BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 43 3858—3861 (1970)

Hydrolysis of Dipeptides with Metal Oxides

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(Received May 14, 1970)

The hydrolysis of three dipeptides, Gly-Gly, Gly-L-Leu, and Gly-L-Phe, with metal oxide catalysts was studied. In most cases, the hydrolysis occurred in the pH range 5—6 accompanied by the formation of metal complexes of amino acids and dipeptides. Copper oxide, nickel oxide, cobalt oxide (III), and alumina have more catalytic activity than copper powder, cuprous oxide, commercial nickel oxide (II), and silica gel. Alumina and silica gel gave no metal complex. It is assumed that the hydrolysis proceeds by means of the formation of an active metal complex of dipeptide or by means of activation on the surface of the catalyst. The order of reactivity of the dipeptides was: Gly-Gly>Gly-L-Leu, Gly-L-Phe.

It has previously been reported[1] that 2-cyanopyridine and pyridine-2-carboxamide are easily hydrolyzed with nickel or copper oxide as a catalyst and that they afford chelate compounds of the acid with nickel or copper. This hydrolysis proceeds when they are refluxed in water without acid or alkali. On the other hand, 3 or 4-cyanopyridine is not hydrolyzed under the same reaction conditions. It is interesting to note that metal catalysts such as nickel or copper have no catalytic activity in this hydrolysis.

To develop this idea, thereupon, we attempted the hydrolysis of some dipeptides with metal oxides in the same manner. It has thus been found that the hydrolysis of dipeptides affords metal chelates of amino acids, along with metal complexes of the dipeptides. It is of interest that the hydrolysis of peptides thus takes place without acid or alkali. It has already been established that specific chelate catalysts,[2,3] an ion-exchange resin[4] or a copper (II) ion[5] can be effectively used for the hydrolysis. The reaction mechanism, especially in relation with metal ion catalyzed hydrolysis, will be discussed.

Results

Hydrolysis of Dipeptides with Metal Oxides. The dipeptides used were glycylglycine (Gly-Gly), glycyl-L-leucine (Gly-L-Leu), and glycyl-L-phenylalanine (Gly-L-Phe). The catalysts used were copper oxide, cuprous oxide, copper powder, nickel oxide-(A) (black), nickel oxide(B) (olive green, II), cobalt oxide (III), aluminum oxide (alumina), and silica gel. Copper powder, cuprous oxide, nickel oxide-(B), and silica gel showed very low activity for the hydrolysis of dipeptides. In general, hydrolysis started in the pH range 5—6. Soon after the reactions with copper oxide, nickel oxide(A), and cobalt oxide catalysts started, the reaction mixture colored, and the coloration was thereafter enhanced gradually. When copper powder, cuprous oxide, and nickel oxide(B) were used, the coloration was very slow. It is obvious that the dipeptides draw out the metal ions from the metal oxides and afford the water soluble metal complexes. The UV spectra of the reaction mixture after thirty minutes refluxing are shown in Table 1. As for the complex of Gly-Gly with copper, it is accordance with the data by Nakahara[6] (λ_{max} 643 mμ, logε=1.92).

TABLE 1. UV SPECTRA OF DIPEPTIDE COMPLEXES (after 30 min refluxing)

Dipeptide	Catalyst	Color	λ_{max} (mμ)
Gly-Gly	copper oxide	blue	643
Gly-Gly	nickel oxide(A)	light blue	376, 626, 1023
Gly-Gly	cobalt oxide	red violet	398, 519
Gly-Gly	alumina	no	—
Gly-Gly	silica gel	no	—
Gly-L-Leu	copper oxide	blue	638
Gly-L-Leu	nickel oxide(A)	light blue	380, 630, 1036
Gly-L-Leu	cobalt oxide	red violet	524
Gly-L-Phe	copper oxide	blue	641
Gly-L-Phe	nickel oxide(A)	light blue	377, 629, 1017

Spectrophotometer: Hitachi Recording Spectrophotometer EPS-3T

1) K. Sakai, T. Ito and K. Watanabe, This Bulletin, **40**, 1660 (1967).

2) A. Nakahara, K. Hamada, Y. Nakao and T. Higashiyama, *Coordin. Chem. Rev.*, **3**, 207 (1968).

3) D. A. Buckingham, J. P. Collman, D. A. R. Happer and L. G. Marzilli, *J. Amer. Chem. Soc.*, **89**, 1082 (1967).

