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Depressing Effect of Salts on Thermal Degradation of Inosine 5′-Monophosphate and Guanosine 5′-Monophosphate in Aqueous Solution

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The thermal degradation of inosine 5′-monophosphate (IMP) and guanosine 5′-monophosphate (GMP) was depressed in the presence of $CaCl_2$, $MgCl_2$, and $MnCl_2$, but not in the presence of NaCl and KCl. The effect of $CaCl_2$, $MgCl_2$, and $MnCl_2$ was mainly to depress the cleavage of the phosphoric ester bond of the nucleotides. These results suggested that the coordination of divalent metal ions with the nucleotides resulted in suppression of the degradation. An infrared analysis of the crystal structure of the metal–nucleotide complex suggested that a ring structure was formed through intramolecular hydrogen bonds between two water molecules and the phosphate moiety in the coordinated IMP and GMP with divalent metal ion. The fixation of the phosphoric ester bond from the formation of this ring structure may cause the depression of hydrolysis of the phosphoric ester bond of the 5′-ribonucleotide.

Monosodium glutamate (MSG) and 5′-ribonucleotides such as inosine 5′-monophosphate (IMP) and guanosine 5′-monophosphate (GMP) are typical umami substances. These substances have become important enhancers in improving the palatability (or flavor) of food cooked at home and in commercial foods. Of these substances, IMP and GMP seem to be less stable thermally than MSG. When IMP and GMP are utilized in home cooking and by the food industry, they may be degraded by heat treatment.

There have been several investigations of thermal degradation of 5′-ribonucleotides (Fujita et al., 1965; Davidek et al., 1972; Lee, 1979; Shaoul and Sporns, 1987; Matoba et al., 1988). 5′-Ribonucleotides were degraded on the first-order reaction by heating (Fujita et al., 1965; Davidek et al., 1972; Lee, 1979; Matoba et al., 1988). Lee (1979) reported that the maximum rate of degradation of IMP was observed near pK_{a_2} (pH 5.87). On the other hand, Fujita et al. (1965), Davidek et al. (1972), Shaoul and Sporns (1987), and Matoba et al. (1988) reported that the rate of degradation of IMP and GMP was increased by lowering the pH. Davidek et al. (1972) reported that the thermal degradation of IMP and GMP was the hydrolysis of the phosphoric ester bond in the nucleotides. Matoba et al. (1988) observed that the cleavage of N-glucosidic bonds of IMP and GMP also occurred.

In general, various components such as carbohydrates, proteins, lipids, and salts are contained in foods. Therefore, these components may interact with the 5′-ribonucleotides to generate undesirable tastes during cooking and food processing. Nguyen and Sporns (1985) observed no considerable influence of the components (casein, glucose, potato starch, salt) in soup on the stability of 5′-ribonucleotides. We also reported that IMP in extract from commercially available umami seasoning powder was stable under heating (Kuchiba et al., 1989). However, the interaction between 5′-ribonucleotides and each food component has not been investigated in detail.

The purpose of this study is to elucidate the effects of salts on the thermal degradation of 5′-ribonucleotides.

MATERIALS AND METHODS

Materials. Inosine 5′-monophosphate (IMP) and guanosine 5′-monophosphate (GMP) were obtained from Kyowa Hakko Kogyo Co. Ltd., Tokyo. All the other reagents were purchased

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from Nacalai Tesque, Inc., Kyoto, and Wako Pure Chemical Industries, Ltd., Osaka.

Thermal Reaction System. The 5′-ribonucleotide and salt were dissolved in buffer (0.01 M sodium acetate at pH 4.0, 0.01 M 3-*N*-morpholinopropanesulfonic acid at pH 7.0), and the solution was adjusted to pH 4.0 or 7.0. The solution was incubated in a sealed Pyrex tube at 90–115 °C. The insignificant change in pH of the solutions during the heat treatment was observed. After heating, the tube was cooled immediately in an ice bath to stop the reaction. The reacted sample was analyzed by high-performance liquid chromatography (HPLC).

All the experiments were repeated once.

HPLC Analysis. A Shimadzu LC-6A liquid chromatograph, equipped with a UV detector, was used to determine 5′-ribonucleotide, nucleoside, and the purine base in the reacted sample. The detector was set at 254 nm. The column used was Cosmosil $5C_{18}$-P (ODS type, 4 × 250 mm), which was purchased from Nacalai Tesque, Inc., Kyoto. A C-R3A integrator (Shimadzu) was used for the calculation of peak areas. The mobile phase was methanol–0.02 M KH_2PO_4 (15:85, v/v). The flow rate was 0.5 mL/min. The temperature of the column was 25–30 °C. Under these conditions, the retention times of 5′-ribonucleotide (IMP, GMP), nucleoside (inosine, guanosine), and base (hypoxanthine, guanine) were 6.5, 10.5, and 8.5 min, respectively. Free phosphoric acid was determined by the method of Bartrett (1959).

