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REDUCTION OF CARBONYL SUBSTRATES BY 3-CARBOXY-N-BENZYL-1,4-DIHYDROQUINOLINE: BIFUNCTIONALITY IN THE GENERAL-ACID CATALYZED NADH MODEL REDUCTION

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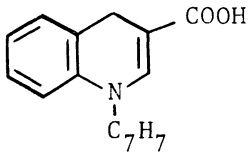
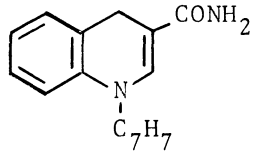
The title NADH analogue acts as a bifunctional reducing reagent under appropriate reaction conditions. The remarkable efficiency of the intramolecular carboxyl group as general-acid was demonstrated.

It has been claimed that some NADH model reductions are subject to general-acid catalysis.[1-4] The investigation involves some important biological implications with regard to the action of glyceraldehyde-3-phosphate dehydrogenase, since it is presumed on the basis of X-ray crystallographic studies that the protonated imidazole of the histidine residue in the active site acts as a general-acid during the reduction process.[5] It thus seems that the mechanism merits further investigation.

It has been noticed that some of the rate acceleration caused by an enzyme may be imitated by intramolecular catalysis.[6] As part of a study to examine the hypothesis, we synthesized a NADH analogue with an intramolecular carboxyl group, 3-carboxy-N-benzyl-1,4-dihydroquinoline(BzlQ(COOH)H) and examined the reactivity toward carbonyl substrates in comparison to the corresponding monofunctional NADH analogue, 3-aminocarbonyl-N-benzyl-1,4-dihydroquinoline(BzlQH). The acid-stable quinoline structure[3,4] was chosen in order to avoid the kinetic complexity which may arise from acid-catalyzed decomposition of 1,4-dihydronicotinamide.[7]

BzlQ(COOH)H: 

BzlQH: 

BzlQ(COOH) was synthesized as follows: 3-aminocarbonyl-N-benzylquinolinium bromide[4] was hydrolyzed to 3-carboxy-N-benzylquinolinium salt($BzlQ^+(COOH)$) under an alkaline condition(chloride salt: mp(dec) 168 °C). $BzlQ^+(COOH)Cl^-$ was

reduced by N-propyl-1,4-dihydronicotinamide in methanol to give BzlQ(COOH)H in 67% yield: mp(dec) 165°C. BzlQ(COOH)H obtained gave satisfactory elemental analyses and spectral (NMR and IR) properties consistent with the assigned structure.

The reduction of hexachloroacetone (HCAc) was conducted at 30°C in acetonitrile, whereas that of trifluoroacetophenone(TFAcPh) at 50°C in ethanol. The reactions were performed under the pseudo first-order condition(excess substrate), and the progress of the reaction was followed spectrophotometrically at 338 nm for BzlQ(COOH)H and 336 nm for BzlQH. As described previously[3,4] the overall reaction rate is given by Eq. 1.[8]

$$v_{obsd} = k_2'[\text{Substrate}]x[\text{BzlQ(COOH)H or BzlQH}]$$

$$= (k_2 + k_{ga}[\text{Acid}])[\text{Substrate}]x[\text{BzlQ(COOH)H or BzlQH}]$$

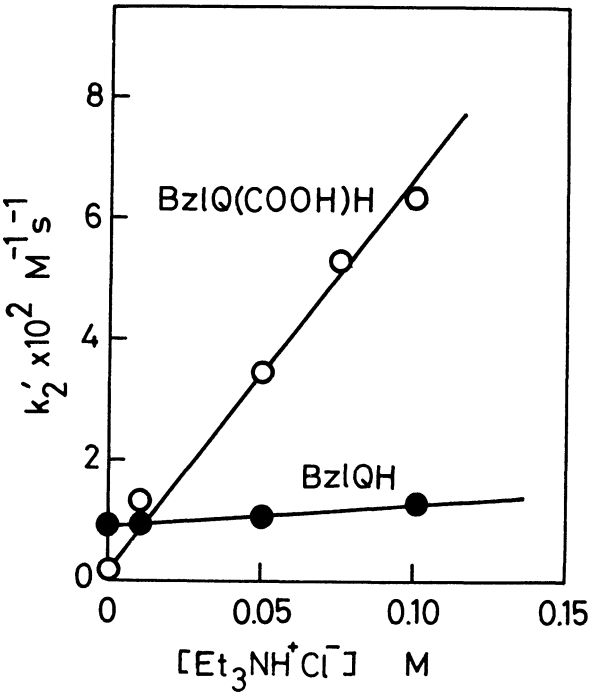
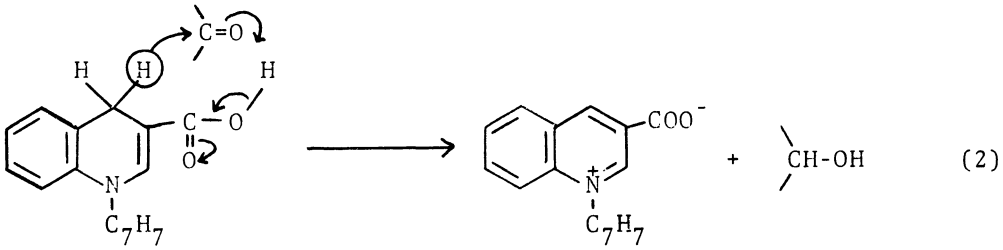


Fig. 1. Reduction of HCAc in acetonitrile at 30°C. [BzlQ(COOH)H] = [BzlQH] = 5.0×10^{-5} M, [HCAc] = 0.5 M.

where k_2 is the second-order rate constant in the absence of acids and k_{ga} the general-acid catalyzed third-order rate constant. The k_{ga} was obtained by plots of apparent second-order rate constant(k_2') against acid concentration ($[CH_3COOH]$=0-1.5 M and $[Et_3NH^+Cl^-]$=0-0.15 M). The typical examples are recorded in Fig. 1. The k_2 and k_{ga} values thus determined are summarized in Table 1.

