

Metal Ion Dependence of the Asymmetric Transamination of Phenylpyruvic Acid by Pyridoxamine in the Presence of β -Cyclodextrin

L. Casella, M. Gullotti, F. Piemontesi, and A. Pintar

Dipartimento di Chimica Inorganica e Metallorganica, Centro CNR, Via Venezian 21, 20133 Milano, Italy

Received September 12, 1988

Transamination reactions of phenylpyruvic acid and pyridoxamine in the presence of metal ions and β -cyclodextrin as a chiral auxiliary have been investigated in neutral aqueous solution. The rate and extent of the transamination, and the asymmetric induction observed in the reaction depend upon the nature of the metal ion. In particular, while Zn^{2+} and Co^{2+} yield preferentially the aldimine complex of L-phenylalanine, Cu^{2+} yields preferentially the complex of D-phenylalanine and Ni^{2+} only the racemic product. It is proposed that the ketimine complexes are bound to β -cyclodextrin through the phenyl group of the keto acid residue and that the stereoselectivity of the reaction is originated by some direct interaction of the hydroxyl groups of the cyclodextrin moiety and the metal ions. Although the extent of asymmetric induction is modest in these simple systems (10—20% optical purities), the present results show that transition metal complexes can play a prominent role in determining the steric course of the asymmetric reaction.

INTRODUCTION

Enzymatic reactions of amino acids catalyzed by the vitamin B₆ group of coenzymes play an important role in metabolism.¹ For instance, the synthesis of most amino acids occurs through transamination reactions from pyridoxamine phosphate and α -keto acids. Other metabolic transformations of amino acids, such as decarboxylation, α,β -elimination, retroaldolization and β -substitution reactions, involve pyridoxal phosphate. All these reactions proceed through the initial formation of a Schiff base, the selectivity of the subsequent reaction being determined by appropriate conformation of the substrate in the enzyme-cofactor-substrate complex and specific orientation of the catalytic groups of the enzyme in the active site.^{1,2}

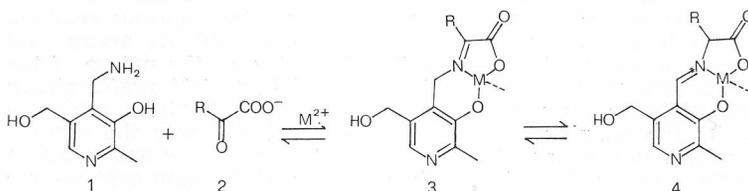
Recent advances in the design of model systems for transaminase enzymes have provided a number of chiral auxiliaries to effect asymmetric transaminations of α -keto acids to amino acids. These synthetic systems enable investigation of the stereochemical requirements for stereoselectivity and provide routes to the highly asymmetric synthesis of some amino acids.³⁻⁷ The chiral auxiliaries employed are usually modified pyridoxamine derivatives,³⁻⁶ but

other optically active molecules acting as additional ligands have been used.⁷ A common feature of all synthetic transaminase systems is the requirement for a metal ion, since this can act as a trap for the intermediate ketimine species and favour the isomerization reaction to the aldimine species.^{8,9} Generally, zinc(II) ions have been routinely used in these investigations, but the possible role of metal ions in the asymmetric reaction has never been taken into account.

We have been interested for some time in pyridoxal model systems involving metal ions¹⁰⁻¹⁴ and wish to report here some preliminary results on the effect of metal ions in the asymmetric transamination for the simple system composed of phenylpyruvic acid and pyridoxamine in the presence of β -cyclodextrin as a source of chirality.

RESULTS AND DISCUSSION

When pyridoxamine (1) reacts with an α -keto acid (2) in the presence of a metal ion, rapid formation of the ketimine complex (3) occurs, followed by a slower isomerization to the aldimine complex (4).^{8,9} The position of the equilibrium $3 \rightleftharpoons 4$ depends on various factors, such as the nature of the



metal ion, the pH and the presence of additional ligands in solution, and can be completely shifted toward the aldimine complex only in nonaqueous medium. The course of the transamination reaction can be easily followed spectrophotometrically through the increase of the intense near-UV absorption of the aldimine complex, near 380 nm, which is well separated from that of the ketimine complex, near 320 nm.

We thought that by performing the reaction in the presence of a chiral cyclodextrin it could be possible to induce optical activity in the amino acid fragment formed upon transamination. A series of preliminary experiments carried out in various conditions, using Zn^{2+} as the metal ion and pyruvic, phenylpyruvic and indolepyruvic acids as representative α -keto acids showed that only with phenylpyruvic acid (2, $R = PhCH_2$) in the presence of β -cyclodextrin was it possible to obtain some optically active aldimine complex 4. α -Cyclodextrin was instead unable to act as a chiral auxiliary in the transamination of any α -keto acid using either Zn^{2+} or Cu^{2+} ions.

