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Complexes between Nucleic Acid Bases and Bivalent Metal Ions. II.[1) Complexes formed by Guanine or Cytosine, and Zinc(II)[2)

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The new 2: 1 guaninium–zinc chloride, 2: 1 cytosinium–zinc chloride, 1: 1 cytosine–zinc hydroxy chloride, or 2: 1 cytosine–zinc chloride complex was obtained from a diluted hydrochloric acid, 70% ethanol, or ethanol solution. The infrared and proton magnetic resonance spectra of these complexes were characterized to assign the binding site of zinc to guanine or cytosine. On the basis of these data, it was suggested that the N9 site of guanine was bound to zinc in the guaninium–zinc chloride complex, and that the N3 site of cytosine was coordinated with zinc in the 2: 1 cytosine–zinc chloride and 1: 1 cytosine–zinc hydroxy chloride. It was indicated that the N3 site of cytosine was protonated in the 2:1 cytosinium–zinc chloride complex.

Keywords——guanine; guaninium chloride; cytosine; cytosinium chloride; zinc complexes; infrared spectra; proton magnetic resonance spectra; lower-field shift; higher-field shift

Introduction

It is of interest that nucleic acid contains various metal ions.[4,5) Interaction of metal ions with nucleic acids includes binding to phosphate oxygen and that to bases of nucleic acids.[6–11) Eichhorn and Shin[11,12) suggested that Zn^{2+} is bound to the bases when Zn^{2+} takes part in the reversible winding and unwinding of deoxyribonucleic acid (DNA), as shown in Fig. 1. Therefore, it is important to study the binding site of Zn^{2+} to each individual base.

The present study was undertaken to investigate the interaction of guanine or cytosine with Zn^{2+}, and to examine the resulting crystals of the new 2:1 guaninium–zinc chloride, 2:1 cytosinium–zinc chloride, 1:1 cytosine–zinc hydroxy chloride, and 2:1 cytosine–zinc chloride complexes. The binding site of Zn^{2+} to guanine or cytosine in the complexes is discussed on the basis of infrared (IR) and proton magnetic resonance (PMR) spectral data.

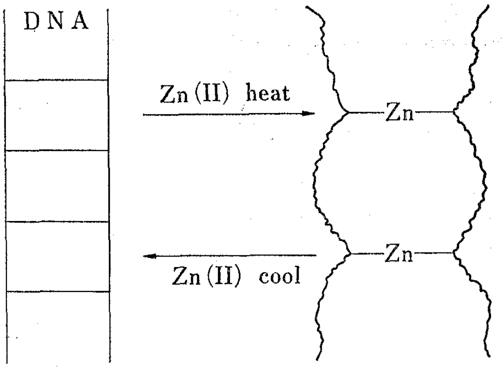

Fig. 1. Interaction of Zinc with DNA

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2) Presented in part at the 94th Annual Meeting of the Pharmaceutical Society of Japan, Sendai, April, 1974.
3) Location: *1–33, Yayoi-cho, Chiba-shi, Chiba, 260, Japan.*
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Experimental

Materials——Guanine (Sigma Chemical Co., U.S.A.) was recrystallized from diluted HCl before use. Cytosine (Sigma Chemical Co.) was recrystallized from H_2O. $ZnCl_2$ was from Koso Chemical Co., Tokyo. EtOH was dried over CaO and distilled before use.

Syntheses——Guaninium–Zinc Chloride (2/1) Complex (2:1 Guaninium–$ZnCl_4$): Guanine (500 mg) was dissolved in 0.3 N HCl (100 ml) with stirring at 50°, $ZnCl_2$ (1.5 g) was added, the mixture was stirred at 70° for 2—3 hr, and the mixture was allowed to stand in a thermostat at 40°. After 3—5 days, colorless columnar crystals of 2:1 guaninium–$ZnCl_4$ were obtained. The complex decomposed at above 350°. When exposed to ultraviolet (UV) ray of 365 nm, the complex gave a blue fluorescence. *Anal.* Calcd. for $(C_5H_6N_5O)_2ZnCl_4\cdot 2H_2O$: C, 21.94; H, 2.95; N, 25.59; Zn, 11.94. Found: C, 21.80; H, 2.95; N, 25.78; Zn, 11.87.

Guaninium–Zinc Chloride (1/1) Complex (Guaninium–$ZnCl_3$): The complex was prepared according to the method of Srinivasan and Taylor.[13]

Guaninium–Copper Chloride (1/1) Complex (Guaninium–$CuCl_3$): This complex was synthesized by the method of Carrabine and Sundaralingam.[14,15]

Cytosinium–Zinc Chloride (2/1) Complex (2:1 Cytosinium–$ZnCl_4$): Cytosine (470 mg) was dissolved in 0.1 N HCl (100 ml) with stirring at 60°, $ZnCl_2$ (1 g) was added, the mixture was heated at 70° for 3 hr with stirring, and allowed to stand at room temperature. After 3—4 days, white columnar crystals of 2:1 cytosinium–$ZnCl_4$ were obtained. The complex decomposed at above 300°. *Anal.* Calcd. for $(C_4H_6N_3O)_2ZnCl_4$: C, 22.27; H, 2.81; N, 19.48; Zn, 15.14. Found: C, 22.11; H, 2.88; N, 19.49; Zn, 14.99.

