Tetrahedron Letters No. 32, pp 3005 - 3008. 0040-4039/79/0801-3005$02.00/0

REGIO-SPECIFIC BEHAVIOUR IN ELECTROCHEMICAL TWO-ELECTRON REDUCTION OF NAD^+ ANALOGS

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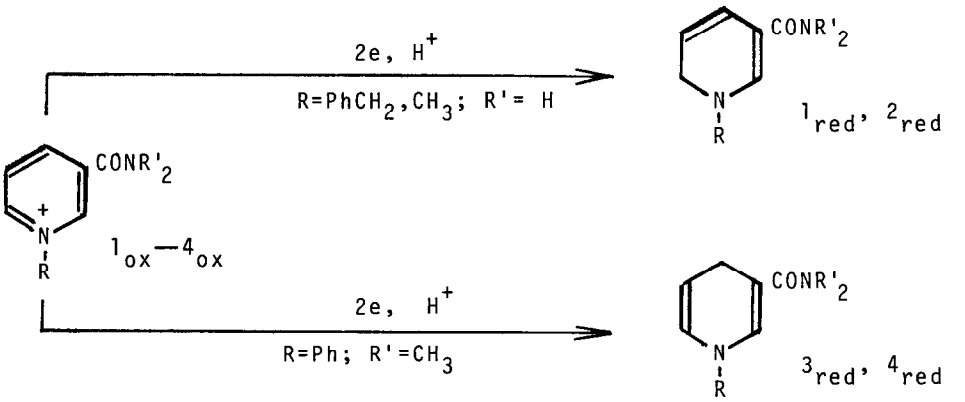
ABSTRACT Conflicting specificities were observed in electrochemical 2e-reductions of NAD^+ analogs: 1-phenyl-3-aminocarbonylpyridiniums gave quantitatively 1,4-dihydronicotinamides whereas 1-benzyl and 1-methyl derivatives gave 1,6-isomers.

As a 1,4-dihydronicotinamide represented by the reduced nicotinamide adenine dinucleotides, NAD(P)H, is formally 2e-reduction product of the corresponding pyridinium cation, electrochemical reductions of NAD^+ and related models have been studied actively[1-3]. However, the structure of the electrolysis product(s) has been postulated only with UV-spectroscopic study which is attended with difficulty and uncertainty because of the similarity of the spectra of 1,4- and 1,6-dihydronicotinamides[4,5]. In the case of NAD^+, the formation of 1,4-NADH was proposed on the basis of the evidence that the product has partially enzymatic activity[2]. However, it is possible that the 1,4-dihydro form arises *via* the isomerization by non-enzymatic transhydrogenation[6] between NAD^+ and 1,6-NADH which might be formed initially in the electrolysis. In model system, the similar isomerization has been observed[7]. Thus, electrochemical behaviour of NAD^+ and related models is still ambiguous[8]. The electrolysis can be utilized for our aim: non-enzymatic conversion from NAD^+ model to the reduced form with 1,4-specificity. Here, we wish to report success of this attempt and remarkable effect of the substituent on the ring nitrogen of nicotinamide.

The electrolyses of NAD^+ model (0.5 mmol) were carried out at -1.80V[9] (*vs.* SCE) in pH 7.4 McIlvaine's buffer (15 ml) with a magnetic stirring in a three-compartment cell using a mercury pool as the cathode[10]. Product analyses were performed with NMR spectrometers. Electrolysis of 1-benzyl-3-aminocarbonyl-

pyridinium, 1_{ox}, for 10 min (*ca.* 90% conversion) in the presence of benzene[11] (15 ml) gave quantitatively a mixture of the 1,4- and 1,6-dihydronicotinamides, 1,4-1_{red} and 1,6-1_{red}, in the ratios of 2/8 and 1/9 in duplication. This difference may be due to the isomerization taking place before the primary product has been extracted with benzene. Since 1,4/1,6 composition at the equilibrium state was reported as 9/1 in acetonitrile[12], the ratio must be smaller than 1/9. The electrolysis in D_2O gave 1,6-1_{red}-$6d_1$ (*ca.* 100% deuterium content), indicating that the 1,6-isomer is a primary product but not an isomerization product. In the case of 1-methyl-3-aminocarbonylpyridinium, 2_{ox}[3] using D_2O, a mixture of the 1,4- and 1,6-dihydronicotinamides, 2_{red}, both of which were deuterated on C_6, was obtained. The incorporation of deuterium onto C_6 of the 1,4-isomer demonstrates that the 1,4-isomer is formed from the 1,6-isomer *via* the isomerization with a large isotope effect[13]. Consequently, it was established that electrochemical 2e-reductions of 1_{ox} and 2_{ox}, commonly used as NAD^+ models, afford inherently the 1,6-dihydronicotinamides.



On the other hand, 1-phenyl-N,N-dimethyl-3-aminocarbonylpyridinium, 3_{ox}, afforded the 1,4-dihydronicotinamide, 1,4-3_{red}, in quantitative yield accompanying a negligible amount of the 1,6-dihydro form. The electrolysis in D_2O giving 1,4-3_{red}-$4d_1$ (*ca.* 100% deuterium content) proved that the 1,4-form was produced directly from the pyridinium cation. Furthermore, 1-phenyl-3-aminocarbonylpyridinium, 4_{ox}, in D_2O also gave the corresponding 1,4- and 1,6-dihydro compounds, 4_{red}, (9:1) containing a deuterium on C_4.

The present results show that the 1,6-specificity in electrochemical 2e-reduction is regarded as an attribute of usual 3-aminocarbonylpyridinium while the phenyl substituted 3-aminocarbonylpyridinium were electrolyzed to give the 1,4-dihydro compounds with complete specificity. That is, the electrolyses of the usual NAD^+ models afford only 1,6-NADH analogs which is different from 1,4-reduction of enzymatic processes, whereas the behaviour of phenyl substituted NAD^+ analogs are similar to them. Furthermore, the result from the electrochemical reduction of 3_{ox} is different from those in chemical reactions with sodium borohydride[13] and alkoxides[14] producing initially the 1,2- and 1,6-dihydro forms. Though the borohydride reduction may perform with hydride attacking onto the pyridinium, the present electrolysis presumably proceeds *via* e-e-H^+ mechanism[2] involving the formation of carbanion. On the other hand, the reduction with an alkoxide involves pyridinyl radical intermediate, which is supported by formation of the dimers as by-products[13]. If the e-e-H^+ mechanism is true in the electrolyses, the opposing behaviour can be explained in terms of electronic and/or steric effect induced by the substituent on the ring nitrogen of the carbanion. It is also possible that the effect of the mercury surface exerted on phenyl group of the phenyl substituted model is particularly important to bring about the 1,4-specificity in the electrolyses. In order to obtain an evidence for convincing explanation of the remarkable observations, further investigations are underway.

References and Notes

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9) Polarographic secound waves of the NAD^+ analogs used were below -1.80V.

10) The anode was a coil of Pt wire and the reference electrode was a saturated calomel electrode (SCE). The catholite was isolated from the anolite with a glass filter (G4) and agar gel.

11) Benzene was added in order to avoid the isomerization between the dihydropyridine(s) arosen and the pyridinium cation.

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(Received in Japan 1 May 1979)