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Effects of pH and Ligands on the Metals-Catalyzed Hydrolysis of Clioquinol Conjugates

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Metals-catalyzed hydrolysis of clioquinol (C) conjugates, namely C-glucuronide (CG) and C-sulfate (CS), was examined to investigate the effects of pH and ligands on the activities. The initial rates of hydrolysis (V_0) of CG and CS were measured as a function of pH in systems containing various metals. The profiles of V_0 of CG varied from metal to metal, but the order of V_0 in the acidic pH region was Cu(II) > Fe(III) > Ni(II) > Zn(II) > Mn(II), Mg(II), Ca(II). Acid hydrolysis of CG in the absence of metals was negligible down to pH 1. The profiles of V_0 of CS were similar to that of acid hydrolysis in the absence of metals, except for the Cu(II) system, which gave a higher V_0. Under the condition [substrate] : [Cu(II)] : [ligand] = 1 : 10 : 10, the hydrolysis in the presence of various ligands was a pseudo-first order reaction (rate constant = k'). All ligands which coordinate to Cu(II) reduced the value of k'. Such metals-catalyzed hydrolysis may be one of the mechanism of hydrolysis of C conjugates to produce C-metal chelates *in vivo*.

Keywords——clioquinol glucuronide; clioquinol sulfate; metal catalyzed hydrolysis; ligand; chemical form; pseudo-first order reaction

The metabolism and the toxicity of 5-chloro-7-iodo-8-quinolinol (clioquinol, chinoform, C) has been widely studied, especially as regards the relationship between biological metals and C, since the green pigment isolated from green urine and green feces of SMON (subacute-myelo-optico-neuropathy)[1] patients has been identified as C-iron (III) chelate.[2-6] The denaturation of nervous tissues due to the lipoperoxidizing effects of C-metal chelates has also been reported.[7,8] On the other hand, Hay *et al.* found catalytic effects of several metal ions in the hydrolyses of 8-quinolyl sulfate[9] and 8-quinolyl-β-D-glucopyranoside.[10] We kinetically examined the hydrolysis of CG and CS, which are also 8-quinolyl derivatives, and found that C-conjugates, namely C-glucuronide (CG) and C-sulfate (CS), were hydrolyzed easily by such metal ions as Cu(II), Ni(II) and Zn(II).[11] If C-conjugates were hydrolyzed easily by these metals in the body, then the resultant C would be chelated with the metals and the amounts of C-metal chelates in the body may increase. However, the role of biological metals and complexation in the hydrolysis of C-conjugates has not yet been confirmed.

It is important to study the metals-catalyzed hydrolysis of C-conjugates as a possible process related to the formation of C-metal chelates in the body. Therefore, as a continuation of our studies on the roles of metals in biological processes, we studied kinetically the effects of pH and ligands on the activities of various metals for the hydrolysis of C-conjugates.

Experimental

Materials——CG, Cs (sodium salt) and C were kindly provided by the Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, University of Tokyo. Metal nitrates of special grade were supplied by Wako Pure

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Chemical Industries Ltd. Other reagents used were all commercial products.

Apparatus——The high performance liquid chromatograph (HPLC) was composed of a Kyowa KHU-16 micro pump, a Kyowa KGC-3-300 Pyrex column packed with Zipax SAX (Du Pont), a Mitsumi SF-1205 UV monitor and a Toa 12B recorder. The thermostating system was composed of a Yamato BT-21 incubator and a Taiyo C-600 circulator. The potentiometric titrations were carried out with a Radiometer RTS-822, a Radiometer REA-270 and a Horiba M-7 pH meter.

Incubation System——Substrate (CG or CS) and metal (or metal-ligand) solutions at fixed concentrations were prepared at each pH with 0.01 N NaOH or $HClO_4$ when buffer solution was not used. A solution of Cu(II)-rabbit serum albumin at a fixed concentration was prepared with a buffer (0.04 M HEPES, pH 7) solution. All solutions were kept at 37 ± 0.2 °C before use. The substrate solution (2 ml) and metal (or metal-ligand) solution (2 ml) were mixed in a cell which contained, depending on the purpose of the experiment, a part or all of the reagents. The pH of the mixture was measured immediately and kept at the initial value with 0.01 N NaOH during the course of incubation.

Determination of CG and CS——The concentrations of CG and CS in samples taken at suitable intervals were determined by HPLC under the conditions described previously,[12] with some modifications.

Results and Discussion

The initial rate (V_0) of the metals-catalyzed hydrolysis of CG at 37 °C was studied as a function of pH. The initial concentration of metal was the same as that of substrate. The acid hydrolysis of CG was negligible in the pH range from 1 to 8. The maximum V_0 with Cu(II) was observed at pH 5—6. The value of V_0 with Fe(III) increased with pH up to 3. When the pH was higher than 3, the V_0 of Fe(III) were not reproducible because of the precipitation of iron hydroxide. The V_0 of Ni(II) increased with pH up to 7. The V_0 of Zn(II) was high in the range of pH from 3 to 7 (Fig. 1). The hydrolyses by the other metals, Mn(II), Mg(II) and Ca(II), were too slow to be detected under the conditions used.

