The Effect of Ni(II), Zn(II), Cu(II), Co(II) and Pd(II) Ions on Racemisation of Hydroxy α-Amino Acids*

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Abstract

The study examined the effect of Ni(II), Zn(II), Cu(II), Co(II) and Pd(II) ions on the rate of racemisation of L-serine and L-threonine. All ions except Ni(II) enhanced the racemisation of serine; the retarding effect of Ni(II) increased with ion concentration. Both Zn(II) and Ni(II) ions retarded the racemisation of threonine.

Introduction

Reactivity increases when metal ions are present with amino acids or their derivatives in base catalysed aldol-type condensations [1], isotope exchange reaction [2], racemisation [3], Schiff base formation [4] and hydrolysis [5]. The enhanced reactivity in these complexes is attributed to the metal ligand bonding [6] and the net charge on the complex [7, 8].

In contrast, we recently reported that Ni(II), when complexed with L-alanine, and Co(III), when complexed with gly–ala or ala–gly, retarded the rate of racemisation of L-alanine [9]. The retarding effect of Ni(II) was further noted in ultrasound-promoted N-benzylation reactions of metal glycinato complexes [10]. We extended the study of metalations on racemisation to the hydroxy amino acids, and now report the effect of Ni(II) and four other metal ions on racemisation of serine and threonine.

Experimental

Syntheses and purification of the complexes were carried out according to previously reported procedures: $[Ni(ser)_2]3.5H_2O$, $[Cu(ser)_2]$, $[Ni(thr)_2]$ and $[Cu(thr)_2]$ [11a]; $[Zn(ser)_2]$ and $[Zn(thr)_2]$ [11b]; $[Pd(ser)_2]$ [11c]; $[Co(ser)_2]$, a stoichiometric solution of Co(II) ion and serine in deionized water was used to adjust the pH to a desired value to carry out the racemisation study. All complexes gave satisfactory analytical and spectral data.

Sample Preparation and Racemisation

In each case, 30 ml of 0.03 M solution of the complex was prepared in deionised water; pH was adjusted to a desired value by adding sodium hydroxide (0.1 M) or hydrochloric acid (0.1 M). Approximately 2 ml aliquots of the solution containing the complex were sealed into pyrex glass tubes and racemised in a constant temperature oil bath for the required time intervals. After racemisation, the tubes were cooled, opened and the complexes decomposed.

Decomposition and Derivatisation to N-Trifluoroacetyl-O-2-propyl Esters

The metal ions were freed from the complexes by adding a few drops of 0.1 M sodium sulphide solution under acidic or basic conditions depending on the nature of the complex. Zn(II) amino acid complexes were decomposed to the free hydroxy amino acids by adding a few drops of 0.1 M ethylenediamine tetraacetic acid (EDTA) solution. The sample was evaporated to dryness under a stream of air at 80 °C. Traces of moisture were removed by azeotropic distillation with dichloromethane followed by vacuum desiccation. To each dried amino acid residue was added 1 ml of 4 N 2-propanol/HCl, and the tubes were sealed. Sealed tubes were heated in an oil bath for 2 h at 110 °C, and the excess 2-propanol/HCl was removed from the tubes by a stream of air at 80 °C. Derivatisation was completed by addition of 0.5 ml of 30% trifluoroacetic anhydride in dichloromethane. After allowing the tubes to stand for 2 h at room temperature, the excess reagent was removed by evaporation. The derivatised amino acid residue was dissolved in 0.5 ml of dichloromethane for GC analysis.

Gas Chromatography

GC analyses were performed on a HP 5880A gas chromatograph (flame ionisation detector) under

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isothermal conditions, using a stainless steel capillary column (200 ft × 0.02 in.) coated with *N*-docosonyl-L-val t-butyl amide chiral phase [12]. A baseline separation of D- and L-enantiomers was obtained in all cases. Threonine exhibited four peaks due to its diastereomeric pair. Reversible first-order rate constants for the racemisation reaction were determined by plotting $0.5 \ln[(1 + D/L)/(1 - D/L)]$ versus time, where D and L represent the concentrations of D- and L-serine, respectively. For threonine, D represents the concentration of D-*allo*-threonine. The equilibrium constant for threonine racemisation was set at 1.0.

