NAD(P)$^+$-NAD(P)H Model. 39. Asymmetric Reduction by 1,4-Dihydronicotinamide Derivative Bound to Protein

Atsuyoshi OHNO,* Satoshi USHIDA, and Shinzaburo OKA
Institute for Chemical Research, Kyoto University, Uji, Kyoto 611

(Received August 18, 1982)

1,4-Dihydronicotinamide derivatives covalently bound to NH_2 or SH group of proteins such as reduced keratin (RK), egg white albumin (EWA), and bovine serum albumin (BSA) have been synthesized and subjected to the reductions of α,α,α-trifluoroacetophenone derivatives. Chiral alcohol is obtained in the reaction with 1,4-dihydronicotinamide modified by the SH group of BSA.

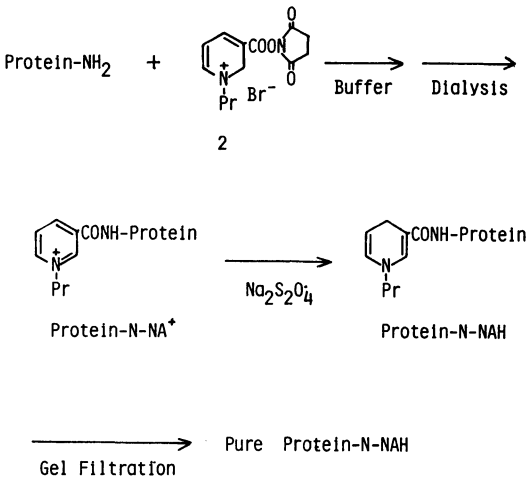
Since our first report on an asymmetric reduction by a model compound of NAD(P)H,[1] many 1,4-dihydronicotinamide derivatives that have chiralities in the molecules have been synthesized.[2,3] Among asymmetric reductions with these model compounds some can compete, in terms of stereospecificity, with enzymic reactions.[3] However, the enzymic reaction is still much more superior to the model reactions in versatility and in reaction rate. Moreover, the stereospecificity in enzymic reactions that require NAD(P)H as a cofactor does not stem from the chirality in NAD(P)H, but from the chiral environment constructed by the enzymes. In fact, NAD(P)H-dependent dehydrogenases are classified into two categories depending on which of hydrogens on the 4-position of 1,4-dihydronicotinamide they utilize during the reduction.[4] The apoenzyme thus plays an important role for the formation of reaction field.

It is interesting to study how far the field effect, exerted by enzymes can be mimicked by organic chemistry, that is, by model compounds. At the same time, it is worthy for organic chemists to study the scope of asymmetric reaction by the use of abundant natural products.

Previously, we reported the syntheses and reactions of 1,4-dihydronicotinamide derivatives covalently bound to protein.[5] In the present work we attempted asymmetric reductions by the use of models covalently linked to proteins. Baba and his coworkers already reported the production of chiral alcohol in the reduction of α,α,α-trifluoroacetophenone (**1**) by 1-propyl-1,4-dihydronicotinamide (PNAH) in aqueous solution of bovine serum albumin (BSA).[6] Kaiser and his coworkers synthesized flavopapain[7] where the active site of the hydrolytic enzyme was utilized for the reaction site of flavin and the modified enzyme was found to exert a dehydrogenase-like property.[8]

Results

Modification of NH_2 Group in Proteins. The NH_2 groups in certain proteins were modified by 1,4-dihydronicotinamide. *N*-Hydroxysuccinimide ester of *N*-propylnicotinic acid (**2**) was employed for the modification as shown in Scheme 1. Thus obtained protein has an absorption maximum at 360 nm which is virtually identical with those of other 1,4-dihydronicotinamide derivatives.[4] This 1,4-dihydronicotinamide derivative of protein can reduce several substrates such as Malachite Green, chloranil, and *N*-methylacridinium iodide in buffered aqueous solution.



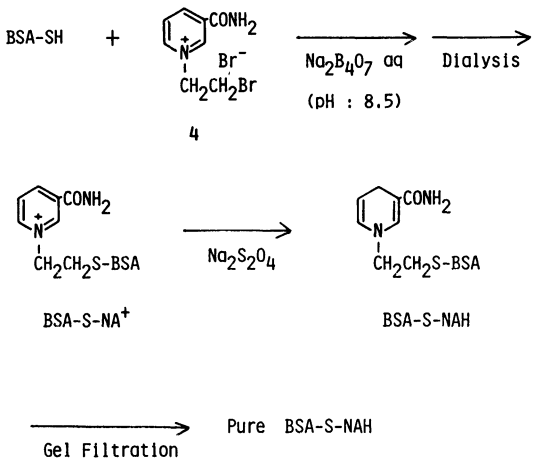
Scheme 1.