The extent of asymmetric induction in the aldimine complexes produced in the presence of β -cyclodextrin was determined by recording circular dichroism spectra of the solutions, through the intensity of the optical activity within the aldimine band at 380 nm. The aldimine complexes 4 prepared from pyridoxal and pure L- or D-phenylalanine provided the standards for evaluating the optical purity of the complex produced in the transamination. No interference in the CD readings at 380 nm by β -cyclodextrin was found

and, in fact, the aldimine complexes prepared from racemic phenylalanine did not exhibit CD activity at 380 nm in the presence of β -cyclodextrin.

TABLE I

Transamination in the Zn^{2+} -pyridoxamine-phenylpyruvate system in the presence of β -cyclodextrin^a

$[Zn^{2+}]$ 10 ⁻³ M	[I] 10 ⁻³ M	[2] ^b 10 ⁻³ M	$[\beta\text{-Cd}]^c$ 10 ⁻³ M	t/h ^d	% 4	% O. p. ^e
1	1	10	1	1.5	55	1
1	1	5	1	1.5	45	2
1	1	2	1	1.5	35	3
1	1	1	1	3.0	30	4
1	1	2	2	1.0	18	5
1	1	2	3	1.0	14	8

^a All runs in neutral aqueous solution the pH ranged between 7.0 and 7.5 according to the amount of phenylpyruvate present.

^b Phenylpyruvate = 2 (R = PhCH₂).

^c β -Cyclodextrin = β -Cd.

^d Time after which the spectral measurement was made.

^e In all cases the amino acid with L configuration is preferentially formed. Optical purity = O. p.

As it is shown by some selected data in Table I, the use of an excess of phenylpyruvic acid accelerates the conversion to the aldimine complex but decreases the optical purity of the amino acid produced. On the other hand, increasing the amount of β -cyclodextrin (β -Cd) increases the asymmetric transamination but markedly decreases the rate of the reaction. With a ratio of $[\beta\text{-Cd}] : [Zn^{2+}] = 5$ the transamination is virtually stopped. Comparative data on the effect of divalent transition metal ions on the rate and asymmetric induction of the transamination are reported in Table II. For practical purposes the reactions were performed in neutral aqueous solutions since in many instances the use of buffering agents produced insoluble precipitates. Cobalt(II) and copper(II) systems had to be studied under an inert atmosphere because they undergo redox reactions in the presence of dioxygen. The observed pseudo-first-order rate constants for the transamination reactions were determined from the spectral curves of the aldimine species; isosbestic points were observed near 280 and 340 nm in all cases except for the Pd(II) system, which does not react appreciably. The spectral changes undergone by the Cu(II) system are reported in Figure 1. The humped curve in the aldimine spectral region is a characteristic of the copper(II) complex,¹² since charge transfer transitions involving the metal ion contribute to the absorption in this range.

Both the rate constants and the position of the equilibrium $3 \rightleftharpoons 4$ show a marked metal ion dependence; Cu²⁺ is the only metal ion for which conversion to aldimine is almost complete in aqueous solution. Attempts to increase the aldimine conversion in other cases by addition of methanol (up to 10% in volume) to the aqueous solutions were partially successful but determined a loss of the optical activity in the product. The solutions of the

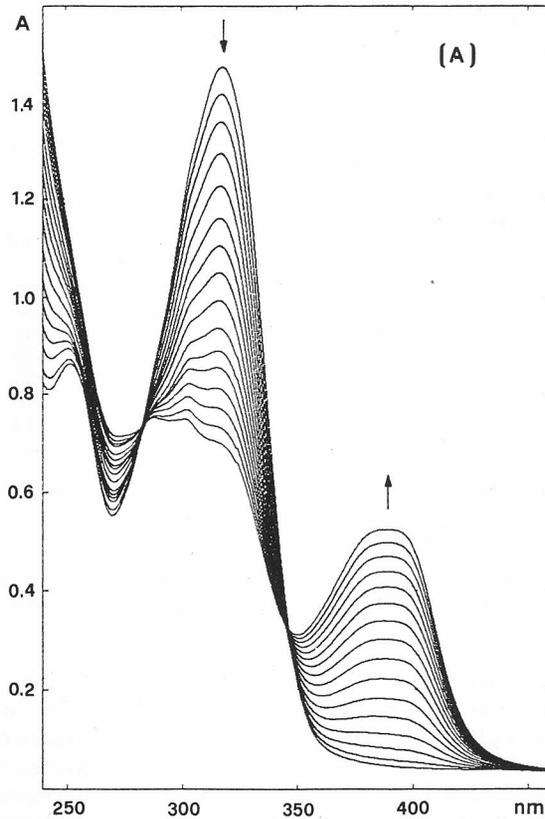
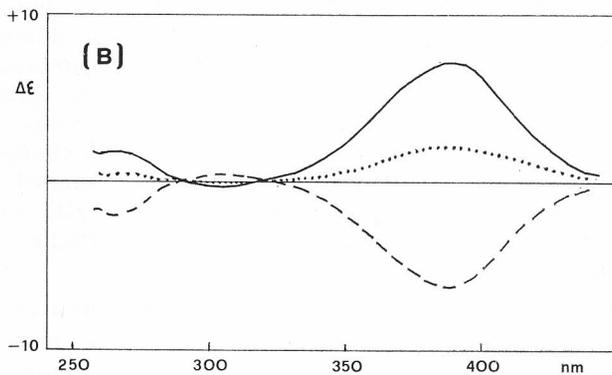


Figure 1. **[A]** Representative electronic spectra recorded during the transamination of the system copper(II)-pyridoxamine-phenylpyruvate in the presence of β -cyclodextrin in neutral aqueous solution under an inert atmosphere. Spectra were taken at 15 min intervals the first curve is the recording taken 9 minutes after mixing the reagents.



[B] Circular dichroism spectra of solution (A) after about 4 hour reaction time (.....) and freshly prepared solutions of *N*-pyridoxylidene-*L*-phenylalaninatocopper(II) (---) and *N*-pyridoxylidene-*D*-phenylalaninatocopper(II) (—), in the presence of β -cyclodextrin.

aldimine complexes undergo slow racemization reactions on standing, as it is also evidenced by some of the data in Tables I and II.

TABLE II

Asymmetric transaminations in the M^{2+} -pyridoxamine-systems in the presence of β -cyclodextrin^a

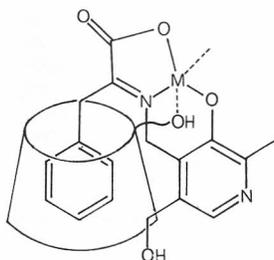
Metal ion	k_{obs}/s^{-1}	% 4 ^b	% O.p.	Configuration
Zn ²⁺	$1.8 \cdot 10^{-4}$	35	5	L
Co ²⁺	$0.9 \cdot 10^{-4}$	30	5	L
Cu ²⁺	$0.8 \cdot 10^{-4}$	95	15	D
		(60) ^c	(22) ^c	(D) ^c
Ni ²⁺	$0.5 \cdot 10^{-4}$	40	0	—
Pd ²⁺	—	5	0	—

^a Conditions: all runs in neutral aqueous solution, pH 7.2, at room temperature; $[M^{2+}] = 10^{-3}$ M, $[1] = 10^{-3}$ M, $[2] = 2.10 \cdot 10^{-3}$ M, $[\beta\text{-Cd}] = 3.10 \cdot 10^{-3}$ M.

^b Equilibrium concentration.

^c Aldimine present after about 4 hour reaction time.

Although the extent of asymmetric induction is generally modest, the data in Table II show that the stereochemical course of transamination is ruled by the metal ion: while Zn(II) and Co(II) yield preferentially the amino acid with L absolute configuration, Cu(II) gives the opposite enantiomer and Ni(II) only the racemic product. This different behaviour must be related to subtle differences in the geometric requirements of the metal ions. It is very likely that the ketimine complex **3** is bound to β -cyclodextrin by forming an inclusion complex through the phenyl group of the keto acid residue. In fact, the phenylpyruvate ion forms an inclusion complex with β -cyclodextrin (as shown by the optical activity generated within the electronic band at 285 nm, $\Delta\epsilon = +0.56 \text{ M}^{-1} \text{ cm}^{-1}$ for a 10^{-3} M aqueous solution of sodium phenylpyruvate in the presence of 1 molar equivalent of β -cyclodextrin), while pyridoxamine does not apparently bind to β -cyclodextrin. The stereochemical control of the reaction by the metal ion must then involve some direct interaction with the hydroxyl group(s) of the cyclodextrin moiety, as shown schematically by structure **5**, the resulting arrangement tending to impose the



5

preference for a given orientation of the prochiral azomethine plane during prototropy according to the coordination needs of the metal ion. This inter-

pretation seems confirmed by the parallel behaviour of the Zn(II) and Co(II) complexes, since these metal ions have similar coordination characteristics.