4) J. R. Whitaker and F. E. Deatherage, *ibid.*, **77**, 3360 (1955).

5) I. J. Grant and R. W. Hay, *Aust. J. Chem.*, **18**, 1189 (1965).

6) A. Nakahara, This Bulletin, **32**, 1195 (1959); O. Yamaguchi, Y. Hirano, Y. Nakao and A. Nakahara, *Can. J. Chem.*, **47**, 3441 (1969).

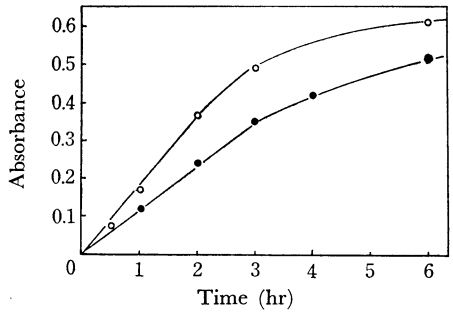


Fig. 1. Formation of dipeptide complexes: dipeptide 0.0023 mol, copper oxide 1.01 g, water 40 m*l*; ○ Gly-Gly, ● Gly-L-Leu. After the sample was diluted to five times with water, the absorbance was measured at 643 mμ for Gly-Gly and at 638 mμ for Gly-L-Leu.

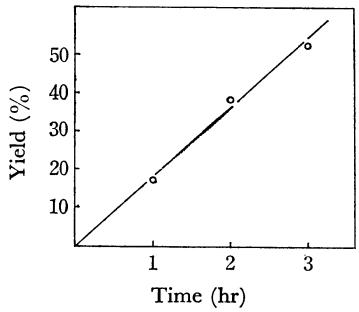


Fig. 2. The yield of Gly-Gly-Cu complex. The yield was calculated on the basis of UV spectrum of Gly-Gly-Cu complex prepared. The amount of glycine hydrolyzed at 3 hr was regarded as negligible.

In an earlier step of the reaction, the reaction solution was treated with ion-exchange resin (Dowex 50×2H type), in order to remove the metal ion, and was then analyzed by paper chromatography. The formation of complexes in the reaction with copper oxide is shown in Fig. 1 and Fig. 2. It is assumed that the formation of the Gly-Gly-Cu complex is *ca.* 50% after 3 hr reaction.

When the refluxing was continued longer than 3 hr, hydrolysis proceeded further in most cases. However, amino acids also formed complexes with metal ions, and usually no free acids were found in the reaction mixtures. The general profiles of the hydrolysis of three dipeptides with various catalysts are shown in Table 2. The pH values in the reactions are also shown in the table. On the whole, the hydrolysis proceeded in the pH range

Table 2. Hydrolysis of dipeptides with metal oxides

dipeptide, 0.0023 mol; water, 40 m*l*; catalyst, 1.0 g; temp, 100°C

Dipeptide	Catalyst	pH[a]		Gly, time (hr)[b]			
		S	E	1	3	9	21
Gly-Gly	copper powder	5.7	6.1	···	tr.	+	++
Gly-Gly	cuprous oxide	5.7	6.0	···	···	+	++
Gly-Gly	copper oxide	5.9	5.9	tr.	+	++	+++
Gly-Gly	nickel oxide(A)	6.0	7.9	+	++	+++	+++
Gly-Gly	nickel oxide(B)	5.9	6.9	···	···	tr.	+
Gly-Gly	silica gel	6.2	7.0	···	tr.	+	++
Gly-Gly	alumina	6.1	7.5	+	++	+++	+++
Gly-Gly	cobalt oxide	6.0	7.3	tr.	+	++	+++

Dipeptide	Catalyst	pH[a]		Gly, time (hr)				Leu, time (hr)			
		S	E	1	3	9	21	1	3	9	21
Gly-L-Leu	copper oxide	5.9	6.0	···	tr.	+	++	···	···	+	++
Gly-L-Leu	nickel oxide(A)	5.9	7.9	tr.	+	++	++	···	+	++	++
Gly-L-Leu	cobalt oxide	5.9	7.6	···	tr.	+	++	···	···	+	++
Gly-L-Leu	alumina	6.2	6.8	tr.	tr.	++	+++	···	···	++	+++

Dipeptide	Catalyst	pH[a]		Gly, time (hr)				Phe, time (hr)			
		S	E	1	3	9	21	1	3	9	21
Gly-L-Phe	copper oxide	5.7	5.8	···	tr.	+	++	···	···	···	+
Gly-L-Phe	nickel oxide(A)	6.0	7.9	···	+	++	++	···	···	+	++

a) pH: S=starting point, E=end point (21 hr refluxing)

b) The relative intensities of the spots on the chromatograms are represented thus: ···=no spot; tr.= trace, just visible; + to +++, increasing intensity of visible spots.
+: The amount of glycine was examined as 2—5% of the glycylglycine.

