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A Kinetic Study of a Zn^{2+}-catalyzed Transamination Reaction between Pyridoxamine Analogs with a Pyridinophane Structure and α-Keto Acids

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The kinetics of the nonenzymatic transamination reaction from pyridoxamine analogs with a pyridinophane structure to α-keto acids catalyzed by Zn^{2+} were investigated by monitoring the changes in the absorption spectra in methanol. It was found that these reactions obeyed first-order kinetics for the formation of the Zn^{2+} chelate of an aldimine. No appreciable change in the reaction rate was observed when the concentration of the α-keto acid was increased, indicating that the isomerization of the ketimine chelate to the aldimine chelate is the rate-determining step. There was a considerable enhancement of the reaction rate when the molar ratio of the zinc ion to the pyridoxamine analogs was reduced from 1/1 to 0.5/1. The reaction rates corresponding to the various α-keto acids employed in the presence of an amount of Zn^{2+} equimolar to the pyridoxamine analog decreased in this order; phenylpyruvic acid>pyruvic acid≈4-methyl-2-oxopentanoic acid>3-methyl-2-oxobutanoic acid, whereas those in the presence of half-molar equivalents of Zn^{2+} to the pyridoxamine analog decreased in this order; pyruvic acid>phenylpyruvic acid ≈4-methyl-2-oxopentanoic acid>3-methyl-2-oxobutanoic acid. Furthermore, the use of the pyridoxamine analog with a linear "ansa" bridge resulted in a larger reaction rate than the use of the one with a branched "ansa" bridge. The solvent isotope effect is also described.

In a series of studies of the vitamin B_6-dependent enzyme mimics, we have succeeded in the asymmetric synthesis of various α-amino acids by employing the chiral pyridoxamine analogs with an "ansa" bridge between the 2- and the 5-positions of the pyridine ring as the donor of an amino group to the corresponding α-keto acids.[1,2] This stereoselective nonenzymatic transamination reaction was catalyzed by Zn^{2+}, like the usual transamination model reactions.[3] The mechanism of the transamination reaction involves the formation of the zinc chelate complex of the ketimine Schiff base (**4**) and the subsequent isomerization to the aldimine Sciff base (**6**) within the complex.[4] In order to obtain further information about the mechanism of this system, we investigated the kinetics of this nonenzymatic transamination reaction.

All the reactions examined here with the racemates of the pyridoxamine analogs proceeded quite smoothly at room temperature, and all could be followed by means of the spectral change in a methanol solution. This paper will describe the influence of the molar ratio of Zn^{2+} to the pyridoxamine analogs, and also that of the bulkiness of the "ansa" bridge in the pyridoxamine analog molecules, on the reaction rates of the nonenzymatic transamination reaction; our results indicate some different aspects from those reported for the reactions using pyridoxamine itself.[3,5]

Experimental

The pyridoxamine analogs, 15-aminomethyl-14-hydroxy-5,5-dimethyl-2,8-dithia[9](2,5)pyridinophane (**1**)[6] and 15-aminomethyl-14-hydroxy-2,8-dithia[9](2,5)pyridinophane (**2**),[1] and pyridoxal analogs, 15-formyl-14-hydroxy-5,5-dimethyl-2,8-dithia[9](2,5)pyridinophane (**7**)[6] and 15-formyl-14-hydroxy-2,8-dithia[9](2,5)pyridinophane (**8**),[7] were prepared as has been reported previously. The other materials were obtained from commercial sources. The UV spectra were recorded with a Hitachi 124 spectrometer.