CS was hydrolyzed in the absence of metals in the acidic pH region, and acid hydrolysis was significant at low pH in comparison with the metals-catalyzed hydrolysis. The value of V_0 with Cu(II) was much larger than that of the metal-free system (which contained only substrate and $NaClO_4$), and the maximum value was observed at pH 5—6. The values with the other metals, Ni(II), Zn(II), Mn(II), Mg(II) and Ca(II), were almost the same as that of the metal-free system, although the value with Fe(III) was slightly larger. However, the difference between metal-added systems and the metal-free system bacame significant at higher concentrations of such metals as Ni(II) and Zn(II).

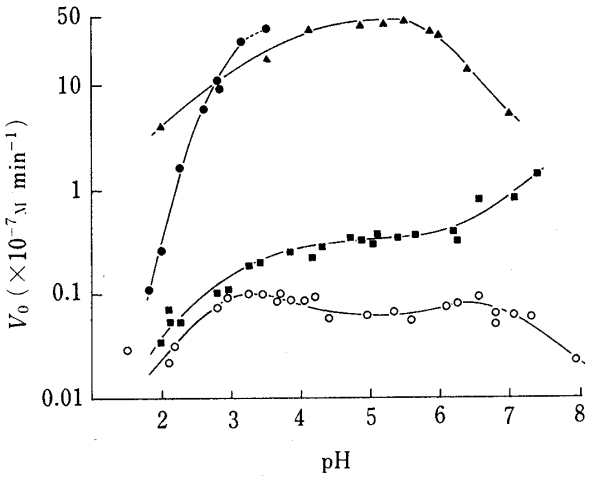

Fig. 1. V_0–pH Profiles for Metals-Catalyzed Hydrolysis of CG

▲, Cu(II); ●, Fe(III); ■, Ni(II); ○, Zn(II). $[CG]_{initial} = [metal]_{initial} = 5 \times 10^{-5}$ M. Incubation temperature, 37 °C.

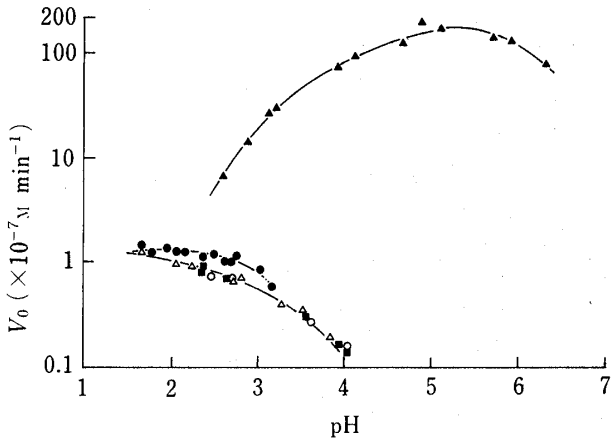

Fig. 2. V_0–pH Profiles for Metals-Catalyzed Hydrolysis of CS

▲, Cu(II); ●, Fe(III); ■, Ni(II); ○, Zn; △, metal free. $[CS]_{initial} = [metal]_{initial} = 5 \times 10^{-5}$ M. Incubation temperature, 37 °C.

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In the Cu(II)-catalyzed hydrolysis of 8-quinolyl sulfate and 8-quinolyl-β-D-glucopyranoside, possible intermediate stages were reported by two groups.[9,10,13] If the metal-catalyzed hydrolysis of C-conjugates is divided into two steps, namely, coordination of the metal to the conjugate and release of glucuronic acid or sulfuric acid from the C-metal chelate, the reaction rate would depend on the amounts of intermediates formed between the metals and substrates. Further, the chemical forms of metals and substrates are affected by change in pH, as are the amounts of the intermediates. Although the values of pH at which the initial rate for each metal system was largest were different, Cu(II) and Ni(II) were more active than the other metals even at neutral pH. The order of the catalyzing activities of metals at neutral pH was almost the same as that reported previously.[11] It is interesting that Fe(III) was very active at lower pH. In addition, it was found that CS was hydrolyzed more easily than CG by Cu(II), but less easily by Fe(III), Ni(II) and Zn(II).