Visible Absorption Spectra of Nickel(II). Visible absorption spectra of nickel(II) were measured by the continuous variation method according to the procedure of Longhi and Drago (1963). 5′-Ribonucleotide solution and nickel(II) sulfate solution were mixed at various molar fractions (total concentration was 4×10^{-2} M). After the mixture was allowed to stand for 30 min, the visible spectra of the mixtures (300–1100 nm) were measured with a Shimadzu UV-visible recording spectrophotometer, Model UV-160.

Preparation of Crystals of Metal–5′-Ribonucleotide Complexes. Equimolar solutions (7×10^{-2} M) of salt ($NiSO_4 \cdot 6H_2O$, $MnCl_2 \cdot 4H_2O$, $CaCl_2$ (anhydride), or $MgCl_2 \cdot 6H_2O$) and 5′-ribonucleotide were mixed slowly. The mixture was left at room temperature. The resulting crystal was filtered off, washed with water and methanol, and dried over P_2O_5 in vacuo. The crystals of the Cu^{2+}–GMP complex were prepared according to the method of Ogawa and Sakaguchi (1971).

Measurement of Infrared Spectra. IR spectra were measured in a solid state (KBr pellets) with a Perkin-Elmer 1740 IR Fourier transform spectrometer.

Representation of the Crystal Structure. The crystal structure was represented by using the personal computer PC-9801 (NEC) with the system Gonnosuke produced by Ando Laboratory, Department of Polymeric Engineering, Tokyo Institute of Technology, Tokyo.

RESULTS AND DISCUSSION

Effects of Salts on the Thermal Degradation of 5′-Ribonucleotide. Figure 1 shows the semilogarithmic plots (a logarithm of the remaining amount of IMP was plotted against heating time) for the thermal degradation of IMP in the presence of $CaCl_2$ (0–0.25 M) at pH 4 and 100 °C. The degradation of IMP in the presence of $CaCl_2$ proceeded according to the first-order reaction as in the absence of salts (control). The degradation rates of IMP decreased significantly with the increasing concentration of $CaCl_2$. The first-order rate constant of IMP in the presence of 0.25 M $CaCl_2$ was 61% of that of the control. The thermal degradation of GMP in the presence of $CaCl_2$ was also depressed, as was observed for IMP (data not shown). These results suggested that $CaCl_2$ depressed the thermal degradation of 5′-ribonucleotides.

The degradation of IMP and GMP in the presence of various salts (0.25 M) was investigated. The first-order rate constants are summarized in Table I. Ca^{2+}, Mg^{2+}, and Mn^{2+} significantly depressed the degradation. Mg^{2+} and Mn^{2+} were more effective than Ca^{2+}. On the other hand, Na^+ and K^+ scarcely depressed the degradation. SO_4^{2-} had a small depression effect on the degradation of IMP and GMP, but this was not the case with the other anions. The effect of each salt on the degradation was similar between IMP and GMP. Overall the rate constants were lower at pH 7 than those at pH 4, but a similar trend of the effect of each salt was observed at both pH values.

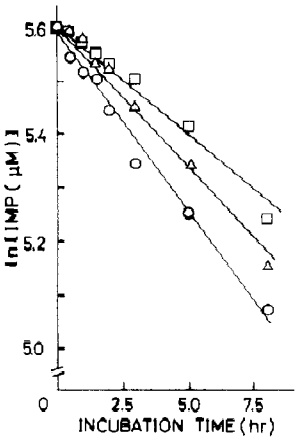

Figure 1. Effect of $CaCl_2$ on thermal degradation of IMP (100 °C, pH 4.0): ○, control (no addition of salts); △, 0.05 M $CaCl_2$; □, 0.25 M $CaCl_2$.

Table I. Effect of Salts on the Thermal Degradation of Inosine 5′-Monophosphate (IMP) and Guanosine 5′-Monophosphate (GMP)[a]

	k_{obsd},[b] $\times 10^{-2}$ h^{-1}			
	pH 4		pH 7	
salt[c]	IMP	GMP	IMP	GMP
control[d]	7.2 ● 0.07	10.9 ± 0.12	4.8 ± 0.17	7.7 ± 0.87
NaCl	6.7 ± 0.21	9.9 ± 0.34	4.4 ± 0.20	6.4 ± 0.18
KCl	6.8 ± 0.21	9.8 ± 0.31	4.1 ± 0.08	6.8 ± 0.20
$CaCl_2$	4.4 ± 0.16	6.3 ± 0.21	1.3 ± 0.05	1.3 ± 0.01
$MnCl_2$	3.6 ± 0.21	3.5 ± 0.14	ND[e]	ND
$MgCl_2$	3.8 ± 0.13	5.0 ± 0.75	0.5 ± 0.15	0.4 ± 0.08
CH_3COONa	6.5 ± 0.23	10.2 ± 0.20	NA[f]	NA
Na_2SO_4	5.6 ± 0.13	8.2 ± 0.58	3.2 ± 0.13	6.6 ± 0.21
NaH_2PO_4	6.6 ± 0.32	9.1 ± 0.07	NA	NA