Cytosine–Zinc Hydroxy Chloride (1/1) Complex (Cytosine–Zn(OH)Cl): Cytosine (470 mg) was dissolved in 70% EtOH (100 ml) with stirring at 60°, $ZnCl_2$ (1 g) was added, the mixture was boiled under reflux for 3—4 hr, and allowed to stand in a thermostat at 40°. After 2—3 days, colorless colunmar crystals of cytosine–Zn(OH)Cl were obtained. The complex decomposed at above 350°. *Anal.* Calcd. for $(C_4H_5N_3O)Zn(OH)Cl$: C, 21.08; H, 2.66; N, 18.44; Zn, 28.68. Found: C, 21.00; H, 2.59; N, 18.64; Zn, 28.53.

Cytosine–Zinc Chloride (2/1) Complex (2:1 Cytosine–$ZnCl_2$): Cytosine (470 mg) was dissolved in EtOH (100 ml) with stirring at 65°, $ZnCl_2$ (1 g) was added, the mixture was boiled under reflux for 5 hr, and allowed to stand in a thermostat at 40°. After 2—3 days, white micro columnar crystals of 2:1 cytosine–$ZnCl_2$ were obtained. The complex decomposed at above 300°. *Anal.* Calcd. for $(C_4H_5N_3O)_2ZnCl_2$: C, 26.79; H, 2.82; N, 23.44; Zn, 18.23. Found: C, 26.83; H, 2.82; N, 23.37; Zn, 18.09.

Cytosine–Copper Chloride (2/1) Complex (2:1 Cytosine–$CuCl_2$): The complex was prepared according to the method of Melzer.[16]

Measurement of IR Spectra——The spectra of these complexes were measured on a Hitachi Model EPI-295 spectrophotometer, as a KBr disk or in 10% $DCl+D_2O$ (DCl) and EtOD solutions. The spectra in DCl and EtOD solutions were obtained by using As_2Se_3 cell (0.1 mm).

Measurement of PMR Spectra——Guanine, guaninium chloride (guanine hydrochloride), and guaninium-zinc chloride complexes were each dissolved to 0.1 M concentration (for the ligand in the complexes) in 20% $DCl+D_2O$, CF_3COOH (trifluoracetic acid (TFA)), or $(CD_3)_2SO$ (dimethyl sulfoxide (DMSO)-d_6). Cytosine, cytosinium chloride (cytosine hydrochloride), and the 2:1 cytosinium–$ZnCl_4$, cytosine–Zn(OH)Cl, and 2:1 cytosine–$ZnCl_2$ complexes were each dissolved to 0.1 M concentration (for the ligand in the complexes) in D_2O, TFA, DMSO-d_6, or DMSO-d_6+4% H_2O. Their chemical shifts were measured on a JEOL Model NM4H-100 spectrometer operated at 100 MHz, at 24°. Internal references used were DSS (in D_2O, 20% $DCl+D_2O$, or TFA) and TMS (in DMSO-d_6 or DMSO-d_6 +4% H_2O). PMR solvents used were commercial products (from Sigma Chemical Co.).

Results and Discussion

New guaninium–, cytosinium–, or cytosine–zinc chloride complexes were obtained from an acidic or ethanol solution. IR and PMR spectra of these complexes were characterized to assign the binding site of zinc to guanine or cytosine.

Infrared Spectra

Guanine–Zn^{2+} Complexes——The IR spectrum of 2:1 guaninium–$ZnCl_4$ was characterized by investigating the characteristic bands on complexation of the guaninium–$ZnCl_3$ and –$CuCl_3$ in which the metal is bound to N(9) position of the guanine ring protonated at N(3) and N(7).[13–15]

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TABLE I. Relevant Infrared Absorption Bands of Guanine, Guaninium Chloride, and Guaninium–Metal Complexes in KBr Disk (in 300—3500 cm^{-1} region)

Guanine	Tentative assignment	Guaninium chloride	2:1 G–Zn[a)]	1:1 G–Zn[b)]	1:1 G–Cu[c)]
	νOH	3450 s	3420 s		3500 s
3320 s	νNH_2	3390 s	3380 s	3375 s	3350 s
	νNH_2	3330 s	3330 s	3300 s	3290 s
	νNH_2		3250 m	3250 s	3240 s
3160 s	νNH_2	3170 s	3170 s	3180 s	3185 s
		3040 s	3050 sh	3050 sh	3060 s
3000 s	νNH, CH	3000 s	3010 m	3000 sh	3000 sh
2900 s	νNH	2920 s	2925 s	2920 m	2925 m
2850 s	νNH	2875 s			
	νN(3)-H		2825 m	2830 m	2825 m
		2750 s	2760 m	2760 m	2760 m
2700 s	νN(9)-H	2600 m			
1705 s	νC=O	1715 s	1720 s	1734 s	1732 s
1680 s	δNH_2 scissoring	1668 s	1665 s	1685 s	1670 s
	$\nu C=N^+$	1615 s	1642 m	1632 m	1635 m
	$\nu C=N^+$		1612 m	1608 m	1620 m
1587 m	$\nu C=C+\nu C=N$	1594 s	1595 m	1592 m	1592 m
1578 m	$\nu C=C+\nu C=N$	1580 m	1580 m	1580 m	1580 m
1563 m	δNH	1560 m	1557 m	1557 m	1562 m
			1545 sh	1543 sh	1543 sh
	δNH	1518 m	1517 m	1515 m	1520 m
1477 m	Ring vib.	1470 m	1475 m	1476 m	1482 m
1464 m	Ring vib.		1455 m	1452 m	1450 m
1418 m	Ring vib.	1395 m	1399 m	1390 sh	1390 w
1375 m	Ring vib.	1370 m	1370 m	1374 m	1374 m
	Ring vib.	1340 m	1336 w	1340 w	1320 w
			1310 w	1309 w	1304 w
1263 m	νC–N		1255 w	1255 w	1257 w
	νC–N	1240 w	1236 w	1236 w	1240 w
1215 m	Ring vib.	1182 m	1200 w	1203 m	1202 m
1172 m	Ring vib.	1145 w	1154 m	1156 m	1156 m
1100 sh	δNH_2 rocking	1070 w	1095 w	1080 w	1080 w
1042 w		1047 w	1039 w	1038 w	1048 w
	Ring vib.		972 w	973 w	972 w
	Ring vib.	930 w	930 w	930 w	929 w
880 m	δNH		885 sh	889 w	900 w
851 m	δNH	850 m	857 m	849 m	850 w
	δNH	840 m	834 m	830 sh	830 sh
781 m	δCH,	774 m	769 m	765 m	765 m
730 w		741 w	735 w	730 w	730 sh
	Ring vib.	713 w	726 w	720 sh	718 w
705 m	Ring vib.		709 w	709 w	705 w
689 m	Ring vib.	682 m	687 w	673 w	678 sh
649 m	δNH_2 wagging	648 m	642 m	651 m	656 m
608 m	Ring vib.	610 w	607 m	606 m	609 m
560 w	Ring vib.	550 m			
544 w	Ring vib.	530 m	546 w	538 m	542 m
515 w	Ring vib.		523 sh	525 sh	520 w
506 w	Ring vib.	506 m	508 sh	510 w	509 w
	Ring vib.		498 m	492 m	494 m
350 w	Ring vib.	350 sh	353 w	350 w	355 w
	νN–Metal		319 w	319 w	328 w

