

Title	NADH Model Studies : 1,4-Dihydronicotinamide Covalently Bonded to a Cyclic Peptide, Bacitracin (Commemoration Issue Dedicated to Professor Tatsuo Yamamoto on the Occasion of his Retirement)
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Citation	Bulletin of the Institute for Chemical Research, Kyoto University (1980), 58(3): 379-381
Issue Date	1980-05-31
URL	http://hdl.handle.net/2433/76893
Right	
Type	Departmental Bulletin Paper
Textversion	publisher

Communication

NADH Model Studies: 1,4-Dihydronicotinamide Covalently Bonded to a Cyclic Peptide, Bacitracin

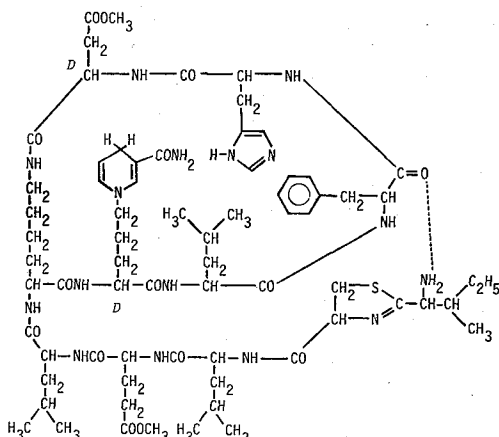
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Received March 21, 1980

KEY WORDS: NADH model compound/ Bacitracin!

Dehydrogenase catalyzed hydrogen transfer between NAD(H) coenzyme and substrates has still been a mystery despite extensive works on the enzyme active site structure and the reaction mechanism as well as coenzyme model studies. The latter approaches are: search for potential substrates which can be reduced or oxidized without enzymes, optimization of experimental reaction parameters for rate enhancement, and introduction of a microenvironment similar to the local structure of enzyme active site into the NADH model compound for activation of 1,4-dihydronicotinamide. The details are well reviewed by Kill and Widdowson¹⁾ and Sigman *et al.*²⁾

These studies have added much to new development of dihydropyridine chemistry. However, we might not be able to rely for ever on such approaches based on approximations of active sites of enzyme as sophisticated models since the function of dehydrogenase enzyme is essentially dependent on highly organized structure of protein. Now it is important to survey effect of any peptide or protein on chemical activation of NADH model compound as well as to survey what stereochemical



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effects of the peptide are expected as chiral environment in asymmetric induction involving the NADH model.³⁾

Hopefully, for the purpose, ensembling both 1,4-dihydronicotinamide part and substrate at the intended site of the peptide through non-covalent bindings would be desirable. However, this seems not feasible by the synthetic techniques presently available. Therefore, we decided to attach dihydronicotinamide covalently to a peptide as a first approximation. A well-known antibiotics, bacitracin was chosen as a promising candidate since the peptide is known to form a coordination complex with zinc ions through chelation with imidazole and thiazoline groups, so that the bacitracin system may provide a model for metal ion activation of enzymes.⁴⁾

Two carboxyl groups of bacitracin were esterified with methanol according to Swallow and Abraham.⁵⁾ The methyl ester hydrochloride was made salt-free by IRA-400 in methanol, and the methanol solution was treated with Zincke salt.⁶⁾ Thus, nicotinamide nucleus was introduced into ornithine side chain of the peptide. The nicotinamide hydrochloride (oxidized form of the NADH model compound) was reduced with sodium hydrosulfite to give the reduced form as light yellow powder. UV, λ_{\max} : 354 nm (ϵ , 4190, methanol) with fluorescence characteristic of dihydronicotinamide. $[\alpha]_D^{20} - 19.6^\circ$ (c , 1.575, methanol). 2.4 g (37 %). Mp. 230–235°C (decomp.).

Using this model compound, we attempted reduction of benzaldehyde to benzyl alcohol in the presence of zinc chloride, cobalt chloride and magnesium perchlorate as catalyst in a mixture of dry methanol and dry acetonitrile (2:1) at 60°C for a week. However, the reduction product, benzyl alcohol, could not be detected by v.p.c. analysis. On the other hand, the NADH model could reduce ethyl benzoylformate in the presence of magnesium perchlorate at room temperature for 20 days in dry methanol. The chemical yield of ethyl mandelate was 41.3 % and the product configuration was *R* in 5.4 % enantiomeric excess. The same reduction using an excess of magnesium perchlorate in acetonitrile for 12 days at room temperature afforded the product in 26.9 % chemical yield and 1.9 % enantiomeric excess with *R* predominant. Thus, indeed the asymmetric synthesis was successfully effected by the peptide, but the asymmetric bias observed was rather moderate under the conditions employed here. The use of methanol in the former run and of an excess of metal salt in the latter was necessary for complete dissolution of the NADH model. As Guthrie⁷⁾ pointed out, a less polar environment may be required for more effective electrostatic interaction responsible for a tight transition state in stereochemical-determining step. In fact, two examples are known in which the use of excess metal salt and of hydroxylic solvent considerably depressed the enantiomeric excess in asymmetric NADH model reactions.⁸⁾ So that the poor performance found for the present system may probably be attributed to the inadequate reaction conditions of necessity employed here. Accordingly, one of the future problem to be solved is how to realize the present NADH model reaction in homogeneous, non-polar media.

Although the present study did not afford any exciting result, this constitutes

the first example of NADH model covalently bonded to a natural cyclic peptide and will certainly help design novel models of growing interest⁹⁾ in this field.

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