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Complexes between Nucleic Acid Bases and Bivalent Metal Ions. III.[1] Syntheses and Spectral Analyses of Cytosine-Calcium Chloride Complexes[2]

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New 2:1 cytosine–$CaCl_2$ and 1:1 cytosine–$CaCl_2$ complexes were obtained from 70% ethanol and water solutions, respectively. The infrared and proton magnetic resonance spectra of the complexes were characterized in comparison with those of many cytosine–metal complexes. On the basis of these data, it is suggested that calcium coordinates with the N(3) and C(2)=O sites of cytosine in the cytosine–$CaCl_2$ complexes.

Keywords——cytosine–metal complexes; calcium; coordination site; IR spectra; complexation sensitive spectral band; PMR spectra; lower-field shift

Introduction

There is a wealth of published information on the interaction of various metal ions with nucleic acids.[4] Studies on metal complexes with nucleic acid components have produced interesting results in the field of bimolecular interactions.[5] Transition metal ions are known to be bound to various sites of the nucleic acid base, but little is known concerning the interactions of alkaline earth metal ions with the bases. A few proton magnetic resonance (PMR) studies have suggested the interaction of $CaCl_2$ with the base in solution, but were unable to distinguish between the binding of Ca^{2+} to the base[6] and the interaction of a chloro anion with the imino or amino proton.[7] Therefore, it is of interest to isolate the Ca-complex and to investigate the mode of interaction in detail by means of physico-chemical measurements.

The present study was undertaken to investigate the interaction of Ca^{2+} with cytosine, and to examine the crystals of the new cytosine–calcium chloride (2/1) and (1/1) complexes obtained. To assign the binding site of Ca^{2+} in the complexes, the infrared (IR) and PMR

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4) J. Shack, R.J. Jenkins, and J.M. Thompsett, *J. Biol. Chem.*, **203**, 373 (1953); H.S. Loring and D.S. Waritz, *Science*, **125**, 646 (1957); G. Zubay and P. Doty, *Biochim. Biophys. Acta*, **29**, 47 (1958); G. Felsenfeld and S. Huang, *Biochim. Biophys. Acta*, **34**, 234 (1959); W.E.C. Wacker and B.L. Vallee. *J. Biol. Chem.*, **234**, 3257 (1959); J. Eisinger, R.G. Shulman, and W.E. Blumberg, *Nature* (London), **192**, 964 (1961); G.L. Eichhorn, *Nature* (London), **194**, 474 (1962); W.F. Dove and N. Davidson, *J. Mol. Biol.*, **5**, 467 (1962).
5) R.M. Izatt, J.J. Christensen, and J.H. Rytting, *Chem. Rev.*, **71**, 439 (1971); G.L. Eichhorn (ed.), "Inorganic Biochemistry," Vol. 2, Elsevier Scientific Publishing Company, Amsterdam, 1973, Chapter 33; G.L. Eichhorn, J.M. Rifkind, and Y.A. Sin, "Bioinorganic Chemistry-II," Advances in Chemistry Series 162, ed. by K.N. Raymond, American Chemical Society, Washington, 1977, pp. 304—311; D.J. Hodgson and L.G. Marzilli, "Progress in Inorganic Chemistry," Vol. 23, ed. by S.J. Lippard, John Wiley and Sons, Inc., 1977, pp. 211—378.
6) *a*) F. Jordan and B.Y. McFaruhar, *J. Am. Chem. Soc.*, **94**, 6557 (1972); *b*) S. Shimokawa, H. Fuli, J. Sohma, and K. Hotta, *J. Am. Chem. Soc.*, **95**, 1777 (1973); *c*) T. Yokono, S. Shimokawa, H. Fuli, and J. Shoma, *Nippon Kagaku Kaishi*, **1973**, 201; *d*) J. Granot and D. Fiat, *J. Am. Chem. Soc.*, **99**, 70 (1977).
7) C.H. Chang and L.G. Marzilli, *J. Am. Chem. Soc.*, **96**, 3656 (1974).

spectral shifts on metal coordination in cytosine–$Cu(II)Cl_2$,[8] –$ZnCl_2$,[1] –$CdCl_2$,[9] –HgCl,[9] –$Co(II)Cl_2$*, and –$Co(II)(NSC)_2$[10] complexes were studied.