The effect of ligand compounds on the metals-catalyzed hydrolysis was studied. The ligands used in this experiment were chosen from three categories: oxycarboxylic acids, amino acids and other complexing agents. Cu(II), which showed the highest catalytic activity, was used at pH 5.5, where the activity of Cu(II) was maximum (Fig. 1, Fig. 2). The initial ratio of [substrate] : [(Cu(II)] : [ligand] was 1 : 10 : 10. Under these conditions, the hydrolysis was a pseudo-first order reaction. The pseudo-first order rate constant (k') was used a measure of the effect of ligands. As shown in the left column in Table I, ligands tended to reduce k'. The stability constants of the complexes between Cu(II) and the ligands[14] are listed in the right column. In the cases of oxycarboxylic acids and diamines, k' tended to become smaller with increase of the stability constant in each group, for both CG and CS. No relationship was found in the case of amino acids (Table I). When rabbit serum albumin was added to a mixture of Cu(II) and a substrate, CG or CS, the ratio of hydrolyzed substrate decreased with increase of the concentration of albumin (Fig. 3). These results indicate that biological ligands

TABLE I. Pseudo-First-Order Rate Constants (k') for Hydrolyses of CG and CS by Cu(II)–Ligand Systems

Ligand	k' ($\times 10^{-2}$ min^{-1})		Stability constant of[14] Cu(II)–ligand complex log K_1 (°C, μ)
	CG	CS	
—	5.8	>8.2	—
Acetic acid	5.8	>8.2	2.05 (30, 0.1)
Lactic acid	5.1	>8.2	2.06 (30, 0.1)
Phthalic acid	2.0	8.2	3.28 (30, 0.1)
Tartaric acid	1.1	3.2	3.10 (20, 0.1)
Oxalic acid	0.5	2.2	4.49 (20, 0.1)
Citric acid	0.1	1.2	5.95 (25, 0.5)
Tryptophan	5.8		8.29 (20, 0.37)
Valine	2.6		8.11 (25, 0.1)
Glutamine	2.6		7.38 (25, 0.1)
Glycine	2.5		8.23 (25, 0.1)
Alanine	2.1		8.25 (25, 0.1)
Histidine	1.1	4.4	10.22 (25, 0.1)
Ethylenediamine	1.6	>8.2	10.44 (25, 0.1)
Nitrilotriacetic acid	<0.1	0.8	13.3 (25, 0.1)
Ethylenediaminetetraacetic acid	<0.1	0.2	18.87 (25, 0.1)

$[CG \text{ or } CS]_{initial} = 1 \times 10^{-4}$ M; $[Cu(II)]_{initial} = [ligand]_{initial} = 1 \times 10^{-3}$ M; $\mu = 0.1$ ($NaClO_4$); pH 5.5; incubation temperature, 37 °C.

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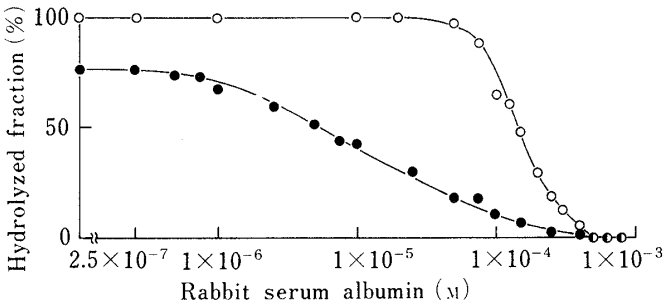

Fig. 3. Effect of Rabbit Albumin on Cu(II)-Catalyzed Hydrolyses of CG and CS

●, CG; ○, CS; $[CG]_{initial}$ or $[CS]_{initial} = 5 \times 10^{-4}$ M; $[Cu(II)]_{initial} = 5 \times 10^{-4}$ M; pH 7.0 (0.02 M HEPES buffer); incubation temperature, 37 °C; incubation time, 3 h.

which can form complexes with metal ions decrease the amount of free metal ions available for the hydrolysis of CG and CS, which have weak affinities for albumin. In practice, hydrolysis with Cu(II), whose concentration in plasma was calculated to be higher than that of albumin, was observed in rabbits following the administration of C and Cu(II). The details will be presented in the following paper.[15]

The first order rate (Table I) suggested that the concentration of metal ion remained almost constant, suggesting the possible existence of a coupled reaction $[ML] \rightleftarrows [M]+[L]$, where M is a metal ion, L is a ligand and ML is the complex. The amounts of iron, zinc and copper in a healthy person are reported to be 4—5 g, 1.4—2.3 g and 80—120 mg respectively.[16] Thus, free metal ions, even though at very low concentrations, might be continuously available in the body for the hydrolysis of clioquinol conjugates from metals bound to biological components. Therefore, the concentration of C-metal chelate might well increase slowly in the body.

It was reported that many SMON patients had been given C and metal drugs.[17] The participation of metals in the hydrolysis of C-conjugates may thus have significant consequences in such patients. Recently, the authors have observed the metal-catalyzed hydrolysis of C-conjugates *in vivo* and noted the occurrence of green urine in rabbits administered C and copper or iron. The details will be reported elsewhere.

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