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MODEL OF FORMATE-DEPENDENT BIOLOGICAL PROCESSES

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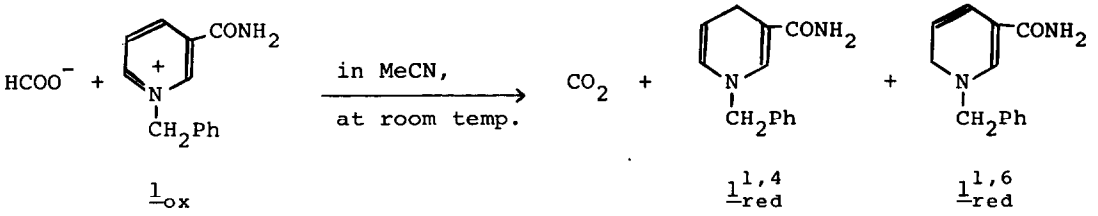
Formic acid or formate is known to be oxidized enzymatically in mammalian cells, plant tissues, and bacteria, in conjugation to reduction of substrates such as NAD^+, hydrogen peroxide, ferredoxin and other electron acceptors.[1] It is also used as a reducing agent in chemical reactions.[2] Taking into account the significance of hydrophobic character at a reaction site in many enzymatic systems, we are naturally interested in studying the mimetic reactions of formate-dependent biological processes with the formate anion in non-aqueous media with or without crown ethers or related compounds. We now wish to report non-enzymatic reduction of NAD^+ and biomimetic reductions of NAD^+-model and other electron acceptors.

A model compound of NAD^+, 1-benzyl-3-carbamoylpyridinium, $\underline{1}_{ox}$, chloride, in aqueous solution is not reduced at room temperature by formic acid or its anion. However, a solution of dry acetonitrile (130 ml) containing the perchlorate salt of $\underline{1}_{ox}$ (3.0 mmol), potassium formate (3.0 mmol) and 18-crown-6 ether (6.0 mmol) at room temperature under anaerobic condition afforded 1-benzyl-3-carbamoyl-1,4-dihydropyridine, $\underline{1}^{1,4}_{red}$, in 80% yield after 90 hr. At the same time, the formation of the 1,6-dihydro isomer, $\underline{1}^{1,6}_{red}$, with the yield of 20% was also observed. These products were identified by their nmr spectra. Carbon dioxide was isolated as barium carbonate in a reasonable yield.

Similar treatment of NAD^+ with formate anion, arisen from large excess of potassium formate and 18-crown-6 ether, in acetonitrile for 2 days was found to



$\underline{1}_{ox}$ $\underline{1}^{1,4}_{red}$ $\underline{1}^{1,6}_{red}$

afford NADH, which was recognized with uv-absorption (349 nm) and fluorescence emmision (430 nm) spectra.

The analyses with high pressure liquid chromatography (HPLC) revealed that combinations of $HCOO^-M^+$ ($M^+ = Na^+, K^+, NH_4^+$, and H^+) and crown ethers, (2,2,2)-cryptate, triethylamine, or polymeric phosphoramide (5a and 5b)[3] were also effective for the biomimetic reductions of $\underline{1}_{ox}$. In each case, the formation of the 1,6-dihydro isomer in 20% yield was recognized in addition to the 1,4-isomer. The results are summerized in Table 1.

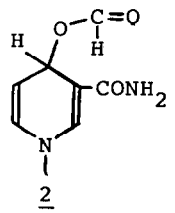
However, the second order rate constant, 4.9×10^{-4} M^{-1} sec^{-1}, for the formation of the 1,4-isomer in the reduction with $HCOOH\text{-}Et_3N$ (1:1.55 molar ratio) at 40°C was one third of that, 1.7×10^{-3} $M^{-1}sec^{-1}$, obtained with the HCOOK-18-crown-6 ether system (1:10 molar ratio).[4] This difference indicates that the naked formate anion is more effective than the ion-paired one.

Further, we found that the naked formate reduces readily phenazine methosulfate, methylene blue, tetrazolium perchlorate, and perchlorate of methyl viologen to give their corresponding *leuco* forms. The formate dehydrogenase from *Escherichia coli* catalyzes the reduction of these electron acceptors.[5]

Gizzi and Joullié reported the reduction of 1-benzyl-3-carbamoylquinolinium chloride by $HCOOH\text{-}Et_3N$ at elevated temperature.[6] The reduction afforded the corresponding 1,4-dihydro compound with the direct hydrogen transfer from the formate to the chloride. These results predict that a reaction site of formate-dependent enzymatic processes lies in hydrophobic surroundings. The present finding makes it also possible to propose that the naked formate is elaborated by chelation of a counter cation with the enzyme.