[a] IMP or GMP solution (0.27×10^{-3} mol dm^{-3}) was incubated for 0, 0.5, 1.0, 1.5, 2.0, 3.0, 5.0, and 7.5 h. [b] Observed rate constant. [c] Concentration of added salt was 0.25 mol dm^{-3}. [d] No addition of salts. [e] Not detected. [f] Not analyzed.

Figure 2 shows the changes in concentration of IMP and its degradation products (inosine and hypoxanthine) during heating in the presence of various salts (0.25 M) at pH 4 and 100 °C. The amount of inosine produced from IMP in the presence of $CaCl_2$ and $MnCl_2$, which had depressed the degradation of IMP and GMP, was smaller than that in the control (no addition of salts). The amount of IMP remaining after heating differed among the added salts: $MnCl_2 > CaCl_2 > Na_2SO_4 > NaCl \approx$ control. And, the amount of inosine produced after heating in the presence of $MnCl_2$ was the smallest, followed by the amounts in the presence of $CaCl_2$, Na_2SO_4, NaCl, and finally the control. This order was essentially the same as that of the amount of remaining IMP, described above. These results suggested that the main effect of the salts on the depression of thermal degradation of 5′-ribonucleotides was to depress the hydrolytic cleavage of the phosphoric ester bond. The amounts of hypoxanthine produced in the presence of $CaCl_2$ or $MnCl_2$ were almost the same as that in the control. Hypoxanthine was formed not only from inosine but also from

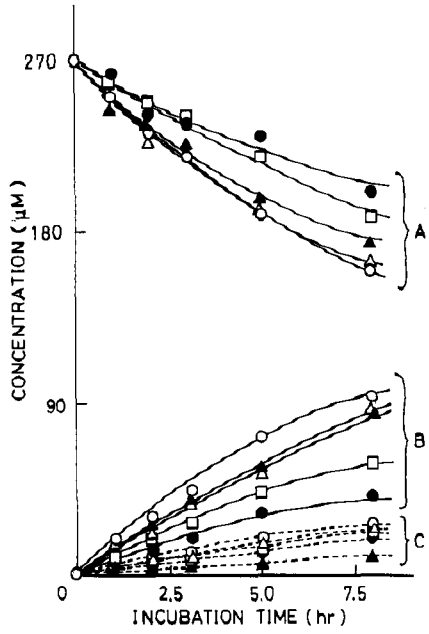

Figure 2. Effect of salts on thermal degradation of IMP (100 °C, pH 4.0): (A) IMP; (B) inosine; (C) hypoxanthine. Key: ○, control (no addition of salts); △, 0.25 M NaCl; □, 0.25 M $CaCl_2$; ●, 0.25 M $MnCl_2$; ▲, 0.25 M Na_2SO_4.

Table II. Effect of Salts on the Thermal Degradation of Inosine and Ribose 5-Phosphate[a]

salt[b]	k_{obsd},[c] h^{-1} A	k_{obsd},[c] h^{-1} B
control[d]	9.1 ± 0.20	5.5 ± 0.16
NaCl	10.9 ± 0.07	6.5 ± 0.12
$CaCl_2$	ND[e]	7.2 ± 0.07
$MnCl_2$	15.6 ± 0.05	12.9 ± 0.27
Na_2SO_4	10.4 ± 0.16	2.3 ± 0.58

[a] Key: A, ribose 5-phosphate; B, inosine. Ribose 5-phosphate and inosine solution ((2–3) × 10^{-3} mol dm^{-3}) was incubated for 0, 0.5, 1.0, 1.5, 2.0, 3.0, 5.0, and 7.5 h, at 100 and 115 °C, respectively. [b] Concentration of added salt was 0.25 mol dm^{-3}. [c] Observed rate constant. [d] No addition of salts. [e] Not detected.

IMP. But, the rate of hypoxanthine formation from inosine was faster than that from IMP (Matoba et al., 1988). The amount of inosine produced in the presence of $CaCl_2$ or $MnCl_2$ was remarkably smaller than that in control. These results suggested that these salts accelerated the cleavage of the N-glucosidic bond. In the presence of Na_2SO_4, the amounts of hypoxanthine produced after heating were much smaller, though the amounts of inosine produced were very similar to that in the control (no addition), suggesting that SO_4^{2-} has a depressing effect on the cleavage of N-glucosidic bond of IMP and GMP. Therefore, Na_2SO_4 depressed the degradation of 5′-ribonucleotides by a mechanism different from $CaCl_2$, etc. (Table I; Figure 2).