a) 2:1 guaninium–$ZnCl_4$.
b) 1:1 guaninium–$ZnCl_3$.
c) 1:1 guaninium–$CuCl_3$.

The relevant infrared absorption bands are presented in Table I. In guanine, absorption bands due to the ring vibration were observed in the region of 300—1500 cm^{-1}.[17–19] Especially, the bands at 350, 506, 515, 689, 705, and 781 cm^{-1} were assigned to the skeletal ring vibrations of guanine.[18,19] These bands were clearly observed in the 2:1 guaninium–$ZnCl_4$, guanium–$ZnCl_3$, and guaninium–$CuCl_3$, indicating that the 2:1 guaninium–$ZnCl_4$ contains the guanine skeleton.

In IR spectrum of the 2:1 guaninium–$ZnCl_4$, many bands appeared newly in the region of 300—1500 cm^{-1}, as in the guaninium–$ZnCl_3$ and –$CuCl_3$. Of the new absorption bands, those at near 720, 930, 1240, and 1390 cm^{-1} were comparable to the ring vibration of guaninium chloride as shown in Table I. On the other hand, the new bands at near 490, 970, 1250, and 1300 cm^{-1}, not present in guanine and guaninium chloride, were assigned to the ring deformation or stretching vibration of the complexes. Moreover, the band assigned to the N–Metal stretching vibration[20,21] was observed at 328 cm^{-1} in the guaninium–$CuCl_3$, at 319 cm^{-1} in the guaninium–$ZnCl_3$, and at 319 cm^{-1} in the 2:1 guaninium–$ZnCl_4$. These facts suggest protonation and binding of the metal to either N(3), N(7), or N(9) site of guanine ring in the 2:1 guaninium–$ZnCl_4$, as in the guaninium–$ZnCl_3$ and –$CuCl_3$.

Guanine is protonated at N(7) site firstly and at N(3) site secondarily.[22] In the 2:1 guaninium–$ZnCl_4$, N(7) site is protonated, because the δ N(7)-H bands were observed at 834 (δ N(7)-H out–plane) and 1517 cm^{-1} (δ N(7)-H in–plane), as in guaninium chloride,[23] and guaninium–$ZnCl_3$ and –$CuCl_3$ complexes (Fig. 2, A). Moreover, N(3) site is suggested to be protonated in the 2:1 guaninium–$ZnCl_4$, since the νN(3)-H band[24] in the guaninium–$CuCl_3$ was observed in the 2:1 guaninium–$ZnCl_4$ and guaninium–$ZnCl_3$ (Table I).

Of the absorption bands in the region of 1600—1800 cm^{-1}, those of strong intensity at 1665 and 1720

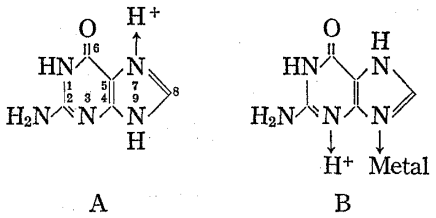
A B

Fig. 2

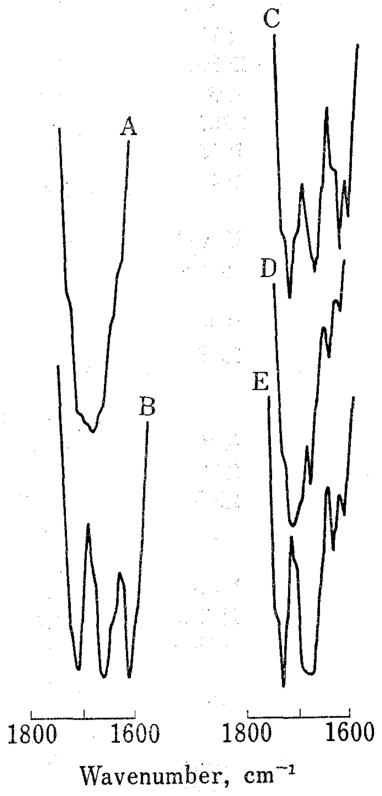

Fig. 3. Infrared Absorption Spectra in the Region of 1600—1800 cm^{-1} in KBr Disk

A, guanine; B, guaninium chloride; C, 2:1 guaninium–$ZnCl_4$; D, guaninium–$ZnCl_3$; E, guaninium–$CuCl_3$.

