Macrocyclic Enzyme Model System. Kinetic Activity of [20]Paracyclophane Bearing 1,4-Dihydronicotinamide and 2-Pyridinecarboxylic Acid Moieties as Effected by Zinc Ion†

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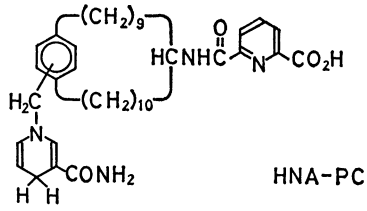
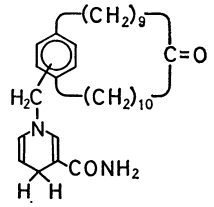
As regards the effect of zinc(II) ion on the reduction of hexachloroacetone, the kinetic activity of a [20]paracyclophane (PCP) bearing 1,4-dihydronicotinamide (HNA) and 2-pyridinecarboxylic acid (Py) moieties, HNA–PCP–Py, has been investigated as an alcohol dehydrogenase model in reference to that of PCP–HNA in the light of their metal-coordination behavior. The reduction ability of PCP–HNA was significantly lowered as it underwent complex formation with zinc. On the other hand, HNA–PCP–Py showed an apparent rate maximum in a relatively lower concentration range of $ZnCl_2$. The kinetic behavior was analyzed on the basis of the formation of two kinds of zinc complexes of HNA–PCP–Py: the 1:1 complex $\left(\begin{array}{c}\text{Py}\\ \text{PCP}\quad \text{Zn}^{\text{II}}\\ \text{HNA}\end{array}\right)$, in which both Py and HNA moieties are simultaneously coordinated to the same zinc ion, showed a decreased reactivity relative to metal-free HNA–PCP–Py; while the 2:1 complex (HNA–PCP–Py–Zn^{II}–Py–PCP–HNA), in which HNA is free from metal-coordination, exercised a much enhanced activity, 7 times as reactive as metal-free HNA–PCP–Py. A plausible reaction mechanism for the enhanced reactivity has been discussed.

The NAD-dependent alcohol dehydrogenase contains zinc ion as an essential cofactor, and catalyzes the conversion of carbonyl compounds into alcohols and the reversed process. Such interconversion reactions are coupled with oxidation-reduction of the coenzyme. It is generally realized that direct coordination of the enzyme-bound zinc ion to an incorporated substrate promotes the hydrogen transfer between the coenzyme and the substrate,[1–3] and that there is no direct metal–coenzyme interaction in the enzyme active site.[3–5] In line with the participation of zinc ion in the enzyme system, metal ions have been observed to catalyze the reduction of *chelating* carbonyl substrates by simple NADH models[6–15] along with detailed kinetic analysis for relevant systems.[7,8,14] Recently, we have pointed out that a good alcohol dehydrogenase model needs to exhibit such a substrate–zinc interaction that arises from an enforced proximity effect even if the substrate has no or least intrinsic capability of coordination with zinc ion.[16] This situation can be attained if the dihydronicotinamide and the zinc ion are placed in an appropriate and fixed orientation owing to some rigid framework. Along this line, we have prepared a macrocyclic enzyme model involving both dihydronicotinamide and 2-pyridinecarboxylic acid moieties, the latter being an intramolecular zinc-binding site, and clarified its zinc-coordination behavior.[16] In the present work, we assessed the reactivities of zinc complexes of the [20]paracyclophane-based alcohol dehydrogenase model (HNA–PCP–Py) in the reduction of a simple carbonyl substrate in relation to that of a related compound having only a dihydronicotinamide moiety, PCP–HNA, in the light of their metal-coordination behavior.



HNA-PCP-Py



PCP-HNA

Experimental

Melting points were measured using capillary tubes with a Yamato MP-1 melting point apparatus (oil-bath type). Infrared spectra and ^1H NMR spectra were taken on a JASCO IR-E spectrophotometer and a Hitachi R-20 spectrometer, respectively. Electronic absorption spectra were obtained with a Union Giken SM-401 high-sensitivity spectrophotometer.