Table 1. Reductions of α,α,α-trifluoroacetophenone derivatives by BSA-N-NAH

pH[a]	Substrate	[BSA] / mmol dm^{-3}	Molar ratio A[b]	Molar ratio B[c]	Yield/%[d]
8.0	**3**	0.34	3.0	11	19
8.0	**3**	0.31	1.9	23	26
6.5	**3**	0.69	0.82	15	27
6.0	**3**	0.37	0.65	12	15
6.0	**1**	0.41	0.66	3	6

a) For modification reaction. b) [dihydropyridine]/[BSA] determined from the absorbance at 360 nm (ε=5900). c) [Substrate]/[BSA]. d) Based on 1,4-dihydropyridine moiety.

The NH_2 groups of *S*-carboxymethylated reduced keratin from human hair (CMRK), *S*-(2-ammonioethyl)ated reduced keratin from wool (AERK), and egg white albumin (EWA) were similarly modified with 1,4-dihydronicotinamide and the proteins were subjected to the reductions of **1** and *m*-trifluoromethyl-α,α,α-trifluoroacetophenone (**3**). The reduction was carried out in an aqueous solution of 0.05 mol dm^{-3} $Na_2B_4O_7$ containing about 2×10^{-4} mol dm^{-3} of 1,4-dihydronicotinamide moiety and $(4—8)\times10^{-3}$ mol dm^{-3} of the substrate. The produced alcohol was converted into the ester of (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid ((*R*)-(+)-MTPA)[9] and the ratio of two diastereoisomers was determined on GLC. Unfortunate-



Scheme 2.

ly, thus obtained alcohols were racemic in all cases.

Modification of NH_2 Group in BSA. BSA, a carrier protein, is known to act as a chiral environment for certain reactions.[10] On this basis we synthesized 1,4-dihydronicotinamide derivative covalently bound to the NH_2 groups in BSA (BSA-N-NAH). As shown in Table 1, modification was carried out under different pH's to change the number of dihydronicotinamide moiety in a molecule of BSA ([dihydronicotinamide]/[BSA]). The alcohols obtained from the reductions of **1** and **3** were, however, again racemic.

Modification of SH Group in BSA. It has been accepted that BSA contains one SH group in one of three hydrophobic domains.[11] We attempted to modify the SH group with 1,4-dihydronicotinamide by using 1-(2-bromoethyl)-3-carbamoylpyridinium bromide (**4**) according to Scheme 2[12] and obtained BSA-S-NAH with a UV-absorption maximum at 355 nm. That only the SH group was modified and that no other residues of BSA such as $-NH_2$ and $-OH$ were influenced under the reaction conditions employed were confirmed by the following three results: 1) The concentration of modified 1,4-dihydropyridine moiety was comparable to that of original SH group in BSA. 2) When a same procedure was applied to BSA whose SH group had been protected by a carboxymethyl group, no product with an absorption maximum at 355 nm was obtained. 3) L-Cystein reacts with **4** under similar conditions to afford the corresponding sulfide, 1-[2-[(2-amino-2-carboxyethyl)thio]ethyl]-3-carbamoylpyridinium salt. After gel filtration BSA-S-NAH was subjected to the reductions of **1** and **3** at pH 9.4. As shown in Table 2, it was found that thus modified BSA induces chirality in the reduction products and that stereoselectivity increases as the molar ratio of the substrate to BSA decreases.

Table 2. Reaction of α,α,α-Trifluoroacetophenone derivatives with BSA-S-NAH[a]

Substrate	[BSA] mmol dm^{-3}	Molar ratio A[b]	Molar ratio B[c]	e.e./%	Config.
1	0.69	0.52	13	5	*R*
3	0.67	0.48	13	5	*R*
1	1.0	0.32	3.1	10	*R*
1	1.1	0.29	3.0	11	*R*
3	1.1	0.32	2.9	17	*R*
3	1.0	0.32	3.1	15	*R*

a) Reaction was run in 0.05 mol dm^{-3} $Na_2B_4O_7$ (pH: 9.4) at room temperature. The yield of alcohol was about 5% based on 1,4-dihydropyridine moiety. b) [Dihydropyridine]/[BSA] determined from the absorbance at 355 nm (ε=5500). c) [Substrate]/[BSA].