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TABLE II. Relevant Infrared Absorption Bands of Cytosine, 2:1 Cytosinium–ZnCl$_4$,* Cytosine–Zn(OH)Cl,** and 2:1 Cytosine–ZnCl$_2$*** in KBr Disk (in 300—3500 cm^{-1} region)

Cytosine	Tentative assignment	2:1 CytH–Zn*	Cyt–Zn**	2:1 Cyt–Zn***
3450 s	νOH		3520 s	
3375 s	νNH$_2$	3400 s	3450 s	3440 s
	νNH$_2$	3300 s	3330 s	3380 s
	νNH$_2$			3247 s
3175 s	νNH$_2$	3200 s	3200 sh	3210 s
	νNH	3150 s		
3100 sh	νCH, νNH	3110 s	3100 s	3083 m
		3020 s	3065 m	3030 w
2980 m	νNH		2970 m	2960 w
2920 m	νNH		2880 m	2892 w
2850 m	νNH	2800 w	2840 m	2800 w
2800 m	νNH	2720 w	2700 m	
2700 m	νNH		2610 m	
1665 s	δNH$_2$ scissoring	1676 s	1681 s	1680 s
1645 s	νC=O	1735 s	1636 s	1645 s
	νC=N$^+$	1625 m	1618 sh	1613 s
1600 sh	νC=C+νC=N			
1575 w	νC=C+νC=N	1580 w	1585 sh	1580 w
	δNH	1570 m		
1539 m	δNH	1543 m	1537 m	1540 m
1503 s	νC=C+νC=N, ring vib.	1510 w	1515 m	1515 m
	Ring vib.	1490 m	1493 m	1507 m
	Ring vib.		1476 m	1476 m
1460 s	Ring vib.	1402 m	1456 m	1447 m
1370 m	Ring vib.	1366 w	1370 m	1369 w
1290 m	νC–NH$_2$ νC–N	1310 sh	1300 w	1296 w
	νC–N		1284 m	1267 m
	νC–N	1250 vw	1245 m	1240 m
1235 s	δCH ring vib.	1220 s	1230 sh	1222 m
1105 w	Ring vib.	1150 w	1109 w	1105 w
978 w	Ring vib.	987 w	992 w	983 w
	Ring vib.	974 w		
880 m	δNH	885 sh	888 w	880 w
	δNH	843 m		
814 m	δNH	810 m	800 w	804 m
793 m	δCH	786 m	783 m	797 m
786 m	δCH			782 m
	Ring vib.	760 m	750 sh	752 w
	Ring vib.		715 w	710 w
	Ring vib.	689 m		
656 m	δNH$_2$ wagging	672 m	660 m	652 m
	Ring vib.		612 m	608 m
601 s	Ring vib.	585 m	586 m	575 m
552 m	Ring vib.	540 m	544 m	546 m
		527 m		
	Ring vib.		442 w	444 w
430 m	Ring vib.	422 m	432 m	434 w
416 w	Ring vib,		420 sh	412 m
	Ring vib.	377 m		
	νZn–Cl	293 m	270 m	285 m

cm^{-1} in 2:1 guaninium–$ZnCl_4$ (at 1685 and 1734 cm^{-1} in guaninium–$ZnCl_3$, and at 1670 and 1732 cm^{-1} in guaninium–$CuCl_3$) were assigned to the δ NH_2 scissoring and γC=O band, respectively, because the former band disappeared in DCl solution, while the latter remained, as in the case of guanine derivatives.[17–19,25–27] The ν C=N$^+$ band not present in guanine appeared newly at near 1610 and 1640 cm^{-1} in these complexes, while the band was observed at 1615 cm^{-1} in guaninium chloride (Fig. 3). The double bond stretching vibrations of the complexes were higher in frequencies than those of guanine and guaninium chloride, as shown in Table I. On the other hand, many ring vibrations due to single bond stretching vibration of the complexes were lower in frequencies than those of guanine, as in the case of guaninium chloride. Especially, the νC–N (internal) band coupled with the νC–N (external) at 1263 cm^{-1} in guanine was split and shifted to a lower-frequency region (at near 1240 and 1250 cm^{-1}) in the complexes, while the band was not split but shifted to a lower-frequency region (at 1240 cm^{-1}). Nakamoto reported that the absorption band due to the C–N stretching vibration shifts to a lower-frequency region on binding of a metal (C–N:→metal).[20,21] Tsuboi reported that protonation at nitrogen of purine ring causes a localization of π-electrons on the ring, and causes higher-frequency shifts in the double bond stretching vibrations.[19,25] Therefore, the higher-frequency shifts in the double bond stretching vibrations and the lower-frequency shifts in the stretching vibrations of the complexes are caused by protonation at N(3) and N(7), and binding of the metal to nitrogen of guanine ring in the complexes. Since the νN(9)-H band[24] in guanine disappeared in the 2:1 guaninium–$ZnCl_4$, as in the guaninium–$ZnCl_3$ and –$CuCl_3$, the N(9) position of guanine ring is suggested to be bound to zinc in the complex, as shown in Fig. 2, B.

