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Studies on the Metal-Nucleotide Complexes. VI.[1] Isolation of Metal-5'-uridylic Acid Complexes and Their Infrared Absorption Spectra

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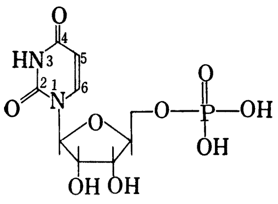
In the previous work of this series,[1,3] we investigated the interaction of metal ions with purine nucleotides by spectral examination of metal complexes isolated. In order to obtain more extensive informations about the interaction of metal ions with nucleic acid, we isolated metal complexes of 5'-uridylic acid (5'-UMP) with zinc, lead, cadmium, and silver from the solution of pH 4.8—6.8.



5'-UMP

Experimental

Apparatus——Infrared absorption spectra were measured with Hitachi Infrared Spectrometer, type EPI S-2 and 215, and pH was measured by Toshiba-Beckman Expandomatic type pH meter.

Reagents——Disodium 5'-uridylate was purchased from Yamasa Co., Ltd. Other reagents were all JIS special grade reagents and used without further purification.

General Procedure for the Preparation of 5'-Uridylic Acid-Metal Complex——To a solution of 5'-UMP-2Na (0.37 g) dissolved in 25 ml of H_2O, a solution of the metal salt in 15 ml of H_2O was added with stirring. The solution was heated at 60°. The crystalline precipitate finally formed was collected by filtration, washed successively with H_2O, EtOH, and ether and dried *in vacuo* over P_2O_5 at 50° for 3 hr.

Zinc Complex: $Zn(NO_3)_2 \cdot 6H_2O$ (0.30 g) was used, heated until a turbidity occurred (15—20 min) and allowed to stand overnight at room temperature.

Cadmium Complex: $Cd(NO_3)_2$ (0.20 g) was used, heated until a turbidity occurred (15—20 min) and allowed to stand until room temperature and the resulting turbidity was filtrated off. The filtrate was stored in a desiccator over silica gel.

Lead Complex: $Pb(NO_3)_2$ (0.33 g) was used, heated for 20 min.

Silver Complex: $AgNO_3$ (0.51 g) was used, heated for 5 min.

5'-Uridylic Acid (5'-UMP Free Acid)——5'-UMP-2Na (1 g) was dissolved in 50 ml of H_2O, passed through a column of Amberlite IRA-122 (H^+ form, 100 ml), and was washed with H_2O. The eluate and washings were combined and evaporated to dryness under diminished pressure below 50°. The product was dried *in vacuo* over P_2O_5 at room temperature. *Anal.* Calcd. for $C_9H_{13}O_9N_2P$: C, 33.34; H, 4.04; N, 8.64: Found: C, 33.83; H, 4.52; N, 8.35.

General Procedure for the Preparation of 5'-Uridylic Acid Salts——5'-UMP free acid (0.33 g) was dissolved in 20 ml of H_2O and the pH of the solution was adjusted. Salts were precipitated by adding EtOH (80 ml) to the above solution, and this mixture was allowed to stand in refrigerator for overnight. The precipitate finally formed was collected by filtration, washed successively with EtOH, and ether, and dried *in vacuo* over P_2O_5 at room temperature.

Dipotassium Salt: The pH of the solution was adjusted to 7.5 with 10% KOH solution. *Anal.* Calcd. for $(C_9H_{11}O_9N_2P)\ K_2 \cdot 4H_2O$: C, 22.86; H, 4.02; N, 5.93: Found: C, 23.01; H, 3.34; N, 5.96.

Barium Salt: The pH of the solution was adjusted to pH 7.5 with solid $Ba(OH)_2$. *Anal.* Calcd. for $(C_9H_{11}O_9N_2P)\ Ba(OH_2)_2$: C, 21.79; H, 303; N, 5.65. Found: C, 22.42; H, 2.96; N, 5.47.