In order to examine the effect of NaCl, Na_2SO_4, $CaCl_2$, or $MnCl_2$ on the cleavages of the phosphoric ester bond and the N-glucosidic bond, the first-order rate constants of thermal degradation of ribose 5-phosphate and inosine at pH 4 were determined by measuring the amount of phosphoric acid released and that of inosine remaining as shown in Table II. Inosine was heated at 115 °C, because the degradation rate of inosine was slow (100 °C for ribose 5-phosphate). The degradation of inosine in the presence of Na_2SO_4 was depressed but was accelerated in the presence of other salts. This observation was consistent with the fact that the amount of hypoxanthine produced in the presence of Na_2SO_4 was small (Figure 2). On the other hand, the degradation (hydrolysis of ester bond) of ribose 5-phosphate was accelerated in the presence of each salt, with $MnCl_2$ being especially effective. This acceleration by metal ions may be explained by the fact that the electronegativity of the oxygen atom forming the phosphoric ester bond increased, because metal ions coordinated with the oxygen atoms of phosphate moiety, as described by Barker (1971).

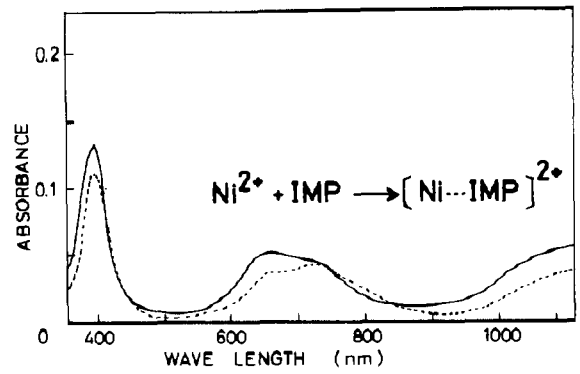

Figure 3. Change in absorbance spectra of Ni^{2+} in the presence and absence of IMP: - - -, 40 mM $NiSO_4 \cdot 6H_2O$; —, 40 mM $NiSO_4 \cdot 6H_2O$ + 40 mM IMP.

From the results described above, it was suggested that Ca^{2+}, Mg^{2+}, and Mn^{2+} interacted with both base and phosphate moiety of IMP to depress the thermal degradation of IMP.

Interaction between 5′-Ribonucleotides and Metal Ions. The interaction between 5′-ribonucleotides and metal ions was examined, because divalent cations depressed the thermal degradation rates of 5′-ribonucleotides.

First, whether or not a divalent cation forms a chelate complex with 5′-ribonucleotides was examined. Ni^{2+} was used as the model metal ion because Ni^{2+} has an absorption at 392, 657, and 723 nm in the visible region, while this is not the case with Mg^{2+}, Mn^{2+}, and Ca^{2+}. Figure 3 shows the visible absorption spectra (350–1000 nm) of Ni^{2+} in the presence and absence of IMP in aqueous solution. The molar absorbance of Ni^{2+} at 723 nm was higher than that at 654 nm in the presence of IMP, while the molar absorbance at 654 nm was higher than that at 723 nm in the absence of IMP, indicating that the presence of IMP caused the change in the absorption spectrum of Ni^{2+}. The spectra in the presence of GMP were also similar to that in the presence of IMP. These results suggest that divalent metal ions can coordinate with 5′-ribonucleotides.

Next, the absorption spectrum of the mixture of $NiSO_4$ and IMP (or GMP) at 654 nm in aqueous solution was measured by the continuous-variation procedure (Longhi and Drago, 1963). Parts a and b of Figure 4 illustrate the curve obtained when the change of absorbance (ΔA) was plotted as a function of the mole fraction of $NiSO_4$. ΔA was maximum when an equimolar amount of the molar ratio of $NiSO_4$ to each 5′-ribonucleotide was present. This result suggests that a 1:1 complex of Ni^{2+} with IMP or GMP was formed.

In order to examine whether or not a 1:1 complex of 5′-ribonucleotide (IMP or GMP) with a divalent cation was formed, an elementary analysis of the crystals of metal–5′-ribonucleotide (Ni^{2+}, Mn^{2+}, Ca^{2+}, Mg^{2+}) complex was performed (data not shown). Each crystal was confirmed to be a 1:1 complex of the metal with 5′-ribonucleotide.