Discussion

The basic idea underlying the present study is to use chiral environment around 1,4-dihydronicotinamide moiety for biomimetic reductions by means of chemical modification on protein, as enzymes do.

When simple proteins, such as RK and EWA, are subjected to modification no chiral products were obtained. In the case of BSA, which is presumed to have much higher degree of functionality than the others, modification of its SH group by 1,4-dihydronicotinamide affords chiral products but modification of its NH_2 group does not. It has been established that BSA contains one SH group in one of three hydrophobic domains. Therefore, by SH modification it must be possible to set a 1,4-dihydronicotinamide moiety into a single chiral circumstance. On the other hand, since BSA and other proteins that we used contain various kinds of NH_2 groups, it seems difficult to carry out the modification selectively. Even if each of modified 1,4-dihydronicotinamide moiety sits itself in a chiral field, the chiralities may be neutralized totally because of a variety of types in modifications. Since NH_2 groups in different environment have different basicities, it is expected that the modification of NH_2 groups under different pH might result in selectively modified protein. However, the attempt was not successful as seen in Table 1.

Alternatively, it is also possible that chiral fields supplied by the protein locate far from the 1,4-dihydronicotinamide moiety, because most of free NH_2 groups stick out of the main domain of the protein.

The present result with BSA-S-NAH is the first example of biomimetic reduction where the chirality in the protein-1,4-dihydronicotinamide complex is transferred, as is observed in enzymic reactions, into a substrate during the reaction in water. Results shown in Table 2 reveal that the reaction between BSA-S-NAH and free substrate does not exert stereoselectivity. On the other hand, the reaction of a substrate trapped on BSA and free reductant ($NaBH_4$ or PNAH) exerts stereoselectivity of about 40% e.e.[6,10] Thus, it seems likely that protein-protein interaction between BSA which binds a substrate and BSA which binds 1,4-dihydronicotinamide is important in the present reaction to determine the stereoselectivity.

There is another possibility; for a bimolecular reaction in micellar system, it is assumed that when one reagent is incorporated into one micelle and the other reagent into another micelle, no reaction takes place because each reagent has no chance to collide each other.[13] Similarly, a substrate incorporated into BSA

might not be able to find a chance to react with 1,4-dihydronicotinamide which is bound to BSA. If this is the case, the substrate which is trapped into a 1,4-dihydronicotinamide-containing crevice of BSA is the only substrate that can undergo the asymmetric reaction. The situation is quite similar to that of ternary complex in an enzymic reaction.

In any case, there is no doubt that the reduction proceeds in a chiral field offerred by such a protein as that which is quite different from dehydrogenases. Although the present result is not satisfactory, it promises further success by appropriate use of crevice in a protein. It should be noted, however, that in the asymmetric reduction with a chiral 1,4-dihydronicotinamide derivative, the interaction between reduced and oxidized forms of the model compound is important in order to exert high stereospecificity.[14,3] If similar situation operates in the present system, high stereoselectivity cannot be expected with BSA-S-NAH because extraordinarily bulky substituent prevents the model compound from the formation of aggregates and more sophisticated design will be necessary for the model compound.

Experimental

Melting and boiling points were not corrected.

Instruments. UV, NMR, and mass spectra were obtained on Union Giken SM-401, JEOL JNMFX-100, and Hewlett Packard 5992B GC/MS spectrometers, respectively. A Yanaco G-1800F was used for GLC analyses. Preparative-scale GLC separations were carried out by using Varian Aerograph Model 920.