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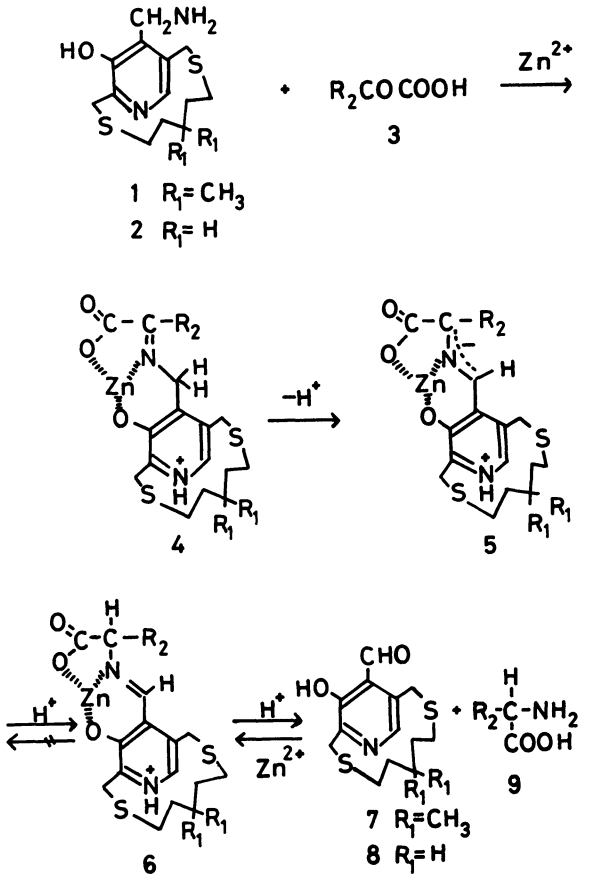

Fig. 1. Nonenzymatic transamination reaction between pyridoxamine analogs and α-keto acid.

Theree methanolic solutions (100 ml each) of a pyridoxamine analog (0.1 mmol), of α-keto acid (0.2—1.6 mmol), and of $Zn(ClO_4)_2 \cdot 6H_2O$ (0.025—0.3 mmol) were prepared. To the pyridoxamine analog solution (10 ml) we added an α-keto acid solution (10 ml) and a zinc perchlorate solution (10 ml) successively; we then immediately diluted them with methanol until the total volume of the solution was 100 ml. The solution was stirred at 23 °C, and aliquots of the solution were taken at

regular time intervals for recording the electronic-absorption spectrum.

Results and Discussion

Spectral Changes. The rates of the changes in the absorption spectra that occur when the pyridoxamine analog (**1**), sodium pyruvate, and zinc perchlorate solutions are mixed simultaneously in the molar ratios of 1 : 4 : 0.5 and 1 : 4 : 1 are shown in Figs. 2 and 3 respectively. When the molar ratio of Zn^{2+} to the pyridoxamine analog (**1**) was 0.5/1, only one peak at 305 nm was formed initially. As the intensity of the 305 nm peak decreased with time, a newly formed peak at 395 nm increased, accompanied by the formation of a couple of isosbestic points at 290 and 340 nm (Fig. 2).

When the molar ratio of Zn^{2+} to the pyridoxamine analog (**1**) was 1/1, the initial spectrum in the reaction had an absorption maximum at 305 nm and a shoulder at 320 nm. As the peak at 305 nm disappeared, the absorption at 320 nm became a single absorption maximum within 3 h. With the gradual decrease in the peak at 320 nm with time, the band at 395 nm is strengthened, with its isosbestic points at 290 and 350 nm (Fig. 3). Although the final spectra were quite similar in both cases, the rate of the absorption spectral change was much smaller in the latter case (Zn^{2+}/**1**=1/1) than in the former case (Zn^{2+}/**1**=0.5/1). The final absorption spectra in both cases were the same as those of the Zn^{2+}–aldimine chelates (**6**) prepared from the pyridoxal analog (**7**), sodium salt of alanine, and zinc perchlorate in the molar ratios of 1 : 4 : 0.5 and 1 : 4 : 1 respectively. These facts suggest that the transformation of the ketimine (**4**) to the aldimine (**6**) was completed within the chelated complex, in each cases including a different amount of Zn^{2+}; the mode of the chelation also seemed to be different.

Matsushima and Martell have reported kinetic studies of the reaction between pyridoxamine, potassium 3-methyl-2-oxobutanoic acid, and zinc acetate to produce Zn^{2+} chelate of pyridoxylidenevaline.[3] They used the absorption band of the Zn^{2+} chelate of pyridoxylidenevaline at 385 nm for these kinetic studies because of its large molecular extinction coefficient, and also because the pyridoxylidenevaline chelate is the only substance that might be present which absorbs at that wavelength. In the same manner, we employed for the present kinetic studies the 395 nm band, which should be assigned to the Zn^{2+}–aldimine chelate (**6**). The plot of $\ln A_\infty/(A_\infty - A_t)$ against the time t (where A_∞ and A_t indicate the optical densities at 395 nm at the completion of the reaction and the time t respectively) gave straight lines. This indicates that these reactions obeyed first-order kinetics for the formation of the Zn^{2+}–aldimine chelate. The first-order rate constant, k_{opsd}, was obtained for the slopes of the straight lines.