Materials. 1-[10(11)-(2-Carboxy-6-pyridylcarbonylamino)[20]paracyclophan-22-ylmethyl]-1,4-dihydronicotinamide (HNA–PCP–Py) and 1-[10(11)-oxo[20]paracyclophan-22-ylmethyl]-1,4-dihydronicotinamide (PCP–HNA) were prepared as described previously.[16] 1-Benzyl-1,4-dihydronicotinamide (BNAH) was prepared according to the published procedure.[17] Dichloromethane was fractionally distilled and stored over 4 Å molecular sieve. Hexachloroacetone was distilled (bp 110 °C/5.3×10^3 Pa) and zinc chloride of the highest quality was fused just before use.

Reduction of Hexachloroacetone with BNAH. A solution of BNAH (0.80 g, 3.7 mmol) and hexachloroacetone (1.0 g, 3.7 mmol) in dichloromethane (25 ml) was placed in a stoppered reaction vessel protected from room light, stirred for 9 h, and allowed to stand for 42 h at room temperature.

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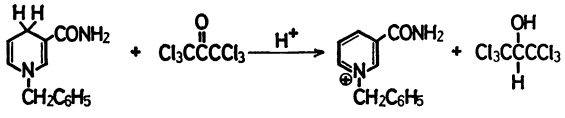
Dilute hydrochloric acid (3%, 10 ml) was added to the solution, and the mixture was extracted with refluxing hexane (450 ml) for 7 h. The solvent was removed *in vacuo* and an oily residue was sublimed at 80 °C/1.1×10^{4} Pa to recover colorless needles of 1,1,1,3,3,3-hexachloro-2-propanol (282 mg, 28%), mp 88.0—88.5 °C (lit,[18] 86—87 °C); IR spectrum being identical with that of the authentic sample obtained by LAH reduction of hexachloroacetone.[18]

The reaction behavior was monitored by ^{1}H NMR spectroscopy. Hexachloroacetone (30 μl, 2.0×10^{-4} mol) was added to a solution of BNAH (31 mg, 1.4×10^{-4} mol) in deuteriochloroform (300 μl), containing 5% toluene as an internal reference, in an NMR tube. After the reaction mixture was allowed to stand at room temperature for 1 h, ^{1}H NMR signals attributable to BNAH disappeared completely on one hand and a proton signal referred to the 2-proton of the alkoxide product (4.90 ppm downfield from Me_4Si) was observed to appear on the other. The latter signal was shifted to 4.81 ppm upon addition of deuterium oxide (30 μl). The signal intensity indicated quantitative formation of the alcohol.

Kinetic Measurements. A 30-μl sample of a stock solution of hexachloroacetone (1.0 mol dm^{-3}) in dichloromethane was injected into a reaction medium [dichloromethane–methanol (100:1 v/v), 3 ml], containing either PCP–HNA or HNA–PCP–Py (1.0×10^{-4} mol dm^{-3}) and an appropriate amount of $ZnCl_2$, placed in a thermostated cell (at 25.0± 0.1 °C) set in the spectrophotometer. The absorption intensity at 370 nm, characteristic of the dihydronicotinamide moiety, was measured to pursue the progress of reaction. An apparent first-order rate constant was obtained from the slope of the first-order plot for an early stage of the reaction. Control experiments indicated that no reaction took place in the absence of the carbonyl substrate. Hexachloroacetone having a broad absorption band at around 300 nm was independently shown to be practically free from spontaneous decomposition in dichloromethane–methanol (100:1 v/v), while underwent rapid decomposition (ketalization) in methanol. Kinetic measurements in methanol were not carried out due to such reason.

