoproteins in proximity to a flavin coenzyme have a significant influence on stereochemistry in the reactions between the flavin coenzyme and a substrate. Thus, we synthesized atropisomeric flavoenzyme models **1**—**8** and investigated the physical properties and stereochemical reactivities of these models.

We have studied the thermal enantiomerization of **1**—**6** kinetically in order to estimate the conformational stability (1). Although the free energy of activation for thermal enantiomerization decreases in the order R = $Bu^t \gg CF_3 > Pr^i > Et > Me \approx CH_2OH$ due to steric effect, the entropy term does not contribute meaningfully to the energy barrier. Furthermore, the difference in the energy barrier between **1**—**3** is small in spite of the difference in size of the substituent of the aryl group at the N(3) position. This is probably because the benzylic protons of the aryl group face toward the flavin skeleton in order to minimize the steric repulsion, which is supported by the results of X-ray crystallographic analyses of these compounds (1—2).

By comparing the geometry of a flavin molecule in the crystal of **3** with that in the crystal of **3**·urea·ethanol that includes hydrogen bonds to the pyrimidine ring of **3**, we have simulated geometrical change observed when an oxidized flavin coenzyme is activated through hydrogen bonding with apoproteins (3). The result has revealed that when hydrogen bonds to the pyrimidine ring of the flavin are formed, both bond lengths of N(1)—C(10a) and C(4a)—C(5) (which are represented formally by a double bond)



1 : R = CH_2OH
2 : R = CH_3
3 : R = CH_2CH_3
4 : R = $CH(CH_3)_2$
5 : R = $C(CH_3)_3$
6 : R = CF_3
7 : R = CO_2H
8 : R = CH_2OTBDM

BIOORGANIC CHEMISTRY —Bioorganic Reaction Theory—

Scope of research

Biochemical reactions are studied from the viewpoint of physical organic chemistry. Namely, the reaction mechanism and stereochemistry of NAD-dependent oxidoreductases are exploed. Stereospecific redox transformations mediated by certain biocatalists such as microbes, enzymes, cultured tissues are also studied. The results will be applied to develop new organic reactions.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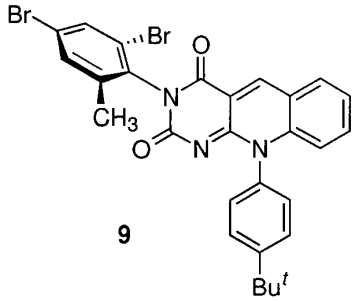
become longer by 0.023 Å, whereas that of C(10a)—C(4a) (which is represented formally by a single bond) becomes shorter by 0.021 Å than those in free **1**, respectively. This indicates that the hydrogen bonding at the pyrimidine ring affects the electronic structure of the flavin greatly: the π-electrons in the conjugated system are shifted to the N(1) position through hydrogen bonding with urea so that the geometry of the oxidized flavin can approach to that of its reduced form and that the electron density at the C(5) position is expected to become low.

It is necessary to determine the absolute configurations of these flavoenzyme models to elucidate the stereochemistry at the transition state in the reactions of these models. Thus, we synthesized **9** that was expected to maintain its conformation for a long time and confirmed that the (—)-enantiomer had the *S* configuration from the X-ray crystallographic analysis by means of anomalous dispersion effect of the bromine atoms (2). Next, (*S*)-(-)-**9** was debrominated by catalytic hydrogenation, and the resultant **2** was subjected to HPLC from which its conformation was determined to be (*R*)-(+). Finally, the (+)-**1** was converted into **2**, and the resultant **2** was subjected to HPLC, which confirmed that the compound was the (—)-enantiomer (1). Consequently, the absolute configuration of (+)-**1** has been assigned as *S*. Furthermore, all absolute configurations of **3**—**8** have been determined on the basis of circular dichroism spectra of **1** and **2**.