Influence of the Concentration of Keto Acid. The results of kinetic runs in which the concentration of the pyridoxamine analog (**1**) was kept at 1.0×10^{-4} M (1 M=1 mol dm^{-3}), while those of the α-keto acids and zinc perchlorate were varied, are shown in Table 1. When the molar ratio of Zn^{2+} to the pyridoxamine

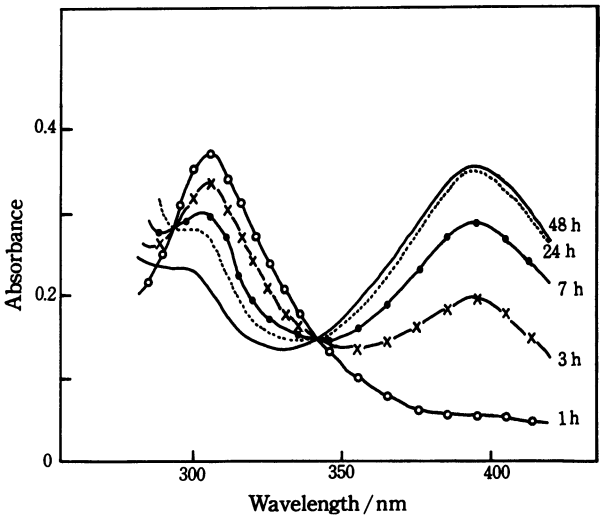

Fig. 2. Changes of electronic absorption spectra with time for a methanolic solution of pyridoxamine analog (**1**) (1.0×10^{-4} M), zinc perchlorate (0.5×10^{-4} M), and sodium pyruvate (4.0×10^{-4} M).

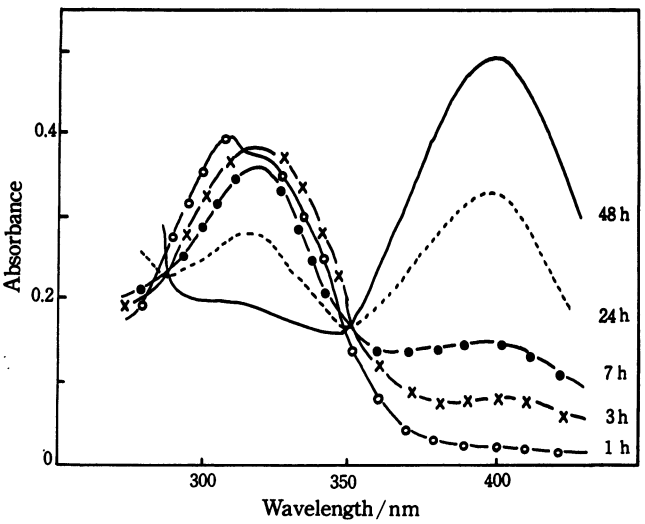

Fig. 3. Changes of electronic absorption spectra with time for a methanolic solution of pyridoxamine analog (**1**) (1.0×10^{-4} M), zinc perchlorate (1.0×10^{-4} M), and sodium pyruvate (4.0×10^{-4} M).

analog (**1**) was 0.5/1, the rate constants increased very slightly with the increase in the α-keto acid concentration from 2.0×10^{-4} M up to 6.0×10^{-4} M, while they were almost constant at higher α-keto acid concentrations ($\approx8.0\times10^{-4}$ M). When the molar ratio of Zn^{2+}/**1** was 1/1, the rate constants were little influenced by the concentration of the α-keto acids. Such an essential independence of the rate constants from the concentration of the α-keto acids indicates that the rate-determining step in the nonenzymatic transamination reaction is not the formation of the Zn^{2+}–ketimine chelates (**4**), but its isomerization to the Zn^{2+}–aldimine chelates (**6**), which is comprised of two reaction stages: the deprotonation of **4** and the protonation of the resulting **5**. The electronic absorption ascribable to **5** has not been observed,[8] suggesting that the protonation of **5** takes