Results and Discussion

Hexachloroacetone is one of the activated carbonyl compounds which can be reduced with 1,4-dihydronicotinamides.[19,20] The reaction of hexachloroacetone with 1-benzyl-1,4-dihydronicotinamide in dichloromethane afforded 1,1,1,3,3,3-hexachloro-2-propanol quantitatively with concomitant formation of the oxidized nicotinamide salt, as shown below.



The reaction between the present cyclophane-1,4-dihydronicotinamide (PCP–HNA or HNA–PCP–Py, 1.0×10^{-4} mol dm^{-3}) and hexachloroacetone (1.0×10^{-2} mol dm^{-3}) in dichloromethane–methanol (100:1 v/v) was investigated kinetically both in the presence and absence of $ZnCl_2$. At lower concentrations of the zinc salt, the addition of water and/or methanol to the C_5–C_6 double bond of the dihydronicotinamide moiety also took place, leading to the formation of a solvolyzed product having an absorption maximum at around 290 nm. Since the solvolyzed product was not detected in the absence of the carbonyl substrate, it must be derived from the nicotinamide–substrate radical pair which was formed through one-electron transfer from the dihydronicotinamide moiety to the substrate.[21,22] The absorption maximum characteristic of the metal-free 1,4-dihydronicotinamide moiety appeared at 354.5 nm and shifted to somewhat longer wavelength as $ZnCl_2$ was added. Thus, the decrease in absorbance at 370 nm was measured to determine the reaction rate. The initial rate constants for reactions with PCP–HNA and HNA–PCP–Py are plotted in Fig. 1 against the total concentration of $ZnCl_2$. As described previously for the coordination equilibrium involving PCP–HNA and zinc ion,[16] the sole existing species in the concentration range of $[Zn^{II}]>1\times10^{-3}$ mol dm^{-3} is a Zn^{II} complex at a 1:1 molar ratio of ligand to metal (PCP–HNA–Zn^{II}) and both the metal-free species and the zinc complex are present at the intermediate zinc

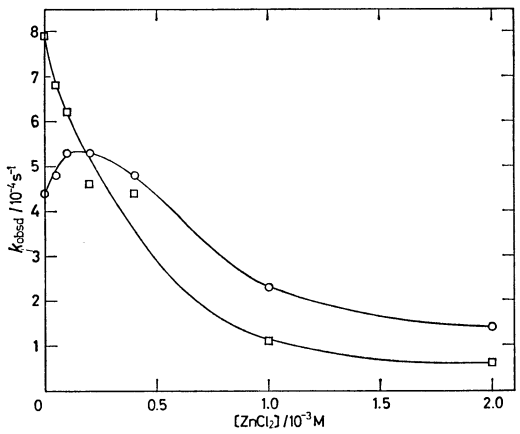


Fig. 1. Correlations between total concentration of $ZnCl_2$ and apparent first-order rate constant for reduction of hexachloroacetone (1.0×10^{-2} mol dm^{-3}) with HNA–PCP–Py (○) (1.0×10^{-4} mol dm^{-3}) and PCP–HNA (□) (1.0×10^{-4} mol dm^{-3}) in dichloromethane–methanol (100:1 v/v) at 25.0 °C.

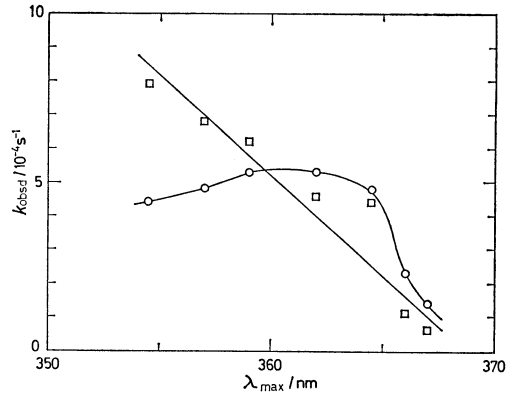


Fig. 2. Correlations between λ_{max} of the dihydronicotinamide moiety and rate constant for reduction of hexachloroacetone (1.0×10^{-2} mol dm^{-3}) in the presence of the same varying amounts of $ZnCl_2$: HNA–PCP–Py (○), 1.0×10^{-4} mol dm^{-3}; PCP–HNA (□), 1.0×10^{-4} mol dm^{-3}; in dichloromethane–methanol (100:1 v/v) at 25.0 °C.