Cytosine–Zn^{2+} Complexes——The relevant infrared absorption bands in KBr disk are listed in Table II. In the spectra of 2:1 cytosinium–$ZnCl_4$, cytosine–Zn(OH)Cl, and 2:1 cytosine–$ZnCl_2$ complexes, their δC–H, δN–H, ring vibration, νC=C, νC=N, δNH_2, and νC=O bands are attributed to the cytosine structure,[17,18] indicating that the complexes retain the cytosine skeleton (Table II).

In cytosine, absorption bands due to the ring vibration were observed in 400—600 cm^{-1} and in 1100—1500 cm^{-1} regions.[17,18,28] In the cytosine–Zn(OH)Cl and 2:1 cytosine–$ZnCl_2$, many bands appeared newly in these regions on complexation, as in the 2:1 cytosine–$CuCl_2$[1] (Table II). These bands not present in cytosinium chloride were assigned to the ring deformation or stretching vibration of the complexes. In particular, the new bands at near 610 and 710 cm^{-1} were observed in many cytosine–metal complexes[29] in which the metal is coordinated with N(3) site of cytosine ring, and those were assigned to the characteristic skeletal ring vibrations of the cytosine–metal complexes. On the other hand, these bands were not

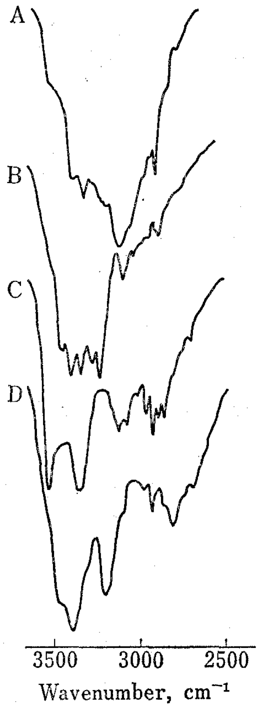

Fig. 4. Infrared Absorption Spectra in the Region of 2500—3500 cm^{-1} in KBr Disk

A, 2:1 cytosinium–$ZnCl_4$; B, 2:1 cytosine–$ZnCl_2$; C, cytosine–Zn(OH)Cl; D, cytosine monohydrate.

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present in the 2:1 cytosinium–$ZnCl_4$, while the absorption bands at 377, 422, 585, 974 and 1227 cm^{-1} were comparable to the ring vibrations of cytosinium chloride. These facts suggest the coordination of zinc with nitrogen of cytosine ring in the cytosine–Zn(OH)Cl and 2:1 cytosine–$ZnCl_2$, and protonation at the nitrogen in the 2:1 cytosinium–$ZnCl_4$.

The ν N(1)-H bands characteristic of cytosine[17,18] were extermely weak and shifted to a lower-frequency region in the 2:1 cytosinium–$ZnCl_4$ and 2:1 cytosine–$ZnCl_2$, whereas the bands were observed in the cytosine–Zn(OH)Cl (like cytosine), as shown in Fig. 4. On the other hand, the δ N(1)–H bands were clearly observed at 810 and 1543 cm^{-1} in the 2:1 cytosinium–$ZnCl_4$, and at 804 and 1540 cm^{-1} in the 2:1 cytosine–$ZnCl_2$, as in the 2:1 cytosine–$CuCl_2$[1] (in Fig. 5), indicating that N(1) position of cytosine ring is bound to proton in the complexes. The variation of the ν N(1)-H bands may be caused by hydrogen bonding in the complexes.[24,30–32]

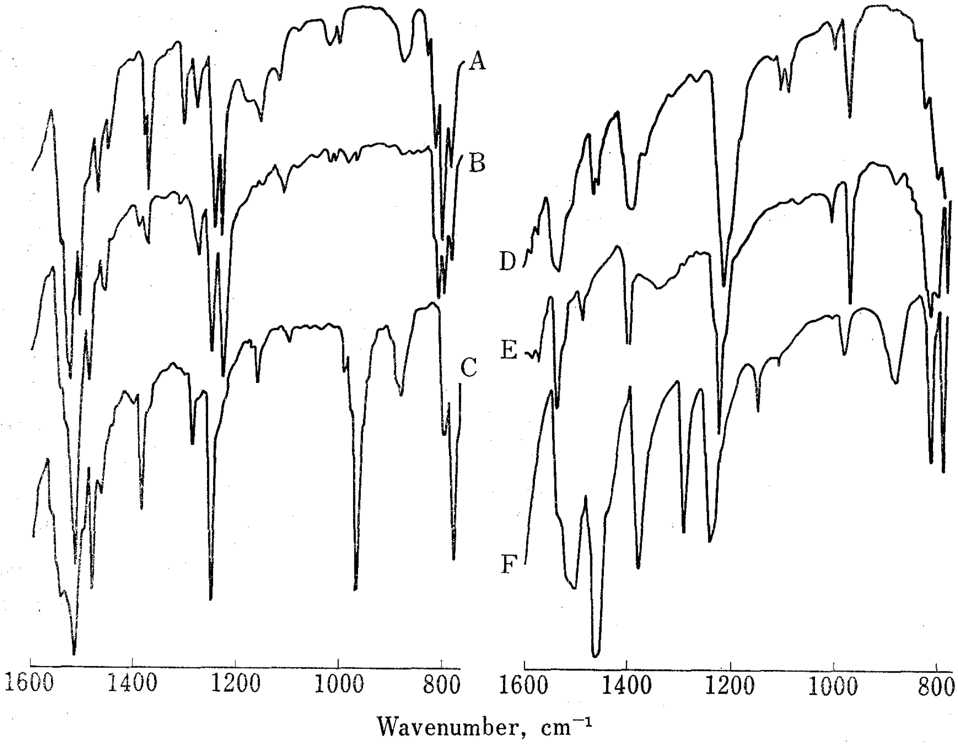

Fig. 5. Infrared Absorption Spectra in the Region of 800—1500 cm^{-1} in KBr Disk

A, 2:1 cytosine-$CuCl_2$; B, 2:1 cytosine-$ZnCl_2$; C, cytosine-Zn(OH)Cl; D, 2:1 cytosinium-$ZnCl_4$; E, cytosinium chloride; F, cytosine monohydrate.