Experimental

Materials——Cytosine (from Sigma Chemical Co., U.S.A.) was recrystallized from H_2O. Metal chlorides were from Koso Chemical Co., Tokyo. EtOH was dried over CaO and distilled before use. Deionized water was redistilled before use.

Syntheses

Cytosine-Calcium Chloride (2/1) Complex (2: 1 Cytosine-$CaCl_2$)——Cytosine (500 mg) was dissolved in 70% EtOH (100 ml) with stirring at 60°, $CaCl_2 \cdot 2H_2O$ (1 g) was added, and the mixture was boiled under reflux for 5—6 hr, then allowed to stand at room temperature. After 4—5 days, colorless columnar crystals of 2: 1 cytosine–$CaCl_2$ complex were obtained. The complex decomposed at above 250°. *Anal.* Calcd for $(C_4H_5N_3O)_2CaCl_2 \cdot 1.5H_2O$: C, 26.67; H, 3.65; N, 23.33; Ca, 11.13. Found: C, 26.37; H, 3.70; N, 23.40; Ca, 11.01.

Cytosine-Calcium Chloride (1/1) Complex (Cytosine-$CaCl_2$)——Cytosine (500 mg) was dissolved in water (50 ml) with stirring, $CaCl_2$–$2H_2O$ (3 g) was added, and the mixture was heated with stirring at 80° for 5 hr, then allowed to stand in a thermostated bath at 40°. After 1 week, colorless columnar crystals of cytosine–$CaCl_2$ complex were obtained. The complex decomposed at above 300°. *Anal.* Calcd for $(C_4H_5N_3O)CaCl_2 \cdot H_2O$: C, 20.01; H, 2.95; N, 17.50; Ca, 16.69. Found: C, 19.82; H, 2.95; N, 17.47; Ca, 16.15.

Cytosine-Cobalt(II) Chloride (2/1) Complex (2: 1 Cytosine-$Co(II)Cl_2$)——Cytosine (500 mg) was dissolved in EtOH (100 ml) with stirring at 60°, $Co(II)Cl_2 \cdot 6H_2O$ (1.5 g) was added, and the mixture was boiled under reflux for 2—3 hr, then allowed to stand in a thermostated bath at 40°. After 2—3 days, blue prismatic crystals of 2: 1 cytosine–$Co(II)Cl_2$ complex were obtained. *Anal.* Calcd for $(C_4H_5N_3O)_2Co(II)Cl_2$: C, 27.29; H, 2.87; N, 23.88; Co, 16.74. Found: C, 27.27; H, 2.86; N, 23.97; Co, 16.61.

Cytosine-Cobalt(II) Thiocyanate (2/1) Complex (2: 1 Cytosine-$Co(II)(SCN)_2$)——The complex was synthesized according to the method of Weiss and Venner.[10a]

Cytosine-Copper(II) Chloride (2/1) Complex (2: 1 Cytosine-$Cu(II)Cl_2$)——This complex was prepared by the method of Melzer.[8a]

Cytosine-Zinc Chloride (2/1) Complex (2: 1 Cytosine-$ZnCl_2$)——The complex was isolated as described in the preceding report.[1]

Cytosine-Cadmium Chloride (2/1) and -Mercury(II) Chloride (1/1) Complexes (2: 1 Cytosine-$CdCl_2$ and 1: 1 cytosine-Hg(II)Cl)——These complexes were synthesized by the method of Sakaguchi and Fujita.[9]

Measurement of Infrared (IR) Spectra——The spectra of cytosine, cytosinium chloride (cytosine hydrochloride), and the complexes were measured on a Hitachi EPI-295 spectrophotometer, in KBr disks and in D_2O, EtOD, or DMSO (dimethyl sulfoxide) solution. The spectra in solution were obtained by using an As_2Se_3 cell (0.1 mm).