5—6 and the pH values increased more or less in the 21 hr reaction.

Since the hydrolysis of dipeptides hardly ever occurs without catalysts in this pH range,[5] it is evident that these metal oxides have a catalytic effect on the hydrolysis. It has been observed that the reactivity of the hydrolysis is dependent upon both the kinds of dipeptides and the kinds of catalysts, and that nickel oxide(A) turns a greenish gray and copper oxide turns brown in part, while the other metal oxides do not change color. Among the metal oxides, alumina is an especially active catalysts. In this case, free amino acid was obtained by the hydrolysis, but no aluminum complex of dipeptide was detected in the reaction mixture. The test was carried out with Gly-Gly (0.3 g) and alumina (1 g) in water (pH 6.3). After refluxing for 6 hr, the catalyst was filtered out and the filtrate was analyzed by the xylenol orange method.[7]

Hydrolysis of Dipeptide Complex. As has been mentioned above, it is apparent that both the formation of dipeptide complexes and the hydrolysis of dipeptides occur during the refluxing with the transition metal oxides. If the hydrolysis could proceed through the complex formation, it may be assumed that the sole dipeptide complex formed is hydrolyzed without catalysts and that it then affords the complexes of amino acids.

In order to examine this assumption, Gly-Gly was refluxed with copper oxide for 45 min, after which the catalyst was filtered out. It was then found by paper chromatography that no free glycine or glycine-Cu complex was present in the blue filtrate. On the other hand, the filtrate (pH 5.7, λ_{max} 643 mμ) was further refluxed and a few samples of it were taken out to be analyzed by paper chromatography. As is shown in Table 3, the hydrolysis of the Gly-Gly-Cu complex took place to give the glycine complex, and free glycine was detected in the solution refluxed for 21 hr. On the other hand, when the Gly-Gly-Cu complex[6] prepared beforehand was refluxed with water under about the same reaction conditions.*1 It gave about the same results (Table 3).

TABLE 3. HYDROLYSIS OF DIPEPTIDE CHELATE

	pH[a]		Gly, time (hr)			
	S	E	1	3	9	21
(1) filtrate	5.7	6.3	···	+	++	++
(2) Gly-Gly-Cu and Gly-Gly	5.6	5.6	···	+	++	—[b]

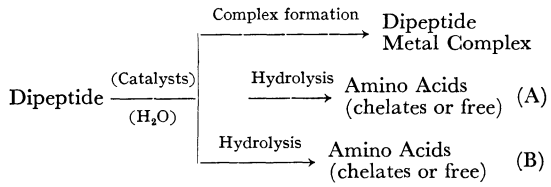
a) S=starting point, E=end point: (1) 21 hr, (2) 9 hr refluxing
b) not measured

7) J. Dvorak and E. Nyvltova, *Mikrochim. Acta*, **1966**, 1082.

*1 Gly-Gly-Cu, 0.0042 g; Gly-Gly, 0.20 g; H_2O, 40 m*l*.

Discussion

As was expected, copper oxide, nickel oxide(A)*2, and cobalt oxide are effective for the hydrolysis of the peptide bond as well as for the specific hydrolysis of 2-cyanopyridine. These transition metal oxides form metal complexes with dipeptides. As a result of our studies, two routes might be proposed for this hydrolysis:



Route A is a mechanism in which metal complexes participate in the hydrolysis of peptides as the ligands; it is supported by the results of the experiment without heterogeneous catalysts (Table 3). In this mechanism, the metal oxides serve only as donors of metal ions to form complexes with dipeptides. As for the hydrolysis shown in Table 3, the concentration of the Gly-Gly-Cu complex is very low; therefore, the reaction velocity may be very slow in comparison with the hydrolysis in the presence of metal oxides. Grant[5] reported the hydrolysis of Gly-Gly with the Cu^{2+} ion in the pH range 3.5—6.0, in which Cu-Gly-Gly$^+$ type of active complex was assumed to participate in the reaction. In the case of our studies, it is also presumed that an analogous type of species might participate in the hydrolysis of Route A.