Results and Discussion

Hydroxy amino acids racemise faster than all other amino acids [13]. The hydroxy group in the side chain of these amino acids clearly plays an important role in enhancing the reactivity toward racemisation.

Reaction rate constants for racemisation of free and complexed L-serine and L-threonine are shown in Table 1. Serine racemised approximately five times faster than threonine, in good agreement with previous findings [13, 14]. Serine, complexed with Zn(II), Co(II), Cu(II) or Pd(II) racemised three to forty times faster than in the free state. Similarly, Cu(II) complexed threonine exhibited a five-fold enhanced reactivity. On the other hand, Ni(II) retarded the racemisation rate of serine. No appreciable racemisation was measured for threonine complexed to Ni(II) or Zn(II). It is noteworthy that Zn(II) enhanced reactivity in serine, while it significantly retarded racemisation of threonine.

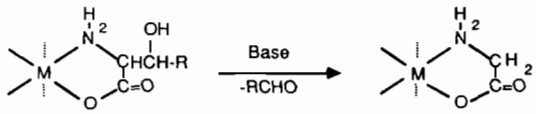
TABLE 1. Reaction rate constants for the racemisation[a] of free and complexed serine and threonine

Amino acid or complex	$10^6 \times k$ (s^{-1})	Glycine formed[b] (%)
Serine	3.42	5.0
$[Ni(ser)_2]3.5H_2O$	2.12	
$[Zn(ser)_2]$	10.00	8.0
$[Cu(ser)_2]$	22.00	5.0
$[Co(ser)_2]$	27.00	15.0
$[Pd(ser)_2]$	144.00	2.0
Threonine	0.62	
$[Ni(thr)_2]$	[c]	2.0
$[Zn(thr)_2]$	[c]	
$[Cu(thr)_2]$	3.0	57.0

[a]Racemisation conditions: pH: 9.0 and temperature 108 °C. [b]As determined by GC. [c]No appreciable racemisation was measured.

The enhancing electronic effects (inductive and resonance) and solvation effects may outweigh the retarding effect due to Zn(II) in the Zn(II)–serine complex. The retarding effect of Ni(II) on the racemisation of these amino acids has importance in geochronology and chemical evolution [15]. Tatsumoto and co-workers [16] demonstrated the metal complex effect in studies of the oxidative deamination of metal-complexed α-amino acids to their corresponding keto acids. The relative catalytic activity due to the various metal ions studied was reported as: Mn(II) > Co(II) > Cu(II) ≫ Ni(II) ≃ 0. These workers pointed out [16] that the catalytic effect of Ni(II) in the oxidative deamination is less significant. It is interesting to note that a similar trend was observed in the case of Ni(II) in the present study toward its reactivity in racemisation.

Another side reaction competes with racemisation in the complexes studied which results in the formation of formaldehyde/acetaldehyde. Aldehyde formation can result from a retro-aldol reaction [17] of serine and threonine, respectively, and is represented in Scheme 1. Presumably the presence



Ser or Thr complex glycine complex

Scheme 1.

of aldehyde results in Schiff base formation, which enhances racemisation rates [4, 6]. If so, a large increase would be expected in the rate of racemisation for $[Cu(thr)_2]$ where 57% of glycine formed in the reaction mixture. This was not observed. Similarly, little glycine was formed (2%) in the $[Pd(ser)_2]$ complex. However, the rate of racemisation of this Pd(II) complex was the fastest of all the complexes studied. These results do not appear to support the hypothesis in this instance that enhanced reactivity is due to Schiff base formation.