Materials. BSA was Fraction V grade from Armour. α,α,α-Trifluoroacetophenone (**1**) (bp 423 K) was prepared according to the literature procedure.[15] *m*-Trifluoromethyl-α,α,α-trifluoroacetophenone (**3**) was prepared from *m*-trifluoromethylphenylmagnesium bromide and trifluoroacetic acid.[16] (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid was purchased from Aldrich Chem. Co. and was converted into the corresponding chloride (MTPACl) by the use of thionyl chloride.[9]

S-Carboxymethylated Reduced Keratin from Human Hair (CMRK). Human hair (5.0 g) was reductively solubilized by sodium mercaptoacetate (5.0 g) in aqueous sodium sulfide (200 cm^3, pH: 12.0). After being kept for 5 d at room temperature under nitrogen atmosphere, 3.15 g of sodium borohydride was added and the solution was allowed to stand for a day. Carboxymethylation of free SH groups was effected by the addition of iodoacetic acid (8.2 g) at pH 8.5. Thus obtained CMRK was subjected to the modification after dialysis against 2 dm^3 of borate buffer for several times.

Reduced Keratin from Wool Whose SH Group Was Protected with (2-Bromoethyl)trimethylammonium Bromide (AERK).[17] Wool (1.7 g) was reductively solubilized using 2-mercaptoethanol (1.2 g) in 100 cm^3 of 8 mol dm^{-3} urea–0.01 mol dm^{-3} Tris in the presence of 0.001 mol dm^{-3} EDTA. The solution was stirred for 3—5 h at room temperature under nitrogen atmosphere. To the solution 3 g of (2-bromoethyl)trimethylammonium bromide was added. After being kept for a day the solution was dialyzed against 2 dm^3 of borate buffer for several times. Thus obtained solution of AERK was subjected to further modification.

N-Hydroxysuccinimide Ester of N-*Propylnicotinic Acid (2).* Dicyclohexylcarbodiimide (10.3 g) was added to a suspension of nicotinic acid (6.15 g) and *N*-hydroxysuccinimide (5.75 g) in 150 cm^3 of dioxane. The precipitate was separated by filtration. The dioxane was removed from the filtrate under reduced pressure to give *N*-hydroxysuccinimide ester of nicotinic acid quantitatively. Thus obtained ester (10 g) was dissolved in propyl bromide. The mixture was refluxed for a day. The precipitate was separated, washed with anhydrous acetone, and kept *in vacuo* to constant weight (9.4 g, 60% yield).

1-(2-Bromoethyl)-3-carbamoylpyridinium Bromide (4). A mixture of nicotinamide (0.60 g) and 1,2-dibromoethane (7 cm^3) was stirred at 313—323 K for 5 d. The solid was collected and extracted with hot methanol. After the removal of the solvent, the crude product was washed with acetone. Twice recrystalization from methanol gave 0.40 g of **4** (mp 465—467 K).

Found: C, 31.13; H, 3.28; N, 8.95%. Calcd for $Br_2C_8H_{10}N_2O$: C, 31.00; H, 3.25; N, 9.04%.

^1H NMR (D_2O, δ from TSP): 4.09 (2H, t, J=6 Hz), 5.22 (2H, t, J=6 Hz), 8.27 (1H, m), 8.80—9.10 (2H, m), 9.28 (1H, bs).

Modification of NH_2 Group in CMRK, AERK, and EWA. To a buffered solution of a protein (20 cm^3) about 100 mg of **2** was added. After the dialysis against 2 dm^3 of 0.05 mol dm^{-3} $Na_2B_4O_7$ for a day modified pyridinium ion was reduced with sodium dithionite (*ca.* 20 mg). Excess dithionite was removed by gel filtration on a column of Bio-gel P-6.

Modification of NH_2 Group in BSA. The same procedure as described above was employed for a 0.05 mol dm^{-3} $Na_2B_4O_7$ solution of BSA (0.5 mmol dm^{-3}). The amount of **2** and pH of the buffered solution were changed in compliance of the cases.

Modification of SH Group in BSA. To a solution of BSA (1.5 mmol dm^{-3}) in 0.05 mol dm^{-3} $Na_2B_4O_7$ (50 cm^3, pH: 8.5), 50 mg of **4** was added. The BSA contained 0.45—0.55 free SH groups in a molecule. The solution was kept at room temperature under nitrogen atmosphere for about 3 d until no SH group could be detected by DTNB-method. After the dialysis against 2 dm^3 of 0.05 mol dm^{-3} $Na_2B_4O_7$ for several times, 50 mg of sodium dithionite was added to the solution of BSA-S-NA$^+$. Excess dithionite was removed by gel filtration on a column of Bio-gel P-6 to afford pure BSA-S-NAH.