There have been various investigations of the crystal structures of the metal–nucleotide complexes. Aoki (1975)

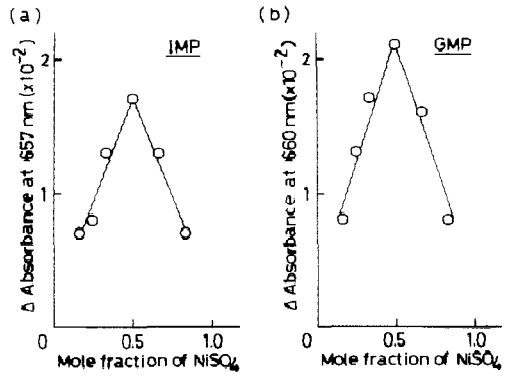

Figure 4. Continuous variation method of the system $NiSO_4$–IMP at a total concentration of 4×10^{-2} M. Δ Absorbance was the absorbance of the mixture of $NiSO_4$ and each 5′-ribonucleotide minus the absorbance of only the equimolar $NiSO_4$ solution. Key: (a) IMP, (b) GMP.

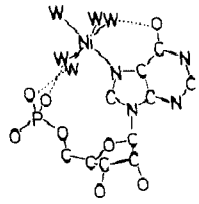

Figure 5. Perspective view of the molecule in the nickel complex. Reprinted with permission from Aoki (1975). Copyright 1975 Nippon Kagakkai. W = water molecule.

elucidated the crystal structure of the 1:1 complex of Ni^{2+} with IMP by X-ray crystallography as follows (Figure 5). Ni^{2+} has six coordination sites occupied by the nitrogen atom N(7) of hypoxanthine moiety and the five oxygen atoms of water ligands. One of these water ligands (W(2)) forms a hydrogen bond with the carbonyl (keto form) oxygen O(6) of the base. The other two water ligands (W(3) and W(4)) also form hydrogen bonds with the oxygens of phosphate (O(8) and O(9)). Thus, the Ni^{2+} interacts indirectly with the phosphate oxygens through water ligands. Therefore, IMP, Ni^{2+}, and water ligands form an 11-membered ring structure through coordination bonds and intramolecular hydrogen bonds. Furthermore, Aoki (1976) reported that a Cu^{2+} ion interacts directly with the two phosphate oxygens of GMP but interacts with the guanine moiety in the same manner as the Ni^{2+}–IMP complex.

Infrared Analysis of Metal–5′-Ribonucleotide Complexes. To clarify the interaction of IMP or GMP with Ca^{2+}, Mn^{2+}, or Mg^{2+}, infrared spectra of the metal–5′-ribonucleotide complexes were measured (Figure 6a,b). It has been known that the absorption bands related to a N(7) of purine base appear at approximately 1485 cm^{-1} ($\nu(C_8{=}N_7) + \delta(C_8{-}H)$) and 1330 cm^{-1} ($\nu(C_8{=}N_7) + \nu(C_8{-}N_9)$) (Calafat et al., 1987). The bands at 1482 and 1328 cm^{-1} in the present spectrum of IMP correspond to those of the purine base, described above. These two bands disappeared or split in the complexes of these metals with IMP (Figure 6a). The changes in these bands for IMP were also observed for GMP, though the latter were not clear (Figure 6b). These results revealed that a N(7) of the purine bases of IMP and GMP was coordinated to the divalent metal ion.

It has been known that the $\nu(PO_3)_{sym}$ band at 980 cm^{-1} and $\nu(P{-}O)$ bands at 785 and 820 cm^{-1} appear (Calafat et al., 1987; Tajmir-Riahi and Theophanides, 1983). The band at 980 cm^{-1} did not shift when Ca^{2+}, Ni^{2+}, and Mn^{2+} ions formed complexes with the 5′-ribonucleotides (Figure 6a,b). But this band of Cu^{2+}–GMP complex