In case the 3-carboxyl group behaves as a general-acid source in an unbuffered solution, a zwitterionic species ($BzlQ^+(COO^-)$) would result (Eq. 2).



(2)

$BzlQ^+(COO^-)$

In general, the formation of such zwitterionic species in nonpolar solvents is presumed to be energetically-unfavorable. On the other hand, Hajdu and Sigman[9] reported that the negatively charged carboxylate which is fixed "closely" near N(1) atom of dihydronicotinamide is able to accelerate the reduction reaction in

Table 1. The rate constants for the BzlQH and BzlQ(COOH)H reduction

Solvent	Reaction	k_2 ($\times 10^3$ $M^{-1}s^{-1}$)	k_{ga} ($\times 10^3$ $M^{-2}s^{-1}$)	
			CH_3COOH	$Et_3NH^+Cl^-$
CH_3CN[a]	BzlQH+HCAc	9.0	3.4	37
	BzlQ(COOH)H+HCAc	1.1	1.7	570
C_2H_5OH[b]	BzlQH+TFAcPh	($<10^{-4}$)	—[c]	0.43
	BzlQ(COOH)H+TFAcPh	0.21	0.46	0.74

[a] 30°C. [b] 50°C. [c] The disappearance of the absorbance of BzlQH did not obey the first-order equation.

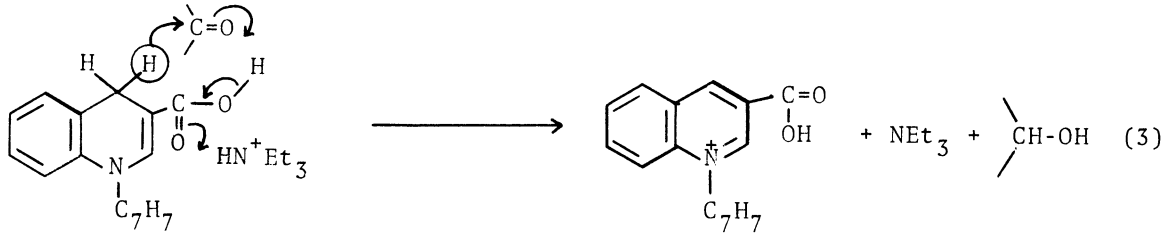
nonplar solvents due to the stabilization of the partial positive charge which develops on the nicotinamide moiety in the transition state.

As shown in Table 1, the k_2 value for BzlQH reduction of HCAc in acetonitrile is 8 times greater than that by BzlQ(COOH)H. This is readily accomodated by the electron-withdrawing nature of COOH stronger than that of $CONH_2$.[10] It is implicated, therefore, that the 3-carboxyl group in acetonitrile acts as a "simple" substituent and the stabilization of the cationic charge by 3-carboxylate is not the case.

Since the zwitterionic species is more solvated in ethanol (polar solvent) than in acetonitrile, Eq. 2 becomes more energetically-favorable. Table 1 indicates that the reduction of TFAcPh by BzlQH was undetected under the present reaction conditions, while it was reduced by BzlQ(COOH)H with the k_2 value of 2.1×10^{-4} $M^{-1}s^{-1}$. Clearly, participation of the 3-carboxyl group as general-acid is allowed in ethanol solvent. Since the k_2 value for BzlQH + TFAcPh is less than 10^{-7} $M^{-1}s^{-1}$, the rate augmentation amounts to more than three orders of magnitude. The efficiency is equivalent to BzlQH reduction in the presence of 0.5 M $Et_3NH^+Cl^-$.

The efficiency of intermolecular acid catalysis is expressed by the k_{ga} value in Table 1. In acetonitrile, the k_{ga} value for BzlQH + CH_3COOH is greater than that for BzlQ(COOH)H + CH_3COOH. The trend is compatible with the above observation that the 3-carboxyl group acts as a simple, electron-withdrawing substituent. On the contrary, the k_{ga} value for BzlQ(COOH)H + $Et_3NH^+Cl^-$ is greater by 15-fold than that for BzlQH + $Et_3NH^+Cl^-$. Also extraordinary is that in BzlQH reduction $Et_3NH^+Cl^-$ catalysis is only 10 times more effective than CH_3COOH catalysis, whereas in BzlQ(COOH)H reduction $Et_3NH^+Cl^-$ catalysis is enhanced, in fact, by 335-fold relative to CH_3COOH catalysis. It is thus conceivable that the 3-carboxyl group participates in the hydrogen transfer step of BzlQ(COOH)H + $Et_3NH^+Cl^-$.

As a tentative mechanism, Eq. 3 may be offered in which the hydrogen transfer is assisted by the 3-carboxyl group which accepts a proton from HN^+Et_3 through the tautomeric form.



(3)

It is interesting to presume why $Et_3NH^+Cl^-$ is able to participate in Bz1Q(COOH)H reduction and CH_3COOH is not. $Et_3NH^+Cl^-$ is classified as a stronger acid than CH_3COOH in nonpolar solvents, while their acidities are comparable in ethanol[2,11] where the k_{ga} values are also comparable (Table 1). It is most probable, therefore, that the acid which adopts the reaction mode of Eq. 3 must have the acidity stronger than the 3-carboxyl group.

A conclusion derived from foregoing summaries is that the efficiency of the NADH model reduction can be improved greatly by introducing the concept of the intramolecular catalysis which is believed to be a main factor of enzymatic reactivities.[6] Further applications are now devoted in this laboratory.

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