Table 1. Specific first-order rate constants for reactions of dihydronicotinamide species with hexachloroacetone (1.0×10^{-2} mol dm^{-3}) at 25.0 °C

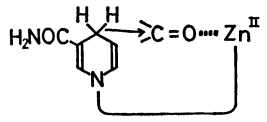
Dihydronicotinamide	Medium	k/s^{-1}
PCP-HNA	CH_2Cl_2-CH_3OH (100 : 1 v/v)	7.9×10^{-4}
PCP-HNA-Zn^{II}	CH_2Cl_2-CH_3OH (100 : 1 v/v)	0.6×10^{-4}
HNA-PCP-Py	CH_2Cl_2-CH_3OH (100 : 1 v/v)	4.4×10^{-4}
	CH_2Cl_2	2.9×10^{-4}
PCP$<^{\text{Py}}_{\text{HNA}}>$$Zn^{II}$	CH_2Cl_2-CH_3OH (100 : 1 v/v)	1.4×10^{-4}
HNA-PCP-Py-Zn^{II}-Py-PCP-HNA	CH_2Cl_2-CH_3OH (100 : 1 v/v)	3×10^{-3}
	CH_2Cl_2	2×10^{-3}

concentration range. The rate constant for the reaction with PCP–HNA monotonously decreases as the concentration of zinc ion is raised until it levels off beyond $[Zn^{II}]>1\times10^{-3}$ mol dm^{-3}. It is apparent that PCP–HNA–Zn^{II} has a reduced reactivity (by a factor of 13) compared with PCP–HNA. A nearly linear correlation between rate constant and λ_{max} of the PCP–HNA species obtained by changing the zinc concentration (Fig. 2) also indicates that the formation of the zinc complex with the dihydronicotinamide moiety of PCP–HNA is responsible for the diminished rate, and the medium effect has no direct concern. Whatever the mechanistic details may be,[13,23–31] the reduction with dihydronicotinamide is an electron-donating process and the deactivation of PCP–HNA upon complex formation with Zn^{II} is readily understood on this basis.[20]

As for the HNA–PCP–Py system, the most remarkable aspect is that the rate constant reaches a maximum and then falls down as the concentration of $ZnCl_2$ increases (Fig. 1). This overall feature is completely reproducible in several sets of kinetic runs. The observed correlation of rate with $ZnCl_2$ concentration may be understood on the following basis: at least two kinds of zinc complexes are present in the reaction system to control the rate, the major one with a diminished (relative to HNA–PCP–Py) reactivity and the minor one with an enhanced reactivity. Figure 2 shows another aspect of the reaction behavior. In spite of an increase in the fraction of dihydronicotinamide–Zn^{II} species as the Zn^{II} concentration increases to a certain extent, the expected reduction in rate is compensated by contribution of rate-enhancement brought about by the active zinc species. In the light of the coordination behavior,[16] the major deactivated species and the minor activated one are referred to the 1:1 complex (PCP$<^{\text{Py}}_{\text{HNA}}>$$Zn^{II}$), in which both Py and HNA moieties are simultaneously coordinated to the same zinc ion, and the 2:1 complex (HNA–PCP–Py–Zn^{II}–Py–PCP–HNA), in which HNA is free from metal-coordination, respectively. Consistent with this view, the highest reactivity is attained (Fig. 1) when the 2:1 complex reaches its maximum concentration at $[ZnCl_2]\cong1\times10^{-4}$ mol dm^{-3}. The rate constant at $[ZnCl_2]=2.0\times10^{-3}$ mol dm^{-3} may represent the specific reactivity of PCP$<^{\text{Py}}_{\text{HNA}}>$$Zn^{II}$. The specific rate constant for the 2:1 complex, HNA–PCP–Py–Zn^{II}–Py–PCP–HNA, was calculated so that the observed rate–$[ZnCl_2]$ profile can be best reproduced by summation of rate contributions of the metal-free species (specific rate constant 4.4×10^{-4} s^{-1}), the 1:1 complex (specific rate constant 1.4×10^{-4} s^{-1}), and the 2:1 complex on the basis of their respective populations: a value of 3×10^{-3} s^{-1} (per HNA–PCP–Py unit) was obtained, larger by factor of 7 than that for the metal-free HNA–PCP–Py. The kinetic behavior of HNA–PCP–Py (5.1×10^{-5} mol dm^{-3}) was also investigated in dry dichloromethane, in which the addition reaction of water and/or methanol to dihydronicotinamide was not detected. The rate–$[ZnCl_2]$ profile, showing again a maximum at relatively lower concentration of zinc, was analyzed in a manner as described above under the assumption that the coordination behavior of HNA–PCP–Py in dichloromethane is the same as that in dichloromethane–methanol (100:1 v/v); the reactivity of HNA–PCP–Py–Zn^{II}–Py–PCP–HNA (per HNA–PCP–Py unit), 2×10^{-3} s^{-1}, 7 times as large as that of HNA–PCP–Py (2.9×10^{-4} s^{-1}). The specific rate constants for reduction of hexachloroacetone are summarized in Table 1.