Table 1. Observed rate constants for Zn^{2+}-catalyzed transamination reaction between **1**[a] and RCOCOONa

R	$[Zn^{2+}]$ / 10^{-4} M	[RCOCOONa] / 10^{-4} M	k_{obsd} / 10^{-5} s^{-1}
CH_3	0.5	2.0	8.0
	0.5	4.0	9.0
	0.5	6.0	10.4(9.6)[b]
	0.5	8.0	9.5
	0.5	16.0	6.7
	1.0	2.0	1.3
	1.0	4.0	1.3
	1.0	6.0	1.4
	1.0	8.0	1.4
$C_6H_5CH_2$	0.5	2.0	6.2
	0.5	4.0	7.5
	0.5	6.0	7.8(7.1)[b]
	0.5	8.0	7.4
	0.5	16.0	4.9
	1.0	2.0	3.1
	1.0	4.0	3.1
	1.0	6.0	3.1
	1.0	8.0	3.7
$(CH_3)_2CHCH_2$	0.5	2.0	4.8
	0.5	4.0	6.4
	0.5	6.0	7.8(7.1)[c]
	0.5	8.0	7.4
	0.5	16.0	5.8
	1.0	2.0	0.9
	1.0	4.0	1.1
	1.0	6.0	1.4(1.1)[c]
	1.0	8.0	1.3
$(CH_3)_2CH$	0.5	2.0	1.2
	0.5	4.0	1.1
	0.5	6.0	1.2
	0.5	8.0	1.2
	1.0	2.0	0.6
	1.0	4.0	0.6
	1.0	6.0	0.7
	1.0	8.0	0.7

a) **1**: 1.0×10^{-4} M. b) (*S*)-**1**: 1.0×10^{-4} M. c) (*R*)-**1**: 1.0×10^{-4} M.

Table 2. Observed rate constants for Zn^{+2}-catalyzed transamination reaction between **2**[a] and RCOCOONa

R	$[Zn^{2+}]$ / 10^{-4} M	[RCOCOONa] / 10^{-4} M	k_{obsd} / 10^{-5} s^{-1}
CH_3	0.5	2.0	10.0
	0.5	6.0	16.0
	1.0	2.0	2.7
	1.0	6.0	3.1
$C_6H_5CH_2$	0.5	2.0	8.2
	1.0	2.0	4.6
	1.0	6.0	5.2
$(CH_3)_2CHCH_2$	0.5	2.0	5.6
	0.5	6.0	8.2
	1.0	2.0	1.0
	1.0	6.0	1.5
$(CH_3)_2CH$	0.5	2.0	1.3
	0.5	6.0	1.4
	1.0	2.0	0.6
	1.0	6.0	0.9

a) **2**: 1.0×10^{-4} M.

place rapidly to give **6**. The observation that the employment of a large excess of α-keto acid reduced the rate constant might be explained by the chelation with the α-keto acid that lowers the concentration of the utilizable Zn^{2+}.

Influence of the Kind of Keto Acid. The rate constants decreased in this order; phenylpyruvic acid> pyruvic acid ≈4-methyl-2-oxopentanoic acid>3-methyl-2-oxobutanoic acid when Zn^{2+} and the pyridoxamine analog (**1**) were used in a molar ratio of 1/1. The largest rate constant of phenylpyruvic acid might be explained by assuming that the electron-withdrawing ability of the phenyl group stabilizes the carbanion intermediates (**5**).[9,10] On the other hand, the employment of half-molar equivalents of Zn^{2+} to **1** resulted in this order; puruvic acid>phenylpyruvic acid≈4-methyl-2-oxopentanoic acid>3-methyl-2-oxobutanoic acid. Since the amount of Zn^{2+} is restricted under these conditions, a rigid octahedral Zn^{2+} intermediate coordinated with 2 mol of the ketimine ligands is formed.[11,12] The reaction rate is influenced more by the steric factor than by the electronic factor in this situation.[13,14] This is the reason why the order of the rate constants between pyruvic acid and phenylpyruvic acid was reversed when Zn^{2+}/**1** ratio was changed from 1/1 to 0.5/1.

Influence of the Bulkiness of the "Ansa" Bridge in the Pyridoxamine Analogs. A comparison of Table 2 with Table 1 shows that the rate constants of the transamination reaction using **2** was larger than those using **1**. The branched-bridge chain in **1** seems to affect the reaction rate more negatively than the linear one in **2**.