Measurement of Proton Magnetic Resonance (PMR) Spectra——The solvent was a commercial product (from Sigma Chemical Co., U.S.A.). Each sample was dissolved to give 0.1 M concentration (for the ligand in the complexes) in DMSO-d_6, D_2O, or TFA (trifluoroacetic acid), and the chemical shifts were measured with a JEOL NM4H-100 spectrometer operated at 100 MHz at 24°, using TMS (in DMSO-d_6) or DSS (in D_2O and TFA) as an internal reference.

Results and Discussion

New 2: 1 cytosine–$CaCl_2$, 1: 1 cytosine–$CaCl_2$, and 2: 1 cytosine–$Co(II)Cl_2$ complexes were isolated from 70% EtOH, water, and ethanol solution, respectively. The IR and PMR spectra were analyzed to assign the binding site of Ca or Co(II).

Infrared Spectra

The relevant infrared absorption bands (KBr disk) are listed in Table I and II, and the bands in D_2O are given in Table III. In the spectra of the cytosine–$CaCl_2$ and –$Co(II)Cl_2$ com-

8) *a*) M.S. Melzer, *Chem. Commun.*, **1967**, 1052; *b*) M. Sundaralingam and J.A. Carrabine, *J. Mol. Biol.*, **61**, 287 (1971).
9) T. Sakaguchi and T. Fujita, *Yakugaku Zasshi*, **97**, 65 (1977). *) The cytosine–$Co(II)Cl_2$ (2/1) complex is a new one, and its synthesis and binding site are described in this paper.
10) *a*) R. Weiss and H. Venner, *Hoppe-Seyler Z. Physiol Chem.*, **350**, 396 (1969); *b*) T. Sakaguchi and M. Tanno, *Nippon Kagaku Kaishi*, **1974**, 1637.

Table I. Some Infrared Absorption Bands of Cytosine Monohydrate, 2:1 Cytosine–$CaCl_2$, 1:1 Cytosine–$CaCl_2$, and 2:1 Cytosine–Co(II)Cl_2 in KBr Disks (in the 400—1800 cm^{-1} Region)

Cytosine	Tentative assignment	$(Cyt.)_2$–$CaCl_2$	(Cyt.)–$CaCl_2$	$(Cyt.)_2$–Co(II)Cl_2
1665m	δ NH_2 scissoring	1675 s	1672 s	1675 s
1645 s	νC=O	1643 s	1637 s	1640 s
1600sh	νC=N+C=C	1618 s	1613 s	1612 s
		1596 s	1590 s	
1539m	δN–H in-plane	1535m	1530m	1540m
1503 s	νC=N+C=C	1511 s	1516 s	1515sh
		1502 s	1509 s	1508 s
	Ring vib.			1476m
1460 s	Ring vib.	1437m	1432m	1445m
	Ring vib.	1428m	1426m	
1370 s	Ring vib.	1365m	1365sh	1368w
	Ring vib.	1356m	1358m	
		1320w	1322w	
1290m	νC–NH_2	1304vw	1305vw	1295vw
	νC–N			1278w
	νC–N	1245vw	1250vw	1240m
1235 s	δC–H, ring vib.	1222m	1218m	1221m
1149m	Ring vib.	1135w	1130w	1145w
1110w	Ring vib., δ NH_2	1108w	1106w	1106w
978w	Ring breathing	998vw	995vw	980vw
880m	Ring vib.			865w
814m	δ N–H out-of-plane	809m	803m	805m
793m	δC–H, ring vib.	796m	791m	796m
786m	δC–H, ring vib.			782m
	Ring vib.	759m	758m	752m
	Skeletal ring vib.	721w	720w	710w
656m	δNH_2 wagging	635m	630m	657m
	Skeletal ring vib.	616m	611m	612m
	Skeletal ring vib.	610m	606m	
601 s	Ring vib.			
567m	Ring vib.	572m	570m	577m
552m	δC=O, ring vib.	556sh	557m	555sh
	δC=O, ring vib.	548m	548m	546m
430w	Ring vib.	433w	430w	435w
416w	Ring vib.	420w	420w	415sh
	Skeletal ring vib.	402w	400w	402w

plexes (in Table I), the δC–H, δN–H, ring vibrations, νC=C, νC=N, δNH_2, and νC=O bands are attributable to the cytosine structure,[11] as in the cytosine–Cu(II)Cl_2, –$ZnCl_2$, –$CdCl_2$, and –Hg(II)Cl complexes[1,9] (in Table II).

