Atropisomeric Flavoenzyme Models with a Modified Pyrimidine Ring

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Abstract: Optically active 5-deazaflavin derivatives (3-aryl-10-(4-tert-butylphenyl)pyrimido[4,5-b]quinoline-2,4(3H,10H)-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

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BIOORGANIC CHEMISTRY —Bioorganic Reaction Theory—

Scope of research
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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.

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 Mg²⁺, 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 Mg²⁺, the syn/anti selectivity is reversed from that observed under the Lewis acid (Mg²⁺)-catalyzed reaction. The association constant of selectivity is reversed from that observed under the Lewis acid catalysis.

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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 Mg²⁺ (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 coenzyme 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