Calcium Salt: The pH of the solution was adjusted to pH 7.5 with solid $Ca(OH)_2$. *Anal.* Calcd. for $(C_9H_{11}O_9N_2P)\ Ca(OH_2)_2$: C, 27.12; H, 3.77; N, 7.03. Found: C, 27.51; H, 3.76; N, 6.76.

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[1] Part V: M. Ogawa and T. Sakaguchi, *Yakugaku Zasshi*, **91**, 750 (1971).

[2] Location: *Yayoi-cho, Chiba-shi.*

[3] M. Ogawa, Y. Urata, and T. Sakaguchi, *Bunseki Kagaku*, **19**, 1244 (1970); *idem*, *ibid.*, **20**, 36 (1971); M. Ogawa, *Yakugaku Zasshi*, **91**, 618 (1971); M. Ogawa and T. Sakaguchi, *Chem. Pharm. Bull.* (Tokyo), **19**, 1650 (1971).

Monosodium Salt: The pH of the solution was adjusted to pH 4.5 with 10% NaOH. *Anal.* Calcd. for $(C_9H_{11}O_9N_2P)$ $Na \cdot 2H_2O$: C, 29.66; H, 3.84; N, 7.69. Found: C, 30.41; H, 4.02; N, 7.74.

Result and Discussion

Isolated Metal-5′-UMP Complexes

The elementary analysis revealed that the ratio of 5′-UMP to metal was 1: 1 in the cases of zinc, lead, and cadmium complexes, while this ratio was 1: 3 in silver complex (Table I).

Table I. Isolated Metal 5′-UMP Complexes

Complexes	Prepared pH	Appearance	Formular	Analysis (%) Calcd. C	H	N	metal	Found C	H	N	metal
Zn-5′-UMP	6.6	colorless needles	$(C_9H_{11}O_9N_2P)Zn(H_2O)_2$	25.60	3.55	6.62	15.4	25.81	3.59	6.96	15.4
Cd-5′-UMP	6.8	colorless needles	$(C_9H_{11}O_9N_2P)Cd(H_2O)$	23.86	2.87	6.19	23.9	23.42	2.92	5.46	23.1
Pb-5′-UMP	5.2	colorless crystalline powder	$(C_9H_{11}O_9N_2P)Pb(H_2O)$	19.73	2.37	5.11	37.9	20.17	2.07	4.87	36.6
Ag-5′-UMP	4.8	light brown powder	$(C_9H_{10}O_9N_2P)Ag_3$	16.72	1.86	4.34	50.19	16.58	1.77	3.67	50.59

Infrared Absorption Spectra of 5′-UMP 2Na, 5′-UMP Na, and 5′-UMP Free Acid

Before the discussion of the spectral changes on the complexation with metal ions, we examined the spectral changes of 5′-UMP itself upon the dissociation of phosphate group, which were observed in the region of 1200—900 cm^{-1} (phosphate group) but not in the region of 1700—1600 cm^{-1} (uracil ring moiety) in these compounds.

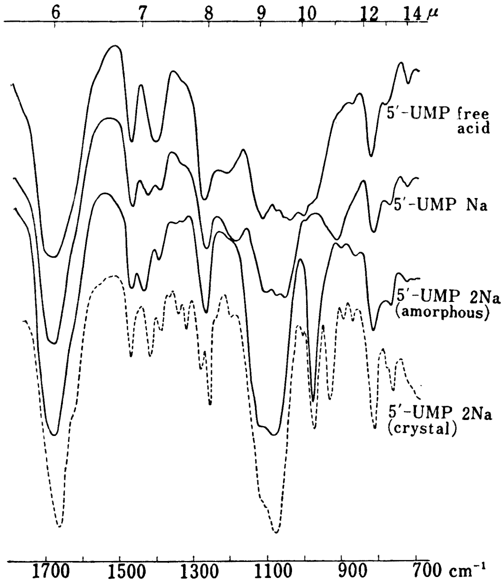

Fig. 1. Infrared Absorption Spectra of 5′-UMP Free Acid, Sodium Salt, and Disodium Salt (Crystal and Amorphous) (KBr Disk)