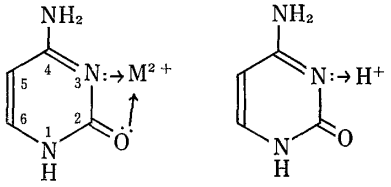
N-H Deformation and C-NH_2 Stretching Modes——As shown in Tables I and II, the N(1)–H out-of-plane and in-plane deformation frequencies of cytosine[11] were almost unaffected by complexation, not only with transition metals but also with calcium, in all cytosine–metal complexes studied in this work. This indicates that a hydrogen atom is located on the N(1) site of cytosine in the cytosine–metal complexes, and therefore that the N(1) site is not affected by metal coordination.[8b,12]

11) *a*) C.L. Angell, *J. Chem. Soc.*, **1961**, 504; *b*) M. Tsuboi and Y. Kyogoku, "Synthetic Procedures in Nucleic Acid Chemistry," Vol. 2, eds. W.W. Zorbach and R.S. Tipson, John Wiley and Sons, Inc., New York, 1973, Chapter 6; *c*) H. Susi, J.S. Ard, and J.M. Purcell, *Spectrochim. Acta*, **29A**, 725 (1973).
12) M.A. Martin and A.L. Beauchamp, *Can. J. Chem.*, **55**, 1213 (1977).

Table II. Some Infrared Absorption Bands of 2: 1 Cytosine–$Cu(II)Cl_2$, 2: 1 Cytosine–$ZnCl_2$, 2: 1 Cytosine–$CdCl_2$, and 1: 1 Cytosine–HgCl (in the 400—1800 cm^{-1} Region)

Tentative assignment	$(Cyt.)_2$–$CuCl_2$	$(Cyt.)_2$–$ZnCl_2$	$(Cyt.)_2$–$CdCl_2$	(Cyt.)–HgCl
δNH_2 scissoring	1680m	1680 s	1670 s	1686 s
$\nu C=O$	1655 s	1645 s	1645 s	1646 s
$\nu C=N+\nu C=C$	1628 s	1613 s	1615 s	1624 s
			1603 s	
$\delta N-H$ in-plane	1550m	1540m	1530m	1550m
$\nu C=N+\nu C=C$	1524m	1515m	1515m	1522 s
$\nu C=N+\nu C=C$	1505m	1507m	1508 s	1500sh
Ring vib.	1470m	1476m	1480m	
Ring vib.	1445w	1447m	1440m	1445m
Ring vib.	1365m	1369w	1366w	1356w
$\nu C-NH_2$	1295vw	1296vw	1305vw	1296vw
$\nu C-N$	1265w	1267w	1280w	1265m
$\nu C-N$	1237m	1240m	1237m	
$\delta C-H$, Ring vib.	1224m	1222m	1222m	1223 s
Ring vib.	1140w	1140sh	1132w	1140w
Ring vib., δNH_2	1105w	1105w	1105w	1102m
Ring breathing	995w	983w	970w	987w
Ring vib.	860w	880w	870w	
$\delta N-H$ out-of-plane	815w	804m	815m	811m
			806m	
$\delta C-H$, Ring vib.	793m	797m	798m	796m
$\delta C-H$, Ring vib.	780m	782m	783m	766m
Ring vib.	765m	752m	757m	753m
Skeletal ring vib.	723w	710w	718w	710w
δNH_2 wagging	670m	652m	660m	650m
Skeletal ring vib.	618m	608m	609m	612m
Ring vib.	578m	575m	580m	565m
$\delta C=O$, Ring vib.	555sh	555sh	560w	558m
$\delta C=O$, Ring vib.	540w	546m	547m	543m
Ring vib., $\nu Cu-N$	450m	444w	442w	
Ring vib.	435w	434w	432w	428m
Skeletal ring vib.	400sh	412m	396m	411m