Route B is a mechanism which is regarded as a heterogeneous catalytic reaction on the solid surface. It is assumed that the amide bond of dipeptide is activated by the adsorption on the catalysts and that the nucleophilic attack of water may occur. Amino acids, the hydrolysis products, then may draw out the metal ions from the catalysts to form the chelates. When alumina or silica gel was used as the catalyst, free amino acids were obtained without forming any chelate. This is also evidence of direct catalytic hydrolysis. When copper powder, cuprous oxide, or nickel oxide(B) was used as the catalyst, the formation of metal complexes with dipeptides was extremely slow, and the hydrolysis proceeded very slowly, too. Route B might also be possible in the case of hydrolysis with transition metal oxides. It can be inferred, however, that the main route is A rather than B in the reactions with copper oxide, nickel oxide(A), and cobalt oxide.

As is shown in Table 2, the reactivity of dipeptide is in the order: Gly-Gly>Gly-L-Leu, Gly-L-Phe.

*2 In our previous paper,[1] NiO in Tables 1 and 2 should be corrected to nickel oxide (black), which is mentioned as nickel oxide(A) in the present paper.

The relatively low reactivity of Gly-L-Leu and Gly-L-Phe may be due to the steric effect of bulky groups in the α-position of the carboxyl group. This effect has also been pointed out in the case of hydrolysis with acid[8] or ion-exchange resin.[4]

Experimental

Materials. The glycylglycine, glycyl-L-leucine, and glycyl-L-phenylalanine were obtained from the Institute for Protein Research, Osaka University. They were examined by paper chromatography and were proved to be free from extraneous ninhydrin-reacting materials. Commercial copper powder, copper oxide (II), nickel oxide (II) (olive green, called nickel oxide(B) in this paper), cobalt oxide (III), alumina (Aluminum Oxide Woelm Neutral, Germany), and silica gel (300 mesh) were washed with distilled water and were then used as catalysts. The nickel oxide(A) (black) was prepared by the careful decomposition of nickel carbonate at 250—300°C and was then washed with ditstilled water. The cuprous oxide was prepared by the controlled reduction of a complex of copper sulfate and Rochelle salt with glucose.

The Hydrolysis of Dipeptides. 0.0023 mol of dipeptide and 1.0 g of a catalyst were refluxed in 40 m*l* of water. During the refluxing, 0.8 m*l* samples of the reaction mixture were taken out after 1, 3, 9 and 21 hr. Each fraction was filtered to remove a small amount of suspended catalysts, after which filtrate was treated with ion-exchange resin (Dowex 50×2H type) in a column (1 cm in diameter). Then, 1.4% ammonia solution as the eluent was passed through the column. Thus, metal ions were removed from the complexes and a solution containing free amino acid and dipeptide was obtained as the eluent. This was then evaporated *in vacuo* to *ca.* 0.3 m*l* for the analysis. In some cases, the cobalt ions of the complexes were precipitated as sulfide with hydrogen sulfide; the filtrate was then also treated as has been described above. In these processes, no hydrolysis of dipeptides took place.

Analysis of Amino Acids and Dipeptides. The analyses of hydrolyzed amino acids and of unchanged dipeptides were carried out by paper chromatography. Toyo filter paper No. 51 and *n*-butanol - ethanol - water (5 : 5 : 3) as the eluent were used. About a 5-μ*l* portion of the sample solution was spotted, and the chromatograms were run for from 12 to 24 hr. Amino acids and dipeptides were developed with a ninhydrin solution and by warming in an air oven at 95°C for 5 min. The results of the analysis (R_f value and coloration) are shown below.

	Gly-Gly	Gly	Gly-L-Leu	Leu	Gly-L-Phe	Phe
R_f	0.07	0.14	0.33	0.54	0.34	0.48
Color	Yellow	Violet	Yellow	Pink	Yellow	Pink

The authors wish to express their thanks to Professor Kazuo Hata and Professor Michio Kobayashi for their kind advice.

8) L. Lawrence and W. J. Moore, *J. Amer. Chem. Soc.*, **73**, 3973 (1951).