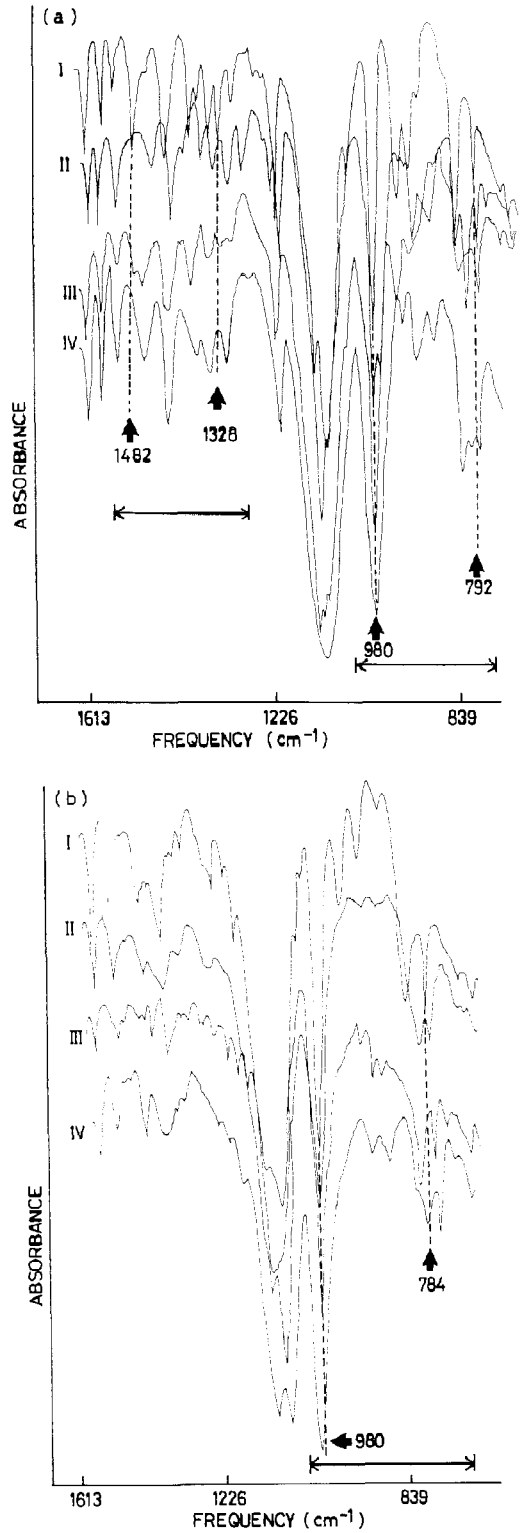

Figure 6. Infrared absorption spectra of 5′-ribonucleotide and metal–nucleotide complexes. (a) IMP and metal–IMP complex: I, IMP; II, IMP–Ni; III, IMP–Ca; IV, IMP–Mn. (b) GMP and metal–GMP complex: I, GMP; II, GMP–Ca; III, GMP–Ni; IV, GMP–Cu. Key: ↔, expanded 150%; (a) 1520–1290 and 995–720 cm^{-1}; (b) 995–700 cm^{-1}.

shifted to a higher frequency (Figure 6b). This showed that the Cu^{2+} was bound directly to the PO_3^{2-} group of GMP, as revealed by X-ray crystallography (Aoki, 1976). When IMP and GMP coordinated to Ca^{2+}, Ni^{2+}, and Mn^{2+}, the $\nu(P{-}O)$ bands at 795 and 820 cm^{-1} shifted (Figure 6a,b), indicating that the structure of the phosphoric ester bond in the 5′-ribonucleotide was changed by coordination with these ions. This evidence suggests that a coordination profile of Ca^{2+} and Mn^{2+} ions with the

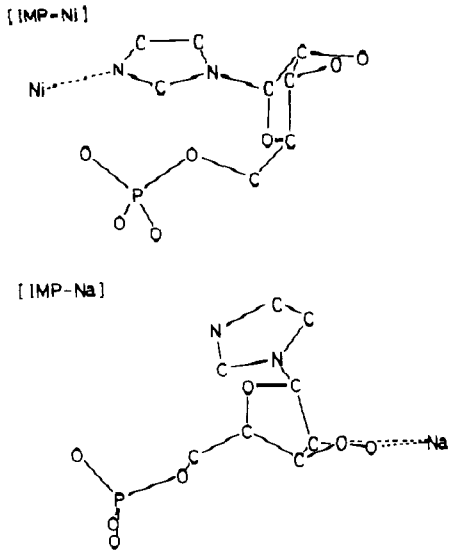

Figure 7. Structures in the neighborhood of the phosphoric ester bond. The structure was represented by using a personal computer with the system Gonnosuke. Positional parameters of the atoms are from the papers of Aoki (1974) and Rao and Sundaralingam (1969).

5′-ribonucleotides is very similar to that of Ni^{2+} with IMP reported by Aoki (1975); that is, these ions formed a ring structure of 5′-ribonucleotides and water ligands with intramolecular hydrogen bonds.

We did not carry out an infrared analysis of the crystal of the Na^+–IMP (GMP) complex, because preparation of the crystal was unsuccessful. Fortunately, there have been some reports on the X-ray crystallography of Na^+–nucleotide complexes. Rao and Sundaralingam (1969) reported that in the Na^+–IMP complex (monosodium) Na^+ binds to two hydroxyl groups of its ribose moiety (O(2′) and O(3′)) and water molecules, but not to the nitrogen atom N(7) of its hypoxanthine moiety. Katti et al. (1981) showed that, in the $2Na^+$–GMP complex (disodium), Na^+ coordinates with hydroxyl oxygens O(2′) and O(3′) of its ribose moiety. On the other hand, Barnes and Hawkinson (1982) reported that, in the $2Na^+$–GMP complex, N(7) of its purine base coordinates with Na^+. These reports on the complexes with sodium ion demonstrate that the Na^+ ($2Na^+$)–5′-ribonucleotide complex does not form a ring structure (PO_3 or PO–metal ion–N(7)) as seen in the Ni^{2+} complex (Aoki, 1975, 1976). The reason why Na^+ and K^+ did not depress the thermal degradation of 5′-ribonucleotide may be associated with the fact that these ions do not coordinate with the 5′-ribonucleotides in the manner of the ring structure as described above (Figure 7).

The NMR studies for Cu^{2+}–AMP, –IMP, and Mn^{2+}–AMP in aqueous solution also suggest preferential coordination of the N(7) atom in the base and the binding or a weak interaction of the phosphate (Berger and Eichhom, 1971a,b; Kotowycz and Hayamizu, 1973).

We think that the complexes formed at room temperature might be fundamentally the same as that at elevated temperature, but this has not been confirmed. The structure of the complexes formed at elevated temperature is a subject for further investigation.

From the above evidence, we propose as follows the possible mechanism of the effect salts have on the depression of thermal degradation of the 5′-ribonucleotides: The formation of an intramolecular ring structure among N(7) of its purine, a divalent ion, water molecules, and PO_3 of its ribose results in the location of the oxygen atom of the phosphoric ester bond inside the ring, thus fixing the moiety of the phosphoric ester bond (Figure 7). Therefore, this ring structure may cause the depression of hydrolysis of the phosphoric ester bond.

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LITERATURE CITED

Aoki, K. Crystallographic studies of interaction between nucleotides and metal ions. I. Crystal structures of the 1:1 complexes of cobalt and nickel with inosine 5′-phosphate. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 1260–1271.

Aoki, K.; Clark, G.; Orbell, J. D. Metal-phosphate bonding in transition metal nucleotide complexes. The crystal and molecular structures of the polymeric copper(II) complex of guanosine 5′-monophosphate. *Biochim. Biophys. Acta* **1976**, *425*, 369–371.

Barker, R. *Organic Chemistry of Biological Compounds*; Prentice-Hall: Englewood Cliffs, NJ, 1971.

Barnes, C. L.; Hawkinson, S. W. Structure of disodium guanosine 5′-phosphate heptahydrate. *Acta Crystallogr.* **1982**, *B38*, 812–817.

Bartrett, G. R. Phosphorus Assay in Column Chromatography. *J. Biol. Chem.* **1958**, *234*, 466–468.

Berger, N. A.; Eichhom, G. L. Interaction of metal ions with polynucleotides and related compounds. XIV. Nuclear magnetic resonance studies of the binding of copper(II) to adenine nucleotides. *Biochemistry* **1971a**, *10*, 1847–1857.

Berger, N. A.; Eichhom, G. L. Interaction of metal ions with polynucleotides and related compounds. XV. Nuclear magnetic resonance studies of the binding of copper(II) to nucleotides and polynucleotides. *Biochemistry* **1971b**, *10*, 1857–1864.

Calafat, A. M.; Mulet, D.; Fiol, J. J.; Terron, A. Chromium(III) interactions with nucleotides. III. *Inorg. Chim. Acta* **1987**, *138*, 105–112.

Davidek, J.; Velisek, J.; Janicek, G. Stability of inosinic acid, inosine and hypoxanthine in aqueous solutions. *J. Food Sci.* **1972**, *37*, 789–790.

Fujita, E.; Kimura, H.; Nakatani, H.; Ishii, K.; Satani, E. Studies on the heat stability of 5′-ribonucleotides. *Eiyo to Syokuryo* **1965**, *18*, 98–102.

Katti, S. K.; Seshadri, T. P.; Viswamitra, M. A. Structure of disodium guanosine 5′-phosphate heptahydrate. *Acta Crystallogr.* **1981**, *B37*, 1825–1831.

Kotowycz, G.; Hayamizu, K. A carbon-13 nuclear magnetic resonance study of nucleotide-metal interactions. Binding of manganese(II) with adenine nucleotides. *Biochemistry* **1973**, *12*, 517–520.

Kuchiba, M.; Kiriyama, C.; Sumiyama, M.; Matoba, T.; Hasegawa, K. 1988. Thermal degradation on IMP in Commercially available umami seasoning extract and stability of IMP in the seasoning powder under various water activities. *J. Home Econ. Jpn.* **1989**, *40*, 677–681.

Lee, J. H. Studies on kinetic of inosine 5′-monophosphate instability. *J. Food Sci.* **1979**, *44*, 946–947.

Longhi, R.; Drago, R. S. Metal-containing compounds of the anion $(C_2H_5)_2NN_2O_2^-$. *Inorg. Chem.* **1963**, *2*, 85–88.

Matoba, T.; Kuchiba, M.; Kimura, M.; Hasegawa, K. Thermal degradation of flavor enhancers, inosine 5′-monophosphate and guanosine 5′-monophosphate in aqueous solution. *J. Food Sci.* **1988**, *53*, 1156–1159.

Nguyen, T. T.; Sporns, P. Decomposition of the flavor enhancers, monosodium glutamate, inosine-5′-monophosphate and guanosine-5′-monophosphate during canning. *J. Food Sci.* **1985**, *50*, 812–814.