On the other hand, the $\nu C-NH_2$ band[11] coupled with the ring stretching mode at 1290 cm^{-1} in cytosine was shifted to a higher-frequency region and was greatly weakened on complexation with a metal (in Tables I and II). These variations arise from metal coordination with the N(3) position of the cytosine ring or protonation at this site (Fig. 1).[1] In cytosinium chloride[13a] and the cytosinium $-ZnCl_4$[1] and $-CuCl_4$[13b] complexes, in which the N(3) site is protonated (in Fig. 1, B), the N(3)–H bands were clearly visible near 840(δNH out-of-plane), 1570(δNH in-plane), and 3150 cm^{-1}(νNH).[1] However, no N(3)–H band was present in the cytosine–metal complexes. Thus, the N(3) site may participate in the coordination with transition metals and with calcium in the cytosine–metal complexes (Fig. 1, A).



A B

Fig. 1. A, Metal Coordination with the N(3) and C(2)=O Sites of Cytosine; B, Protonation at the N(3) Site

13) *a*) N.S. Maudel, *Acta Crystallogr.*, **B33**, 1079 (1977); *b*) K. Ogawa, K. Nishitani, T. Fujiwara, S. Shirotake, and K. Tomita, *Acta Crystallogr.*, **B35**, 965 (1979).

Table III. Double Bond Stretching Vibrations of Cytosine, 2:1 Cytosine–$CaCl_2$, 1:1 Cytosine–$CaCl_2$, 2:1 Cytosine–Co(II)Cl_2, 2:1 Cytosine–Cu(II)Cl_2, 2:1 Cytosine–$ZnCl_2$, and 2:1 Cytosine–$CdCl_2$ in D_2O solution (in the 1500—1800 cm^{-1} Region)

Cytosine	Tentative assignment	2Cyt–Ca	Cyt–Ca	2Cyt–Co	2Cyt–Cu	2Cyt–Zn	2Cyt–Cd
1645 s	C=O	1640 s	1645 s	1640 s	1640 s	1643 s	1640 s
	$C=N^+$	1616 s	1615 s	1622 s	1630 s	1625 s	1620 s
					1620 s		
1603 s	C=N+C=C	1605 s		1608 s	1610 s	1605 s	1605 s
1585 s	C=N+C=C	1590 s	1590 s	1590 s	1590 s	1591 s	1595 s
1560m	C=N+C=C	1580m	1573m	1580sh	1580m	1580sh	1579 s
	C=N+C=C	1562m	1565m	1563m	1570sh	1560sh	1560m
1515 s	C=N+C=C	1515m	1515m	1516m	1518m	1515m	1515sh
1502 s	C=N+C=C	1505 s	1503 s	1502 s	1505 s	1503 s	1501 s

Ring C-N and Double Bond Stretching Modes——The coordination of Cu or Zn with the N(3) position of the cytosune ring[1,8b] caused a lower-frequency shift of the C–N bands (Table II), while it caused a higher-frequency shift of the νC=C+νC=N bands (in Table III). These shifts were also observed in cytosine–$CaCl_2$ complexes (Tables I and III). Since the shifts results from a redistribution of π-electrons in the conjugated C=C, C=N, and C=O system of the cytosine ring,[14,15] it is likely that Ca is coordinated with the N(3) site of cytosine in the cytosine –$CaCl_2$ complexes, as in the cytosine– and CMP–transition metal complexes studied by X-ray crystallography.[8b,12,16]

The νC=O band of cytosine is shifted to a higher-frequency region on protonation at the N(3) site because there is no longer electron migration from the N(3) position to C(2)=O (Fig. 1, B).[11a,b,14,15] As in the case of protonation, the νC=O band should be shifted to a higher–frequency region when a metal is coordinated with the N(3) site.[1] Upon coordination of transition metals with the N(3) and C(2)=O sites of cytosine,[1,8b,12,16] the νC=O band of cytosine remained near 1640 cm^{-1}, whereas the νC=C+νC=N bands were shifted to a higher–frequency region (Fig. 2). Moreover, the νC=O frequencies of cytosine–$CaCl_2$ complexes are comparable to those of the cytosine–transition metal complexes, as shown in Fig. 2 and Table III. In all cytosine–metal complexes studied in this work, the frequency of the νC=O band indicates that the C=O group has a single bond nature, caused mainly by the coordination with the C(2)=O site.