Influence of the Molar Ratio of Zn^{2+} to Pyridoxamine Analogs. According to Matsushima and Martell,[3] the rate constants for the formation of the Zn^{2+} chelate of pyridoxylidenevaline decreased with the decrease in the Zn^{2+} concentration in the range from 2.0×10^{-4} M to 0.5×10^{-4} M (pyridoxamine: 1.0×10^{-4} M and potassium 3-methyl-2-oxobutanoate: 1.0×10^{-3} M). In contrast to their results, the rate constants of our nonenzymatic transamination reaction using the pyridoxamine analogs with a pyridinophane structure such as **1** increased almost linearly as the Zn^{2+} concentration was decreased from 1.0×10^{-4} M to 0.5×10^{-4} M; they then decreased at lower concentrations of Zn^{2+} ($\approx0.25\times10^{-4}$ M). When the concentration of Zn^{2+} was above 1.0×10^{-4} M, the rate constants were constant. The results are illustrated in Fig. 4. The mechanism of such interesting changes in the reaction rates is obscure at this stage. However, they remind us of a similar correlation between the ratio of Zn^{2+}/**1** and the enantiomeric excesses of the α-amino acids that was obtained through the same transamination reaction using chiral **1**[2]; *i.e.*, an enhancement of the enantiomeric excesses was observed with a lowering of the ratio of Zn^{2+}/chiral **1** from 1/1 to 0.5/1, like the enhancement of the reaction rates with the racemate in this paper. The figures in

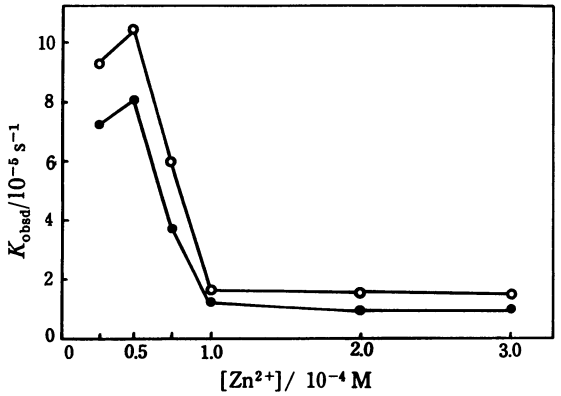

Fig. 4. Variation of k_{obsd} as a function of zinc perchlorate concentration; solutions contained 1.0×10^{-4} M pyridoxamine analog (**1**) with 6.0×10^{-4} M sodium pyruvate (○) and with 6.0×10^{-4} M sodium 4-methyl-2-oxopentanoate (●).

parentheses in Table 1 indicate that there was little difference in the reaction rates between the racemic pyridoxamine analog and the chiral one. This means all discussions of the reaction rates concerning the racemate in this paper are also applicable to the reactions with the chiral one. Consequently, it may be concluded in the chiral system that the enhancement of both the reaction rate and stereoselectivity at the same time can be attained by lowering the ratio of Zn^{2+}/**1**. These interesting phenomena are suggestive of some role of the "ansa" bridge present in the pyridoxamine analog in determining the reaction rate, as this seemed to dominate the enantiomeric excesses of the products in the chiral system.[4]

Solvent Isotope Effect. We have also reported that the α-proton of an amino acid produced through the nonenzymatic transamination reaction was deuteriated when methanol-*d* (MeOD) was used as the solvent.[4] This indicates that the deuterium atom comes directly from the solvent. Furthermore, the pyridoxal analog recovered after the reaction contained no deuterium atom. These results and the aforementioned fact that there was no observable electronic absorption ascribable to **5** strongly support the idea that the rate-determining step of this transamination reaction is the deprotonation of **4** (Fig. 1). In order to measure the solvent isotope effect, the reaction in MeOD was examined. The rate constant was found to be about three times smaller in MeOD than in MeOH. The obtained hydrogen isotope effects, k^H/k^D, were 3.5 for **1** and 2.9 for **2** (1.0×10^{-4} M pyridoxamine analogs, 0.5×10^{-4} M Zn^{2+}, and 6.0×10^{-4} M sodium pyruvate).

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