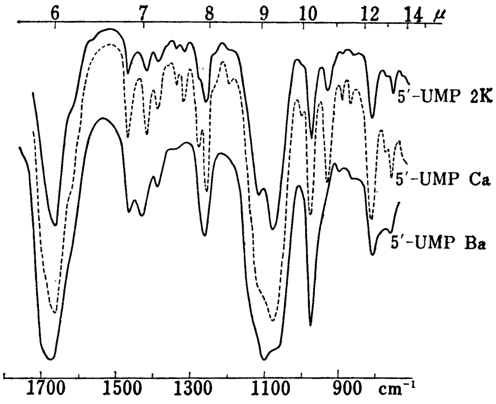

Fig. 2. Infrared Absorption Spectra of 5′-UMP Dipotassium Salt, Barium Salt, and Calcium Salt (KBr Disk)

Infrared Absorption Spectra of 5′-UMP 2K, 5′-UMP Ca, and 5′-UMP Ba Salts

Infrared absorption spectra of 5′-UMP 2K, 5′-UMP Ca, and 5′-UMP Ba salts were measured to examine the coordination of metal ions to the uracil ring moiety. In these compounds, C=O stretching vibration appeared in the region of 1675 cm^{-1} and the spectra in the region of 1200—900 cm^{-1} were similar to that of 5′-UMP 2Na. It was therefore assumed that these alkali and alkali earth metals were bound to phosphate group alone.

In the case of 5′-UMP Ba salt a little broad spectrum was noticed, compared with those of 5′-UMP 2K and 5′-UMP Ca. The spectrum of 5′-UMP Ba salt we prepared showed a spectrum similar to that of the amorphous sample of 5′-UMP 2Na (lyophilized sample), and it was assumed that this change is due to the amorphous structure of 5′-UMP Ba salt.

Infrared Absorption Spectra of the Isolated Metal Complexes

The infrared absorption spectra of the isolated metal complexes are illustrated in Fig. 3 and 4. A strong band appeared in the region of 1730 and 1705 cm^{-1} in the lead complex, and 1690 cm^{-1} in the cadmium and zinc complexes. The strong band appeared in the region of 1640 and 1538 cm^{-1} in complex with silver. The band near 1675 cm^{-1} in the metal salts of 5′-UMP was not seen in these complexes.

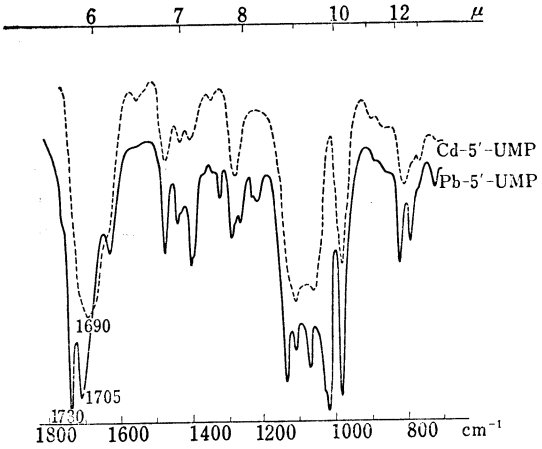

Fig. 3. Infrared Absorption Spectra of Cd-5′-UMP and Pb-5′-UMP (KBr Disk)