Ring Deformation Modes

Cytosine shows the characteristic bands due to the skeletal deformation modes in the region of 400—800 cm.$^{-1}$,[11,15] In this region, interesting bands are present near 400, 610, 710, and 750 cm^{-1} in all the cytosine–metal complexes listed in Tables I and II; these are common to the complexes but not to cytosine. They are absorption bands due to the ligand in the cytosine–metal complexes, because the frequencies were almost unaffected by the kind of metal. The new band near 750 cm^{-1} is present not only in cytosinium chloride but also in cytosinium–$ZnCl_4$ and –$CuCl_4$ complexes (in Fig. 3), and it is assignable to a ring deformation mode. The band is sensitive to the binding of a positively charged atom to the N(3) position of the cytosine ring, like the νC=C+νC=N bands. The other three bands mentioned above are distinguishable from the skeletal deformation bands of cytosinium chloride and the cytosinium–$ZnCl_4$ and –$CuCl_4$ complexes (near 375 and 585 cm^{-1}), as shown in Fig. 3. The metal coordination with the N(3) and C(2)=O sites of cytosine presumably accounts for this difference. It is clear that

14) H.T. Miles, *J. Am. Chem. Soc.*, **79**, 2565 (1957); H.T. Miles, *Proc. Natl. Acad. Sci. U.S.A.*, **47**, 791 (1961).
15) M. Tsuboi, Y. Kyogoku, and T. Shimanouchi, *Biochim. Biophys. Acta*, **55**, 1 (1962); M. Tsuboi, "Basic Principles in Nucleic Acid Chemistry," Vol 1, ed. by P.O.P. Tsu'o, Academic Press, New York, 1974.
16) M.L. Goodgame, I. Jeeves, C.D. Reynolds, and A.C. Skapski, *Biochem. J.*, **151**, 467 (1975); G.R. Clark and J.D. Orbell, *Chem. Commun.*, **1975**, 697; K. Aoki, *Biochim. Biophys. Acta*, **447**, 379 (1976).

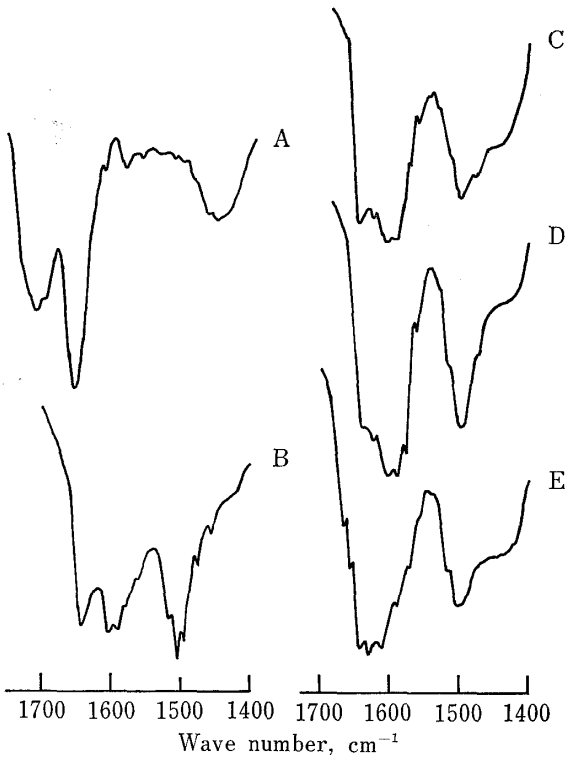


Fig. 2. Infrared Absorption Spectra in the Region of 1400—1700 cm^{-1} in D_2O Solution

A, cytosinium chloride; B, cytosine; C, 1:1 cytosine-$CaCl_2$; D, 2:1 cytosine-Co(II)Cl_2; E, 2:1 cytosine-Cu(II)Cl_2.