The rate-determining step in the racemisation reaction of free and metal-complexed amino acids is the abstraction of the α-proton by base, forming a carbanion [18]. Thus, the rate of racemisation is controlled by the stability of the carbanion intermediate. One explanation for the slow racemisation rate observed for serine complexed to Ni(II) is the ionic nature of the metal ligand bonding [9]. Another possible explanation is that the methine carbon in these Ni(II) amino acid complexes is oriented so that it cannot attain co-planarity, necessary for racemisation to proceed [18]. The data available in hand is insufficient to explain why Zn(II) enhanced the racemisation rate in serine while retarding it in the case of threonine. Additional experiments

are underway to address this question. The enhanced reactivity due to other metal ions is in good agreement with data from previous studies [1–10].

We extended our studies to examine the effect of metal ion on the racemisation of serine as a function of concentration. These experiments were designed in an attempt to understand the effect of metal ions at low concentrations. Table 2 shows the percent D-serine formed in 2 h at 108 °C and pH 9.0 as a function of Cu(II) ion concentration. The stoichiometric ratio of Cu(II) to serine is 1:2. Data presented in Table 2 show ratios equal to and below this. A 1:5 ratio of metal to serine brings about the greatest extent of racemisation. However, even a trace amount of Cu(II) increases the rate of racemisation. The uncomplexed L-serine formed only 2.29% of the D isomer. Racemisation increased three-fold when the ratio of Cu(II) to serine was changed from zero to 0.1:1 (1:10). A further increase (fifteen-fold) was observed in percent D-isomer formation when the ratio of Cu(II) to serine was increased to 0.2:1 (1:5). More than a stoichiometric amount of serine is required to shift the equilibrium toward the complex. When Cu(II) was equal to the stoichiometric amount, 0.5:1 (1:2) the percent D isomer formed dropped to nine-fold. These results illustrate the importance of the concentration of the Cu(II) ion on serine racemisation. This must be true in the case of all other metal ions which exhibit catalytic activity toward racemisation.

TABLE 2. Effect of the concentration of Cu(II) ion on the racemisation[a] of serine

Concentration (molar)		Cu(II):Ser	D Isomer[b] (%)	Glycine[b] (%)
Cu(II)	Serine			
	0.03		2.29	
0.003	0.03	0.1:1	6.66	2.0
0.006	0.03	0.2:1	33.20	12.0
0.015	0.03	0.5:1	20.70	5.0

[a]At 108 °C and pH 9.0 for 2 h. [b]As determined by GC.

TABLE 3. Effect of the concentration of Ni(II) ion on the racemisation[a] of serine

Concentration (molar)		Ni(II):Ser	D Isomer[b] (%)	Glycine[b] (%)
Ni(II)	Serine			
	0.03		6.9	
0.003	0.03	0.1:1	6.5	
0.006	0.03	0.2:1	5.6	
0.015	0.03	0.5:1	3.5	

[a]At 108 °C and pH 9.0 for 6 h. [b]As determined by GC.

A similar experiment was carried out with Ni(II) ion. The percent D-serine formed in 6 h at 108 °C and pH 9.0 as a function of Ni(II) ion concentration is shown in Table 3. From Table 3 it is clear that the % D-serine formed decreased with increasing concentration of Ni(II) ion. Again the retarding effect increased with an increase in Ni(II) ions with respect to the amino acid molecules. This situation is in contrast to what we observed in the case of Cu(II) ions study. It appears that any effect we observe for a particular metal ion is directly proportional to the concentration of that metal ion in the amino acid solution. One limitation is the appropriate stoichiometric ratio for a metal ion to the amino acid concentration.

Conclusions

All metal ions studied increased rates of racemisation of L-serine by three- to forty-fold, except Ni(II), which had a retarding effect on racemisation of both L-serine and L-threonine. Zn(II) enhanced the rate for L-serine, while retarding racemisation of L-threonine. Even at trace levels, Cu(II) ions increased racemisation rates. Increasing the concentration of Cu(II) ions increased the rate of racemisation of serine at first then decreased reactivity as the concentration of Cu(II) was increased. The retarding effect of Ni(II) increased with increasing concentration of metal ions, suggesting that Ni(II) salts would be useful in the peptide syntheses where excessive racemisation is expected.

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