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In order to investigate the selectivity of the faces in which a (net) hydride is transferred, reductions of several flavoenzyme models (**1**, **2**, **6**, and **8**) with 1-benzyl-1,4-dihydronicotinamide (BNAH) were studied (1,4). In the presence of Mg^{2+}, the (net) hydride transfer from BNAH to **2**, **6**, or **8** takes place predominantly in the *anti* face, whereas the selectivity observed in the reaction of **1** is the opposite of that of **2**, **6**, or **8**. Furthermore, in the absence of Mg^{2+}, the *syn/anti* selectivity is reversed from that observed under the Lewis acid (Mg^{2+})-catalyzed reaction. The association constant of **1** with Mg^{2+} is about twice as large as that of **2**, which predicts that the hydroxymethyl group of **1** in the presence of Mg^{2+} plays a significant role in coordinating onto Mg^{2+} to form a ternary complex with BNAH rigidly in the *syn* face. On the other hand, the hydroxymethyl group in the absence of Mg^{2+} is not different from other substituents such as methyl, trifluoromethyl, and [(*tert*-butyldimethylsilyl)oxy]methyl groups in terms of interaction with BNAH in the sense that it is nothing but a sterically interfering group. Consequently, these substituents result in the deactivative *anti* preference rather than a *syn* face reaction.

In addition, we studied asymmetric (net) hydride-transfer reactions between chiral **1** and chiral 1,4-dihydro-2,4-dimethyl-*N*-(α-methylbenzyl)-1-propylnicotinamide (Me_2PNPH) to elucidate the intermolecular arrangement between **1** and an NAD(P)H analog at the transition state of (net) hydride-transfer reactions (1). The results

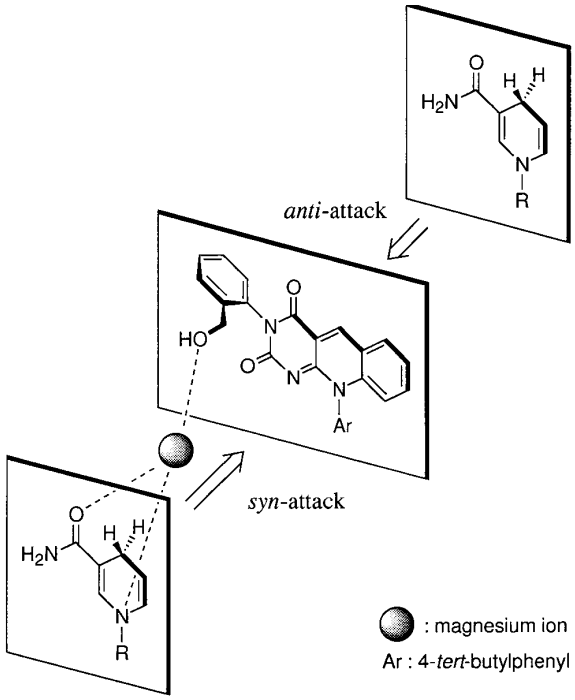

Figure 1. Most predominant intermolecular arrangements between (*S*)-(+)-**1** and an NAD(P)H analog at the transition states of (net) hydride-transfer reactions in the presence (*syn* face) and absence (*anti* face) of magnesium ion, respectively. The conformation of the side-chain carbamoyl group of NAD(P)H analog is drawn arbitrarily.

revealed that the most suitable intermolecular arrangement between **1** and NAD(P)H analog at the transition state of (net) hydride-transfer reactions is the one in which two molecules are arranged with maximum overlap of their molecular planes and the pyrimidine ring of **1** is set in front of the carbamoyl group of the analog, regardless of the presence or absence of Mg^{2+} (Figure 1). The intermolecular arrangement is similar to that reported for FAD and NADPH in the active site of glutathione reductase: the flavin moiety of FAD is stacked onto the nicotinamide ring of NADPH and the pyrimidine ring of the flavin and the carbamoyl group of the nicotinamide face each other. It is of great interest that the intermolecular arrangement can be seen in a model system even though no steric compulsion exists to arrange them in this order.

The present result strongly indicates not only a possibility that there might exist stabilizing effects due to the overlap of molecular planes of a flavin and an NAD(P)H coenzymes but also a possibility that functional groups in an apoprotein in proximity to a flavin coenzyme in the active site of a flavoenzyme have significant influence on the stereoselective interaction with a substrate.

References

1. Ohno A, Kunitomo J, Kawai Y, Kawamoto T, Tomishima M and Yoneda F, *J. Org. Chem.* **61**, 9344-9355 (1996).
2. Kawai Y, Kunitomo J and Ohno A, *Acta Crystallogr., Sect. C*, in press.
3. Kawai Y, Kunitomo J and Ohno A, *Tetrahedron Lett.* **37**, 8905-8908 (1996).
4. Ohno A, Kunitomo J, Kawamoto T, Tomishima M, Bessho K and Yoneda F, *Tetrahedron Lett.* **35**, 9729-9732 (1994).