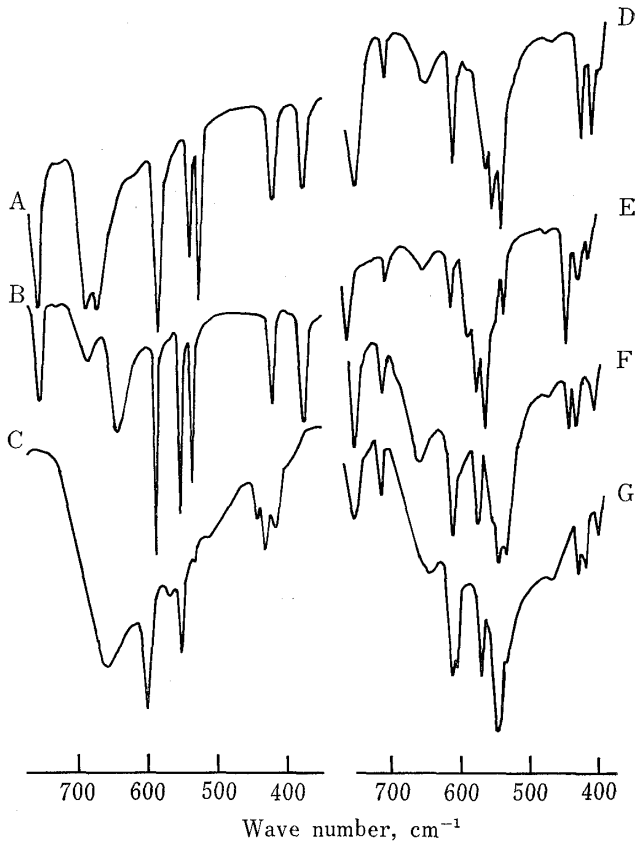


Fig. 3. Infrared Absorption Spectra in the Region of 400—700 cm^{-1} in KBr Disks

A, 2:1 cytosinium-$ZnCl_4$; B, cytosinium chloride; C, cytosine monohydrate; D, cytosine-Hg(II)Cl; E, 2:1 cytosine-Cu(II)Cl_2; F, 2:1 cytosine-Co(II)Cl_2; G, 2:1 cytosine-$CaCl_2$.

the bands near 400, 610, and 710 cm^{-1} are sensitive to metal coordination with the N(3) and C(2)=O sites of cytosine, and they might be assigned to skeletal deformation modes of the cytosine ring in the cytosine–metal complexes.

Proton Magnetic Resonance Spectra

In PMR studies on the complexation of diamagnetic metals with nucleic acid bases, a lower-field shift of the proton resonance on addition of the metal chloride indicates an interaction of the metal with the base.[6,9,17] The interaction in DMSO solution includes binding of the metal to the base[6,17] and an interaction of the chloro anion with the imino or amino proton.[7,18] In the present work, the chemical shifts of cytosine–$CaCl_2$ (2/1) and (1/1) complexes were measured in various solvents, and the shifts were assigned to the binding of Ca^{2+} to cytosine or the interaction of Cl^- with cytosine by referring to the IR results.

In the spectra of cytosine–$CaCl_2$ complexes (Fig. 4), the N(1)–H resonance was observed near 10.5 ppm as a broad signal, indicating the presence of hydrogen on the N(1) site. The C(4)–NH_2, C(5)–H, and C(6)–H resonances of the complexes appeared at lower-field than those of cytosine, as in the case of the other cytosine–metal complexes listed in Table IV. Lower-

17) S.M. Wang and N.C. Li, *J. Am. Chem. Soc.*, **88**, 4592 (1966); S.M. Wang and N.C. Li, *J. Am. Chem. Soc.*, **90**, 5069 (1968); L.S. Kan and N.C. Li, *J. Am. Chem. Soc.*, **92**, 281 (1970).
18) T. Yokono, S. Shimokawa, and J. Sohma, *J. Am. Chem. