Pergamon

Tetrahedron Letters, Vol. 36, No. 36, pp. 6491-6494, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(95)01298-2

The Enzyme/Coenzyme/Metal/Substrate Quaternary Complex Generated in the Transition State of the Reduction of Benzoylformate with NAD(P)H Models[1)]

Yuji Mikata,* Kiyoko Mizukami, Kenji Ikehara

Department of Chemistry, Faculty of Science, Nara Women's University, Nara 630, Japan

and Atsuyoshi Ohno

Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan

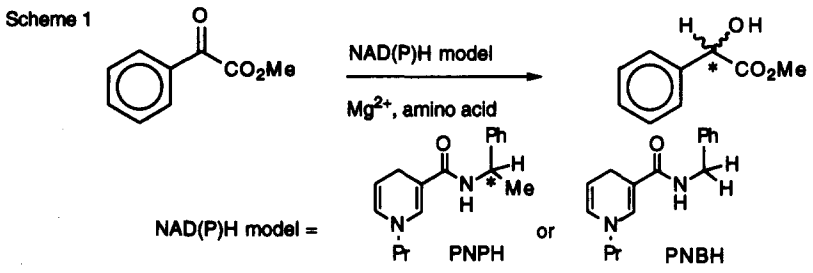
Abstract: Enantioselectivities in the reduction of methyl benzoylformate with NAD(P)H model compounds, *N*-α-methylbenzyl-1-propyl-1,4-dihydronicotinamide (PNPH) and *N*-benzyl-1-propyl-1,4-dihydronicotinamide (PNBH), in the presence of *N*-protected amino acids have been studied. The stereoselectivity was perturbed by the amino acids which have a side chain capable of coordinating onto Mg^{2+}.

Biological $NAD(P)^+$/NAD(P)H redox systems or their models have been extensively studied to elucidate the reaction mechanism as well as to explore the pathway by which excellent stereospecificity is achieved. Many of these studies have tried to mimic the stereospecificity following a pioneering work from our laboratory,[2)] in which a chiral side chain was introduced to the amide nitrogen.[3-6)] Introduction of an enantiotopic methyl group to the C-4 position of the nicotinamide ring is also effective for asymmetric induction with high ee.[7,8)] Recently, we demonstrated that molecular asymmetry with respect to the orientation of the carbonyl group is one of the most effective means to control the stereospecificity of the reaction.[9)]

Molecular recognition by protein is one of the most exciting areas in biochemistry, molecular biology and other fields in biological sciences. Nevertheless, reactions with holoenzyme models have rarely been investigated from the standpoint of organic chemistry because of the complexity that governs the activity of an enzyme. In this context, it will be interesting to study molecular interaction in a small peptide/metal ion/coenzyme model/substrate quaternary reaction system. There has been only limited investigation, however, into asymmetric induction by a noncovalently influencing third reagent in the reaction with the coenzyme or its analogs.[10,11)] Therefore, as the first step in this approach, we studied the reaction of a NAD(P)H model compound in the presence of an amino acid residue. In this paper, we wish to demonstrate the participation of a noncovalently formed enzyme/coenzyme/metal ion/substrate quaternary complex in the transition state to control the stereoselectivity of mimetic reactions. The reaction studied is

shown in Scheme 1.

Scheme 1



The reaction was performed in dry acetonitrile under argon atmosphere in the dark at room temperature for 3 days. The reaction mixture contained 100 mM of substrate and 1 equiv. each of the NAD(P)H model compound, magnesium perchlorate, and an *N*-protected amino acid. The reaction was quenched by adding water to the solution and extracting the organic materials with dichloromethane. A mixture of the product alcohol and the starting material was chromatographed on a silica gel column. Yields of materials (the substrate recovered and the product) and enantiomer excess (ee) of the product were determined on GLC with a chiral column (CP-cyclodextrin-B-236-M-19 (Chrompack®), 25 m × 0.25 mm I.D.). Results are summarized in Table 1, which demonstrates that the amino acid affects the stereochemistry of the reaction.

Table 1. Stereospecificity in the Reduction of Methyl Benzoylformate with NAD(P)H Model Compounds in the Presence of Magnesium Perchlorate and *N*-Boc Amino Acid

	(*S*)-PNPH			(*R*)-PNPH			PNBH		
Amino Acid	Ee (%)[a]	Config.	Conv. (%)[b]	Ee (%)[a]	Config.	Conv. (%)[b]	Ee (%)[a]	Config.	Conv. (%)[b]
none	22	*S*	100	22	*R*	100	0	–	100
Gly	25	*S*	24	23	*R*	36	0	–	17
Val	25	*S*	48	25	*R*	54	5	*S*	51
Thr	24	*S*	6	24	*R*	6	3	*R*	7
Ser	33	*S*	6	7	*R*	7	10	*S*	7
Met	30	*S*	27	21	*R*	26	7	*S*	25
Asp	28	*S*	14	6	*R*	5	12	*S*	7
Phe	31	*S*	28	21	*R*	28	0	–	16
Pro	28	*S*	26	25	*R*	25	0	–	28

a) Error in ee is less than ±3 %.

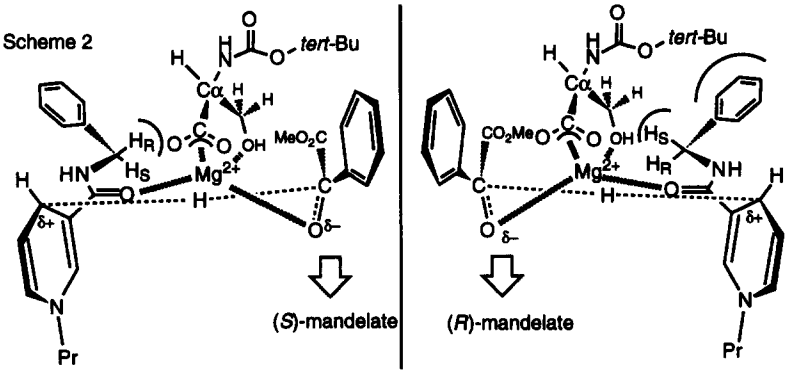
b) After 72 hours.

It is conceivable that the carboxylate part of the *N*-protected amino acid in acetonitrile coordinates onto

a magnesium ion. Consequently, an amino acid added to the reaction mixture retards the reaction in two ways: reduction of Lewis acidity of the magnesium ion and formation of a sterically hindered area around the catalyst.

Serine and aspartic acid are amino acids that contain a polar group in the molecule. The polar group can also coordinate onto the magnesium ion to play a crucial role in selecting a stereochemical reaction course. Namely, the reaction with PNBH, an achiral reducing agent, exerts enantiomeric differentiation in the presence of serine and aspartic acid. A combination of each of these amino acids and (*S*)-PNPH is sterically more favorable than the combination with (*R*)-PNPH, resulting in better enantiospecificity. This observation suggests that the transition state of the reaction is composed of a noncovalently assembled quaternary complex and that the molecular arrangement in the enantiomeric transition state is like that illustrated in Scheme 2. In contrast to this, we have demonstrated that the transition state of the reaction in

Scheme 2



the absence of an amino acid is composed of a substrate/magnesium ion/NAD(P)H analog ternary complex.[12-14] Although precise conformations and geometries of the molecules involved in the transition state are not yet established, we can envision an image of the transition state perturbed by addition of magnesium ion and amino acid in the light of results we have reported[2,7] and the stereochemistry described in this report.

On the other hand, an amino acid with a nonpolar substituent exerts no marked influence on stereochemistry, although the efficiency of the magnesium ion as the catalyst is reduced as can be seen in the decrease in the amount of substrate reacted within an appropriate reaction period. This observation clearly suggests the importance of coordination by a polar substituent of the amino acid onto the magnesium ion. Methionine and phenylalanine are exceptions. Probably, substituents in these amino acids are so large that steric hindrance prevents these amino acids from formation of a quaternary complex with (*R*)-PNPH. Thus, the combination of these amino acids with (*S*)-PNPH only improves the ee.

It should be noted that the apparent enantiospecificity reported herein is not satisfactory, because the

magnesium ion modified by an amino acid is a less effective Lewis acid than a free ion and most of the reaction proceeds under the catalysis of the latter, which results in the formation of an achiral product. It is desired, therefore, to design a peptide ligand which assists the catalytic efficiency of the magnesium ion. Asymmetric reduction of benzoylformate by $NaBH_4$, an achiral reducing agent, in the presence of bovine serum albumin, a chiral reagent, has been reported.[11] In contrast, *N*-protected serine and aspartic acid cannot provide a chiral field in the reduction with $NaBH_4$. This suggests that reducing agents such as PNBH and PNPH only can cooperate with the amino acid to create an asymmetric reaction field.

REFERENCES AND NOTES

1. NAD(P)$^+$-NAD(P)H models. 85.
2. Ohnishi, Y.; Kagami, M.; Ohno, A. *J. Am. Chem. Soc.* **1975**, 97, 4766.
3. Ohno, A.; Ushida, S. *Mechanistic Models in Asymmetric Reductions*.. In *Lecture Note in Bioorganic Chemistry*, Springer-Verlag, Heidelberg, 1986.
4. Inoue, Y.; Oda, J.; Baba, N. *Asymmetric Synthesis*, **1983**, 2, 91.
5. Burgess, V. A.; Davies, S. G.; Skerlj, R. T. *Tetrahedron: Asymm.* **1991**, 2, 299.
6. Combret, Y.; Duflos, J.; Dupas, G.; Bourguignon, J.; Quéguiner, G. *Tetrahedron*, **1993**, 49, 5237.
7. Ohno, A.; Ikeguchi, M.; Kimura, T.; Oka, S. *J. Am. Chem. Soc.* **1979**, 101, 7036.
8. de Kok, P. M. T.; Bastiaansen, L. A. M.; van Liar, P. M.; Vekemans, J. A. J. M.; Buck, H. M. *J. Org. Chem.* **1989**, 54, 1313.
9. Ohno, A.; Tsutsumi, A.; Kawai, Y.; Yamazaki, N.; Mikata, Y.; Okamura, M. *J. Am. Chem. Soc.* **1994**, 116, 8133.
10. Zehani, S.; Gelbard, G. *J. Chem. Soc., Chem. Commun.* **1985**, 1162.
11. Baba, N.; Matsumura, Y.; Sugimoto, T. *Tetrahedron Lett.* **1978**, 4281.
12. Ohno, A.; Kimura, T.; Yamamoto, H.; Kim, S. G.; Oka, S.; Ohnishi, Y. *Bull. Chem. Soc. Jpn.* **1977**, 50, 1535.
13. Ohno, A.; Nakai, J.; Nakamura, K.; Goto, T.; Oka, S. *ibid.* **1981**, 54, 3482.
14. Ohno, A.; Kobayashi, H.; Goto, T.; Oka, S. *ibid.* **1984**, 57, 1279.

(*Received in Japan* 9 *May* 1995; *revised* 7 *July* 1995; *accepted* 8 *July* 1995)