In these complexes, absorption bands due to the NH_2 and C–NH_2 stretching vibrations were higher in frequencies than those of cytosine, as in cytosinium chloride (Table II). Especially, ν C–NH_2 bands of the complexes were weakened and shifted to a higher-frequency region, than that of cytosine (Fig. 5). Since the shift is probably caused by the inductive and mesomeric effects of the ring,[33] N(3) site in the complexes is assumed to be more positively charged than that in cytosine, implying the coordination of zinc with the N(3) or protonation at the N(3). In the 2:1 cytosinium–$ZnCl_4$, N(3) site is protonated, because absorption bands due to N(3)-H were observed at 843 (δ N–H out-plane), 1570 (δ N–H out–

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30) J.A. Carrabine and M. Sundaralingam, *Chem. Commun.*, **1968**, 746.
31) N. Hadijiliadis and T. Theophanides, *Inorg. Chim. Acta*, **15**, 167 (1975).
32) W.C. Hamilton and J.A. Ibers, "Hydrogen Bonding in Solids," W.A. Benjamin, Inc., New York, 1968.
33) L.J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, New York, 1966.

plane), and 3150 cm^{-1} (ν N–H), as in the case of cytosinium chloride (Fig. 6, A).[1,34,35] In contrast, these N(3)-H bands were not observed in the 2:1 cytosine–$ZnCl_2$ and cytosine–Zn(OH)Cl. The ν C–N (internal) band coupled with ν C–NH_2 at 1290 cm^{-1} in cytosine[19] was split and shifted to a lower-frequency region in the 2:1 cytosine–$ZnCl_2$ and cytosine–Zn(OH)Cl. Since no protonation occurs in the complexes, the lower-frequency shift is caused by complexation.[21] Therefore, the N(3) site is suggested to be coordinated with zinc in the 2:1 cytosine–$ZnCl_2$ and cytosine–Zn(OH)Cl, as shown in Fig. 6, B.

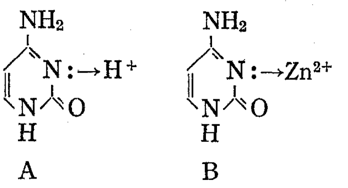

Fig. 6

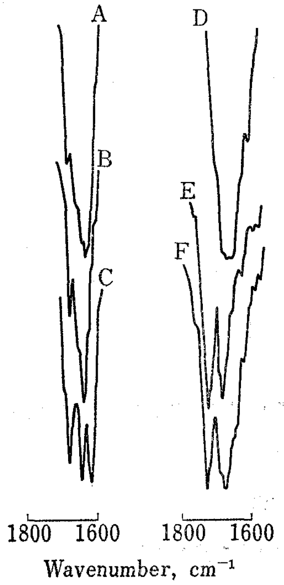

Fig. 7. Infrared Absorption Spectra in the Region of 1600—1800 cm^{-1} in KBr Disk

A, 2:1 cytosine–$CuCl_2$; B, 2:1 cytosine–$ZnCl_2$; C, cytosine–Zn(OH)Cl; D, cytosine monohydrate; E, cytosinium chloride; F, 2:1 cytosinium–$ZnCl_4$.

Table III. Double Bond Stretching Vibrations of Cytosinium Chloride and 2:1 Cytosinium–$ZnCl_4$ Complex* in the 1500—1800 cm^{-1} Region (in EtOD Solution)

Cytosinium chloride	Tentative assignment	2:1 CytH–Zn*
1740 s	νC=O	1730 s
1652 s	νC=N+	1650 s
1584m	νC=C+νC=N	1580m
1550w	νC=C+νC=N	1560w
1520m	νC=C+νC=N	1518m
1508sh	νC=C+νC=N	1510sh

Of the absorption bands in the region of 1600—1800 cm^{-1}, strong ones at 1618, 1636, and 1681 cm^{-1} in the cytosine–Zn(OH)Cl, and at 1613, 1645, and 1680 cm^{-1} in the 2:1 cytosine–$ZnCl_2$ were assigned to the ν C=N+, the ν C=O, and the δ NH_2 scissoring, respectively, since the last band disappeared in EtOD solution (the other bands remained), as in the case of cytosine derivatives.[19,36,37] The ν C=O frequency of the complexes is comparable to that of cytosine but is lower than that of cytosinium chloride. Since ν C=O band shifts to a higher-frequency region when the lone-pair electrons at N(3) position in cytosine take part in the binding,[19,37] the lowering of the band of the 2:1 cytosine–$ZnCl_2$ (than that of cytosinium chloride) suggests the coordination of zinc with the C(2)=O site of cytosine in the complexes, as in the case of the 2:1 cytosine–$CuCl_2$[1,30] (Fig. 7). In the cytosine–Zn(OH)Cl, however, it is difficult to distinguish the coordination of zinc or hydrogen bonding of C(2)=O with hydroxyl anion.