For the systems containing indolepyruvic acid the failure to give asymmetric transamination may be simply due to the large size of the indole residue. This is certainly trapped into the β -cyclodextrin cavity,^{3f,16} as shown by the optical activity generated within the indole chromophore in inclusion experiments, but probably it keeps the cyclodextrin moiety too far from the metal centre to give significant interaction with its hydroxyl groups.

In conclusion, we have shown that even in such simple transamination systems as those described here metal ions can play a prominent role in determining the steric course of the reaction. We anticipate that upon appropriate functionalization of the cyclodextrin, *e. g.* by covalently linking strong donor groups for metal ions, it will be possible to increase the immobilization of the ketimine complex with respect to the cyclodextrin residue and thereby improve the asymmetric synthesis.

EXPERIMENTAL

All reagents were of the highest commercial grade available. Electronic spectra were recorded on a Perkin Elmer Lambda 5 spectrophotometer. Circular dichroism spectra were recorded on a Jasco J-500C dichrograph, calibrated with a solution of isoandrosterone in dioxane ($\Delta\epsilon = +3.31 \text{ M}^{-1} \text{ cm}^{-1}$ at 304 nm).

Reaction solutions were prepared by mixing pyridoxamine dihydrochloride (0.01 mmol), sodium hydroxide (0.02 mmol), appropriate amounts of cyclodextrin and sodium salt of the α -keto acid, and metal acetate (0.01 mmol) in water (final volume 10 ml). The solution was immediately transferred into the optical cell and spectral readings were initiated. The reaction solutions of the copper(II) and cobalt(II) complexes were prepared and handled under an atmosphere of purified nitrogen; the optical and circular dichroism spectra of these solutions were obtained in quartz cells fitted with Schlenk connections. The accuracy of the optical purity determinations was within $\pm 10\%$.

TABLE III

Optical and CD data for the aldimine bands of complexes 4 in neutral aqueous solution in the presence of β -cyclodextrin^a

		Vis-UV		CD	
		λ_{max} (nm)	ϵ ($\text{M}^{-1} \text{ cm}^{-1}$)	λ_{max} (nm)	$\Delta\epsilon$ ($\text{M}^{-1} \text{ cm}^{-1}$)
Zn ²⁺	L	380	6500	372	-9.30
	D	380	6400	374	+8.90
Co ²⁺	L	372	4650	368	-5.40
	D	372	4600	370	+5.50
Cu ²⁺	L	380	4700	390	-6.80
	D	383	4900	390	+7.20
Ni ²⁺	L	388	6000	378	-6.70
	D	388	5800	379	+6.50

^a The spectra of Co²⁺ and Cu²⁺ complexes were obtained under nitrogen.

The kinetic analyses of the spectral data were performed following the increase of absorbance at 380 nm. Plots of $\ln(A_0 - A_\infty)/(A_t - A_\infty)$ against time t (where A_0 , A_∞ and A_t indicate the optical densities at 380 nm at the beginning, at the end and at the time t of the reaction, respectively) gave straight lines. Pseudo-first-order rate constants, k_{obs} , were obtained from the slopes of these lines.

Freshly prepared aqueous solutions of the N-pyridoxylidene-L- and D-phenylalanine complexes of zinc(II), copper(II), cobalt(II) and nickel(II) in the presence of β -cyclodextrin to be used as standards for evaluating the extent of asymmetric transaminations were obtained according to the following procedure. To a methanol solution of the preformed Schiff base (0.01 mmol), the metal acetate (0.01 mmol) and methanolic 0.1 M sodium hydroxide (0.01 mmol) were added. The solution was heated for 1 hour and then evaporated to dryness under vacuum at room temperature. The residue was dissolved in an aqueous solution (10 ml) containing β -cyclodextrin (0.03 mmol) and electronic and circular dichroism spectra were immediately recorded. In all cases the electronic spectra showed absence of ketimine species. All the operations involving cobalt(II) and copper(II) systems were performed under an atmosphere of purified nitrogen. The relevant spectral data are collected in Table III.