UV-Absorption of Modified Protein. Thus obtained modified protein exerts an absorption maximum at around 360 nm due to 1,4-dihydronicotinamide moiety. The amount of 1,4-dihydronicotinamide moiety in the protein was determined by titration with *N*-methylacridinium iodide on monitoring the absorption at around 360 nm.

Reactions of α,α,α-Trifluoroacetophenone Derivatives. To a solution of modified protein **1** or **3** was added. The solution was stirred for 1—2 weeks until UV-absorption at around 360 nm disappeared. The mixture of the products and starting material was extracted with ether.

Determination of Enantiomer Excess. The ether solution of extracted reaction mixture was concentrated to 0.1 cm^3 under atmospheric pressure and the enantiomeric alcohols were separated by preparative GLC on a PEG column and collected as a solution of carbon tetrachloride (0.1—0.2 cm^3). The alcohol was subjected to esterification by (*S*)-(+)-MTPACl[9] and the mixture of diastereomeric esters was submitted to the GLC analysis. Since the esterification is diastereotopic reaction, it should be confirmed that all the starting alcohol is esterified. Otherwise, GLC analysis does not give correct result.

Diastereomeric esters of **5** and **6** (**7** and **8**, respectively) were separated on GLC (10% DEGS, 1 m). The peaks were identified by comparison with those of authentic **7** and **8**. The

structures of **7** and **8** were also identified by GC/MS analyses. The value of enantiomer excess was determined from the ratio of peak areas for the diastereomeric esters. The ratio for racemic **7** and **8** were 50 : 50, respectively.

References

1) Y. Ohnishi, M. Kagami, and A.Ohno, *J. Am. Chem. Soc.*, **97**, 4766 (1975).

2) J. W. ApSimon and R. P. Seguin, *Tetrahedron*, **35**, 2797 (1979); T. Endo, H. Kawasaki, and M. Okawara, *Tetrahedron Lett.*, **1979**, 23; N. Baba, J. Oda, and Y. Inouye, *Bull. Inst. Chem. Res., Kyoto Univ.*, **58**, 379 (1980).

3) A. Ohno, M. Ikeguchi, T. Kimura, and S. Oka, *J. Am. Chem. Soc.*, **101**, 7036 (1979); b) M. Seki, N. Baba, J. Oda, and Y. Inouye, *ibid.*, **103**, 4613 (1981).

4) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," W. A. Benjamin Inc., New York, N. Y. (1966), Vol. 2, p. 301.

5) A. Ohno, S. Ushida, and S. Oka, *Tetrahedron Lett.*, **21**, 2969 (1980).

6) N. Baba, Y. Matsumura, and T. Sugimoto, *Tetrahedron Lett.*, **1978**, 4281.

7) H. L. Levine and E. T. Kaiser, *J. Am. Chem. Soc.*, **100**, 7670 (1978).

8) H. L. Levine and E. T. Kaiser, *J. Am. Chem. Soc.*, **102**, 343 (1980).

9) J. A. Dale, D. L. Dull, and H. A. Mosher, *J. Org. Chem.*, **34**, 2543 (1969).

10) T. Sugimoto, T. Kokubo, Y. Matsumura, J. Miyazaki, S. Tanimoto, and M. Okano, *Bioorg. Chem.*, **10**, 104, 311(1981).

11) H. H. Hull, R. Chang, and L. J. Kaplan, *Biochim. Biophys. Acta*, **400**, 132 (1975); J. R. Brown, *Fed. Proc.*, **35**, 2141 (1976).

12) Similar reagent is reported for the modification of SH group: H. A. Itano and E. A. Robinson, *J. Biol. Chem.*, **247**, 4819 (1972).

13) C. A. Bunton, "Applications of Biochemical Systems in Organic Chemistry," ed by J. B. Jones, C. J. Sih, and D. Perlman, John Wiley and Sons, Inc., New York, N. Y. (1976), Part 2, Chap. 4, pp. 731—814.

14) A. Ohno, T. Kimura, S. Oka, and Y. Ohnishi, *Tetrahedron Lett.*, **1978**, 757.

15) J. D. Park, H. A. Brown, and J. R. Lacher, *J. Am. Chem. Soc.*, **73**, 709 (1951).

16) R. Stewart and R. van der Linden, *Can J. Chem.*, **38**, 399 (1960).

17) J. H. Bradbury, *Adv. Protein Chem.*, **27**, 111 (1973).