Ogawa, M.; Sakaguchi, T. Studies on the metal-nucleotide complexes and their infrared absorption spectra. *Yakugaku Zasshi* **1971**, *91*, 750–754.

Rao, S. T.; Sundaralingam, M. Stereochemistry of nucleic acids and their constituents. V. The crystal and molecular structure of a hydrated monosodium inosine 5′-phosphate. A commonly occurring unusual nucleotide in the anticodons of tRNA. *J. Am. Chem. Soc.* **1969**, *91*, 1210–1217.

Shaoul, O.; Sporns, P. Hydrolytic stability at intermediate pHs of the common purine nucleotides in food, inosine-5′-monophosphate, guanosine-5′-monophosphate and adenosine-5′-monophosphate. *J. Food Sci.* **1987**, *52*, 810–812.

Tajmir-Riahi, H. A.; Theophanides, T. Platinum(II) and magnesium(II) nucleotide complexes. Synthesis, FT-ir spectra, and structural properties. *Can. J. Chem.* **1983**, *61*, 1813–1822.

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Lipase of *Penicillium caseicolum*

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The lipase activity of *Penicillium caseicolum* was tested in a cell-free broth of the organism and in a partially purified preparation of the enzyme. The optimum reaction pH of the enzyme was 9.0 and the optimum temperature 35 °C. A mixture of $CaCl_2$ and sodium taurocholate enhanced the activity of the enzyme toward butter oil. The heat inactivation of this lipase was exponentially related to temperature. A z value of 16 °C and an inactivation energy of 32 200 cal/mol were calculated for this enzyme. Triglycerides of lower molecular weight fatty acids were better substrates than those of higher molecular weight fatty acids for this lipase.

Lipases are widely distributed in nature. By hydrolyzing lipids, lipases contribute significantly to the development of flavor, pleasant or unpleasant, in many foods, especially dairy products (Schwimmer, 1981). Of particular interest to the cheese industry are the lipases produced by microorganisms (Chandan et al., 1969; Shahani et al., 1976). Two fungi commonly used in the manufacture of Camembert and Brie cheeses are *Penicillium camemberti* and *Penicillium caseicolum* (or *Penicillium candidum*). While some work on the lipases of these molds has already been published (Lamberet and Lenoir, 1976), much remains to be learned about them. This study deals with certain aspects of the catalysis by *P. caseicolum* lipase.

MATERIALS AND METHODS

A culture of *P. caseicolum*, obtained from G. Roger Laboratories, Paris, France, was grown in mycological broth (Difco Labs, Detroit, MI) containing 1% corn oil at room temperature with rotary shaking (120 rpm) for 3 days. The growth medium was filtered, and the cell-free broth was assayed for lipase activity. For the determination of optimum pH for the enzyme, the reaction was carried out a seven pH levels in the range 5.0–11.0. The substrate solution contained 10% gum arabic, 2 mM $CaCl_2$, and 10% tributyrin or 10% butter oil, both emulsified by homogenization. The pH was adjusted by adding 0.1 N NaOH. Substrate solution (5 mL) was transferred into the reaction vessel of a pH-Stat (Metrohm, Herisan, Switzerland), the temperature was adjusted to 35 °C, 0.2 mL of cell-free broth was added, and the reaction was allowed to proceed for 5–10 min. The volume of 0.02 N NaOH consumed, to keep the pH constant, was recorded automatically on a strip chart, and the slope of the recorded line (which was straight for the first few minutes) was taken as a measure of the reaction rate. The lipase activity was expressed in micromoles of fatty acid(s) liberated per minute.

For the determination of optimum temperature, the reaction system just described was set to pH 9.0 and the reaction was run at temperatures varying from 25 to 55 °C.

The effect of sodium taurocholate, sodium deoxycholate, and $CaCl_2$ on the lipase hydrolysis was also studied. The salts were added by themselves or in combination at concentrations vary-

Table I. Relative Activity of *P. caseicolum* Lipase toward Natural and Synthetic Lipids[a]

lipid	rel rate of hydrolysis
tributyrin	100
tricaproin	47
tricaprylin	40
tristearin	23
triolein	26
trilaurin	34
trimyristin	16
tripalmitin	9
corn oil	34
sunflower oil	33
grape seed oil	30
almond oil	29
safflower	29
soybean oil	28
peanut oil	28
butter oil	24
sesame oil	24
lard	23
hazelnut oil	22
walnut oil	21
olive oil	18

[a] Reaction mix: 5 mL containing 0.2 mg of purified enzyme, 10% lipid, 10% gum arabic, 8 mM in $CaCl_2$, 8 μM in sodium taurocholate. pH 9.0, 35 °C. Actual lipase activity against tributyrin: 6.2 μmol of FFA/mg of protein per min.

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