On the other hand, in the 2:1 cytosinium–$ZnCl_4$, the bands at 1676 and 1735 cm^{-1} are assigned to the NH_2 acissoring and C(2)=O stretching vibration, respectively, since the former disappeared in EtOD solution. The ν C=O frequency of the complex was higher than that of

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34) E.D. Becker, H.T. Miles, and R.B. Bradley, *J. Am. Chem. Soc.*, **87**, 5575 (1965).
35) R.R. Shoup, H.T. Miles, and E.D. Becker, *J. Am. Chem. Soc.*, **89**, 6200 (1967).
36) H.T. Miles, *J. Am. Chem. Soc.*, **79**, 2565 (1957).
37) H.T. Miles, *Proc. Natl. Acad. Sci. U.S.A.*, **47**, 791 (1961).

cytosinium chloride in solid state, while it was lower by 10 cm^{-1} than that of the latter in EtOD solution (Table III). As in this case, 4:1 cytosinium–copper complex[1)] was lower by 13 cm^{-1} in EtOD solution. This fact implies the coordination of zinc with C(2)=O site of cytosine in the 2:1 cytosinium–$ZnCl_4$.

TABLE IV. Proton Field Shifts of Guaninium Chloride, 1:1 Guaninium–$ZnCl_3$,* and 2:1 Guaninium–$ZnCl_4$** from DSS in 20% $DCl+D_2O$ and TFA

Solvents	Guaninium chloride	1:1 G-Zn*	2:1 G-Zn**
20% $DCl+D_2O$	912 Hz	910 Hz	908 Hz
TFA	886 Hz	881 Hz	878 Hz

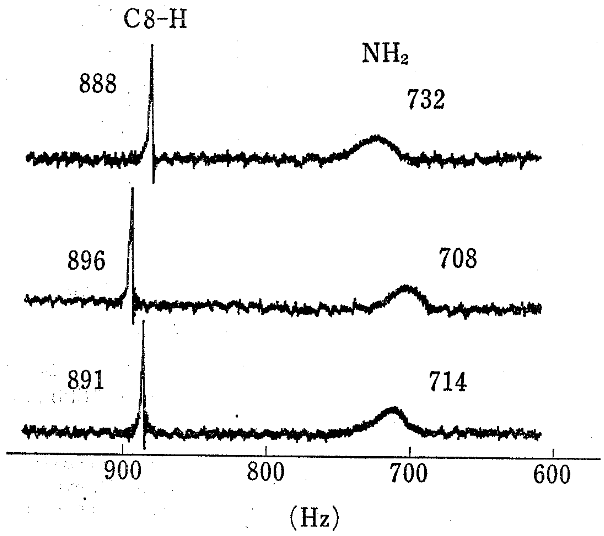

Fig. 8. Proton Magnetic Resonance Spectra in DMSO-d_6

Upper, guaninium chloride; center, guaninium-$ZnCl_3$; lower, 2:1 guaninium-$ZnCl_4$.

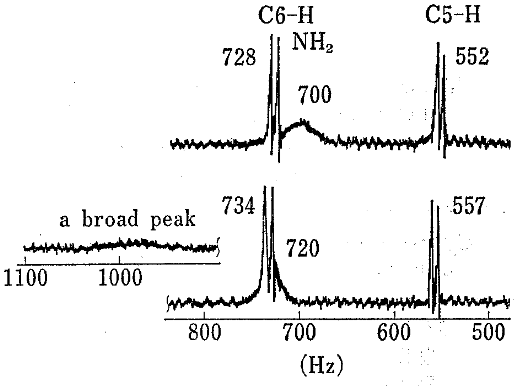

Fig. 9. Proton Magnetic Resonance Spectra in DMSO-d_6

Upper, cytosine monohydrate; lower, cytosine-Zn(OH)Cl.

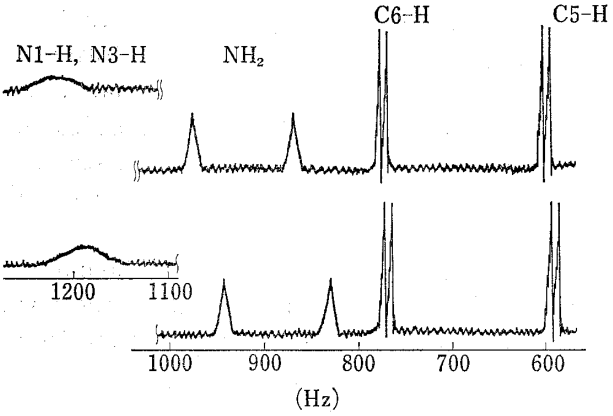

Fig. 10. Proton Magnetic Resonance Spectra in DMSO-d_6

Upper, cytosinium chloride; lower, 2:1 cytosinium-$ZnCl_4$.

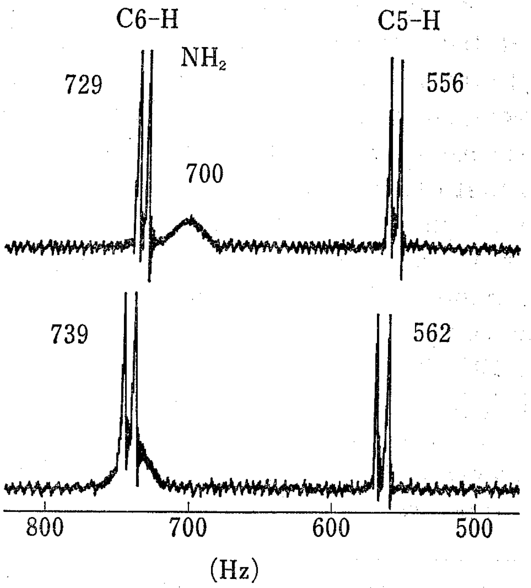

Fig. 11. Proton Magnetic Resonance Spectra in DMSO-d_6+4% H_2O

Upper, cytosine monohydrate; lower, 2:1 cytosine-$ZnCl_2$.