The rate enhancement by HNA–PCP–Py–Zn^{II}–Py–PCP–HNA, even though not extremely large, should not be underestimated in reference to the more marked rate enhancement brought about by Zn^{II} in reduction of 2-pyridinecarbaldehyde,[6–12] its Schiff base,[12] 1,10-phenanthroline-2-carbaldehyde,[13] and pyridoxal phosphate[15] with dihydronicotinamide and its derivatives. These substrates are *specific* because, by virtue of their metal-chelating abilities, they readily form zinc complexes which are undoubtedly more susceptible to reduction with dihydronicotinamides. Not being limited to the nicotinamide chemistry, many investigators working on the metal-ion catalysis often choose *specific* substrates so as to be suited for given catalysts, which then undergo chelation with such substrates. However, we rather take a different approach; a catalyst should be chosen so as to be effective for a given *non-specific* substrate. It is generally realized that the role of zinc ion in the reduction of a carbonyl compound with alcohol dehydrogenase is to polarize the carbonyl group through metal-coordination and consequently facilitate the hydride transfer (in a formal sense) from the 1,4-dihydronicotinamide moiety of coenzyme NADH to the carbonyl carbon.[1–3] Hexachloroacetone, an electron-deficient carbonyl compound, may have the least tendency to coordinate to a metal ion. On the basis of these considerations, the ultimate goal of the present study has been to clarify whether or

not the zinc ion accelerates the reduction of hexachloroacetone, a *non-specific* substrate as regards the metal-coordination tendency, by a reaction mechanism schematically shown in **1**. The acceleration effect pro-



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vided by the zinc ion, involved in the 2:1 complex (HNA–PCP–Py–Zn^{II}–Py–PCP–HNA), is the first successful example along this line, to the best of our knowledge. As for the overall rate effect provided by the zinc ion on the present reaction, the major complex formed with HNA–PCP–Py ($PCP\langle^{Py}_{HNA}\rangle Zn^{II}$) reduced the reactivity of HNA–PCP–Py itself. In order to improve this situation, a ligand for coordination with the zinc ion must have significantly larger metal-binding ability than the dihydronicotinamide moiety. Furthermore, dihydronicotinamide and ligand moieties need to be constrained in a certain limited space so that the cooperation of the former and the zinc ion bound to the latter toward a substrate molecule is geometrically possible under the condition: the zinc-binding with the ligand should not result ultimately in metal-bridging between the dihydronicotinamide and ligand moieties.

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