REFERENCES

1. D. Dolphin, R. Poulson, and O. Avramović (Eds.), *Vitamin B₆ Pyridoxal Phosphate — Chemical, Biochemical and Medical Aspects*, Wiley-Interscience, New York, 1986.
2. a) V. C. Emery and M. Akhtar, in *Enzyme Mechanisms*, M. I. Page, A. Williams (Eds.), The Royal Society of Chemistry, London, 1987, p. 345.
b) J. C. Vederas and H. Floss, *Acc. Chem. Res.* **13** (1980) 455.
3. a) R. Breslow, M. Mammond, and M. Lauer, *J. Amer. Chem. Soc.* **102** (1980) 421.
b) R. Breslow and A. W. Czarnik, *Ibid.* **105** (1983) 1390.
c) J. Winkler, E. Coutouli-Argyropoulou, R. Leppkes, and R. Breslow, *Ibid.* **105** (1983) 7198.
d) S. C. Zimmerman, A. W. Czarnik, and R. Breslow, *Ibid.* **105** (1983) 1694.
e) S. C. Zimmerman and R. Breslow, *Ibid.* **106** (1984) 1490.
f) W. Weiner, J. Winkler, S. C. Zimmerman, A. W. Czarnik, and R. Breslow, *Ibid.* **107** (1985) 4093.
4. R. Breslow, A. W. Czarnik, M. Lauer, R. Leppkes, J. Winkler, and S. C. Zimmerman, *J. Amer. Chem. Soc.* **108** (1986) 1969.
5. a) I. Tabushi, Y. Kuroda, M. Yamada, H. Higoshimura, and R. Breslow, *J. Amer. Chem. Soc.* **107** (1985) 5545.
b) I. Tabushi in *Chemical Reactions in Organic and Inorganic Constrained Systems*, R. Setton (Ed.), Reidel Publishing Company, 1986, p. 1.
6. a) Y. Tachibana, M. Ando, and H. Kuzuhara, *Chem. Lett.* (1982) 1765.
b) Y. Tachibana, M. Ando, and H. Kuzuhara, *Bull. Chem. Soc. Jpn.* **56** (1983) 2263.
7. a) K. Bernauer, R. Deschenaux, and T. Taura, *Helv. Chim. Acta* **66** (1983) 2049.
b) R. Deschenaux and K. Bernauer, *Ibid.* **67** (1984) 373.
8. A. E. Martell, *Adv. Enzymol.* **53** (1982) 163.
9. R. H. Holm, in *Inorganic Biochemistry*, vol. 2, G. L. Eichorn (Ed.), Elsevier, New York, 1973, Chapter 31.
10. a) L. Casella and M. Gullotti, *J. Amer. Chem. Soc.* **103** (1981) 6338.
b) L. Casella and M. Gullotti, *Ibid.* **105** (1983) 803.
11. L. Casella, M. Gullotti, and G. Pacchioni, *J. Amer. Chem. Soc.* **104** (1982) 2386.
12. L. Casella and M. Gullotti, *Inorg. Chem.* **22** (1983) 2259.
13. L. Casella and M. Gullotti, *Inorg. Chem.* **25** (1986) 1293.
14. A. M. Villa, L. Casella, and P. Fantucci, *Bioorg. Chem.* **16** (1988) 133.
15. a) J. S. Pagington, *Chem. Brit.* (1987) 455.
b) K. Ohkubo and H. Nagamura, *Isr. J. Chem.* **25** (1985) 241.
16. R. Breslow, *Science* **218** (1982) 532.

SAŽETAK

Ovisnost asimetrične transaminacije fenil-piruvične kiseline piridoksaminom o metalnim ionima u prisutnosti β -ciklodekstrina

L. Casella, M. Gullotti, F. Piemontesi i A. Pintar

Istražene su reakcije transaminacije fenil-piruvične kiseline i piridoksamina u prisutnosti metalnih iona i β -ciklodekstrina kao kiralnih pomoćnih sredstava u neutralnoj vodenoj otopini. Brzina i stupanj transaminacije, kao i asimetrična indukcija opažena u toj reakciji ovisni su o prirodi metalnih iona. Posebno je zanimljivo da su ioni Zn^{2+} i Co^{2+} dali pretežno aldiminski kompleks L-fenilalanina, ion Cu^{2+} daje pretežno kompleks D-fenilalanina, a ion Ni^{2+} samo racenični produkt. Pretpostavljeno je da su ketiminski kompleksi vezani na β -ciklodekstrin preko fenilne skupine keto-kiselinskog ostatka, a da je stereoselektivnost uzrokovana neposrednom interakcijom hidroksilnih skupina ciklodekstrinske jedinice i metalnih iona. Iako je u ovom jednostavnom sustavu asimetrična indukcija bila skromna (10—20% optičke čistoće), ovi rezultati pokazuju da kompleksi prijelaznih metala mogu igrati prominentnu ulogu u određivanju steričkog toka asimetričnih reakcija.