It is well known that in all living matters NAD(P)H exists overwhelmingly in the 1,4-dihydro form[7] and it can be assumed that the reduction of NAD^+ catalyzed by formate dehydrogenase includes the complete regiospecificity for the formation of 1,4-NADH in analogy with most of NAD^+-dependent dehydrogenases. In this sence, the present mimicry is not sufficient for an excellent model, but is of interest in connection of the mechanism of enzymatic reductions.

The isomerization between the 1,4- and 1,6-isomers catalyzed by $\underline{1}_{ox}$[8] was found to lead the equilibrium composition of 91 and 9 for the former and the latter, respectively, as shown in Table 1. Thus, it is evident that the isomer distributions observed in the present reductions are all controlled kinetically.



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The preferential formtion of the 1,4-isomer in the reduction of $\underline{1}_{ox}$ with formate or dithionite may be interpreted as follows: these anions attack the 4-position of $\underline{1}_{ox}$ forming a σ-adduct, such as 2, followed by rapid decarboxylation or elimination of sulfur dioxide.[11] The susceptibility of the 4-position to

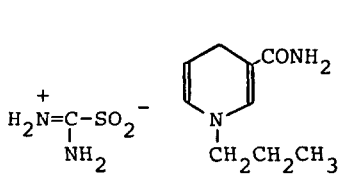
the nucleophile attack is well known.[12]

On the other hand, since boron has large affinity to nitrogen, the reduction of $\underline{1}_{ox}$ with borohydride ion seems to proceed with initial formation of a boron-ring nitrogen bonding. The four-center-type mechanism is most plausible for this reduction.

Table 1. Ratios of the 1,4- and 1,6-dihydro isomers in reduction of 1-benzyl-3-carbamoylpyridinium cation[a] by various reducing agents at room temperature.

Reducing Agent	Media	Ratio of $\underline{1}^{1,4}_{red}$ / $\underline{1}^{1,6}_{red}$
$NaBH_4$	Na_2CO_3 / aq. MeOH	67 / 33 [e]
Bu_4NBH_4	MeCN	67 / 33 [e]
$Na_2S_2O_4$	Na_2CO_3 / aq. MeOH	99.9/0.1
compound $\underline{3}$ [b]	Na_2CO_3 / aq. MeOH	99.9/0.1
HCOOH	Et_3N / MeCN	80 / 20
$HCOO^-M^+$ (M^+= K^+, Na^+ or NH_4^+)	18-crown-6 or 15-crown-5 ether, (2,2,2)cryptate, compound $\underline{5}$a or $\underline{5}$b / MeCN	80 / 20
compound $\underline{1}^{1,4}_{red}$	MeCN	91 / 9
compound $\underline{1}^{1,6}_{red}$ [c]	MeCN	91 / 9
compound $\underline{4}$ [d]	MeCN	91 / 9

a) Chloride and perchlorate salts were used in aqueous and non-aqueous media respectively. b) This is a new system for the reduction of pyridinium cation. Reducing power of this compound is larger than that of sodium dithionite.[9] c) Containing 30% of the 1,4-isomer. d) Containing ca. 3% of the 1,6-isomer. e) The values at the initial stage[10] when 1/4 of the equivalent molar amount of the reducing agent was used.



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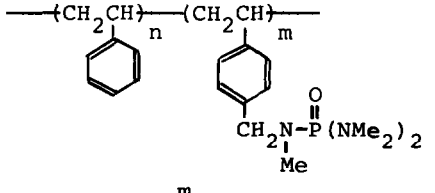
$\underline{4}$



$\underline{5}$a : $\frac{m}{n+m} = 0.29$

$\underline{5}$b : $\frac{m}{n+m} = 0.47$

The elucidation of the origin of the different regiospecificity for mimetic reductions may provide an information of the mechanism of exclusive regiospecificity in enzymatic reduction of $NAD(P)^+$. The research toward this end is in progress.

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10) In spite of many previous works, the borohydride reduction of pyridinium cations have been equivocal. We found that a mixture of $\underline{1}_{ox}$ and sodium borohydride ($\leq 1/4$ equimole) gave $\underline{1}^{1,4}_{red}$ and $\underline{1}^{1,6}_{red}$ without or with negligible small amounts of the 1,2-isomer and tetrahydro derivatives. The isomer ratio in the initial mixture (~1 min) was 30/70 and then increased up to 91/9 at equilibrium state.

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