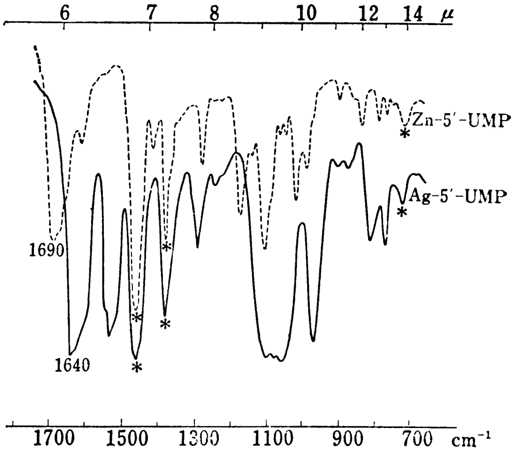

Fig. 4. Infrared Absorption Spectra of Zn-5′-UMP and Ag-5′-UMP (Nujol Mull)

In all the isolated metal complexes and salts, a strong band was seen near 970 cm^{-1}, which was assigned to PO_3^{2-}. It was concluded from the elementary analyses that the isolated metal complexes did not contain NO_3^-. It was ascertained that these metals were also bound to the phosphate group in 5′-UMP. The question arises whether the uracil ring moiety participates in the metal coordination. The carbonyl group in positions 2 and 4, and nitrogen atom at 3 are the feasible coordination sites. In the case of silver complex, the possibility that the coordination sites should be the carbonyl group in position 2 or 4, was presumed from low frequency shifts of these group in infrared absorption spectrum.

From this result and elementary analysis, it was assumed that the reasornable structure for silver complex of 5′-UMP will be alternative structres of Ia and Ib or II involving 2 moles of 5′-UMP as shown in Chart 1.

The spectral changes in lead complex in the region of 1730—1600 cm^{-1} will be due to the metal interaction with the uracil ring moiety of 5′-UMP, but it is still not clear which site of the uracil ring participated in the coordination.

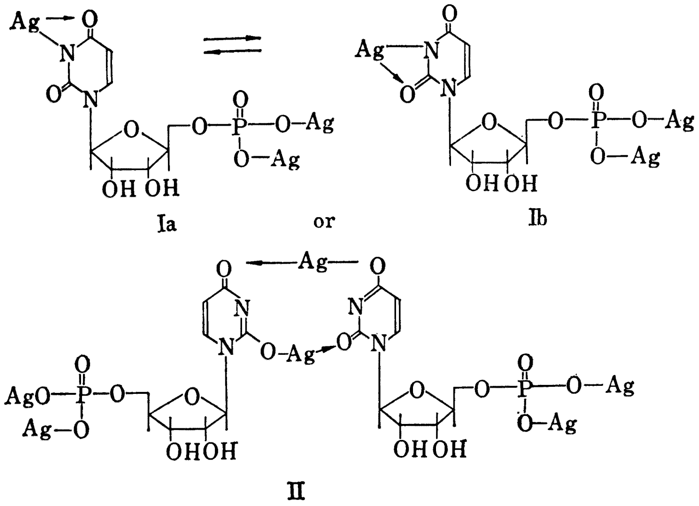

Chart 1. Proposed Structure of Ag-5'-UMP Complex

In the cases of zinc and cadmium, only a little spectral changes were noticed in the region of 1700—1600 cm^{-1}, and that these metals seemed to be predominantly bound to the phosphate group in 5'-UMP.

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Formation and Characterization of 5'-Deoxy-5',6-epimino-5,6-dihydro-2',3'-O-isopropylideneuridine

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The susceptibility of uracil and cytosine to nucleophilic attack at the 5,6-double bond constitutes an important chemistry of their ring systems. It has recently been shown that the addition of an external nucleophile is involved in the mechanism of cleavage of uracil by hydroxylamine,[2)] in deuterium or tritium exchange process of uracil,[3)] in the mode of action of thymidine synthetase,[4)] in the synthesis of triazolopyrimidines from 5-nitrouracil,[5)]

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1) Location: *a) Wako-shi, Saitama; b) Honkomagome, Bunkyo-ku, Tokyo.*

2) J.H. Phillips and D.M. Brown, *Progr. Nucleic Acid Res. Mol. Biol.*, 7, 349 (1967).

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