Proton Magnetic Resonance Spectra

In PMR studies on the nucleosides–diamagnetic metal complexes, Li *et al.*[38,-40] found that the addition of $ZnCl_2$ to nucleosides in DMSO results in a lower–field shift of the proton in the ligand, and determined the binding site of a metal to the ligand from the lower–field shift. In the present work, the binding site of Zn^{2+} to guanine or cytosine was examined on the basis of the shift of proton resonance in the new complexes.

Guanine–Zn^{2+} Complexes——The spectra of guaninium chloride, guaninium–$ZnCl_3$, and 2:1 guaninium–$ZnCl_4$ in DMSO-d_6 are given in Fig. 8. Spectra of the complexes showed a lower–field shift of the C(8)–H resonance when compared with that of guaninium chloride (guanine is insoluble in DMSO). The lower–field shift of the C(8)–H resonance suggests the possibility that Zn^{2+} is bound to either N(7) or N(9) of the imidazole ring in guanine. In the spectra of complexes in 20% $DCl+D_2O$ and TFA solutions, the higher–field shift of C(8)–H was observed, as shown in Table IV. The higher–field shift implies the binding of $ZnCl_4^{2-}$ or $ZnCl_3^-$ to the N(7) or N(9) site. This interpretation may be understood by referring to the structure of guaninium–$ZnCl_3$.[13,14]

Cytosine–Zn^{2+} Complexes——The spectra of cytosine, cytosinium chloride, cytosine–Zn(OH)Cl, and 2:1 cytosinium–$ZnCl_4$ in DMSO-d_6 are given in Figs. 9 and 10, and those of cytosine and 2:1 cytosine–$ZnCl_2$ in DMSO-d_6+4%H_2O (since the complex is insoluble in DMSO) in Fig. 11. The spectra of 2:1 cytosine–$ZnCl_2$ and cytosine–Zn(OH)Cl showed a lower–field shift of C(4)–NH_2, C(5)–H, and C(6)–H resonances when compared with those of cytosine. Since the binding of a positively charged atom to N(3) site of cytosine ring causes a lower–field shift of the proton resonances,[34,35,38-40] the shift of these resonances suggests the binding of zinc to the N(3) site of cytosine in the complexes.

In PMR study on the cytosine–$ZnCl_2$ complex, Wang and Li[38,39] reported that the C(5)–H and C(6)–H are shifted to a lower field by an equal extent in the complex. However, the present data showed that the shift of C(6)–H to a lower field is greater than that of C(5)–H, as in Fig. 11. In cytosinium chloride, in which only the N(3) site is protonated, the shift of C(5)–H to a lower field is greater than that of C(6)–H, when compared with those of cytosine. Therefore, the present data suggest the coordination of zinc with C(2)=O site of cytosine in 2:1 cytosine–$ZnCl_2$. This is quite consistent with the interpretation from IR spectral results of the complex.

The spectrum of 2:1 cytosinium–$ZnCl_4$ showed the geminal amino coupling, like cytosinium chloride, and a higher–field shift of C(4)–NH_2, C(5)–H, and C(6)–H resonances when compared with those of cytosinium chloride, as shown in Fig. 10. The geminal amino coupling and the N–H resonance indicate that N(3) position of cytosine is protonated in the complex. This fact shows that the N(3) site of cytosine has a stronger affinity for proton than for zinc. The higher–field shift of C(5)–H and C(6)–H was observed in D_2O, as in DMSO-d_6 (in Table V). However, it is difficult to elucidate the binding site of zinc in the complex from only the higher–field shift.

Table V. Proton Field Shifts of Cytosinium Chloride and 2:1 Cytosinium–$ZnCl_4$* from DSS in D_2O

Samples	C5-H	C6-H
Cytosinium chloride	616 Hz	773 Hz
2:1 CytH–$ZnCl_4$*	605 Hz	761 Hz

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38) S.M. Wang and N.C. Li, *J. Am. Chem. Soc.*, **88**, 4592 (1966).
39) S.M. Wang and N.C. Li, *J. Am. Chem. Soc.*, **90**, 5069 (1968).
40) L.S. Kan and N.C. Li., *J. Am. Chem. Soc.*, **92**, 281 (1970).

In the spectra of these complexes in TFA, no variation was observed in C(4)–NH_2, C(5)–H, and C(6)–H resonances, when compared with those of cytosine, indicating decomposition of these complexes in a strong acid.

Conclusion

In view of these IR and PMR spectral data, it is suggested that the N(3) site of cytosine is coordinated with zinc in 2:1 cytosine–$ZnCl_2$ and cytosine–Zn(OH)Cl complexes, and that the C(2)=O site is coordinated with zinc in the 2:1 cytosine–$ZnCl_2$ complex, while the N(3) site of cytosine would be protonated in the 2:1 cytosinium–$ZnCl_4$. These results give us an important information that the N(3) site of cytosine is strongly bound to proton rather than to zinc under an acidic condition. In the 2:1 guaninium–$ZnCl_4$, it is suggested that zinc is bound to the N(9) site of guanine.

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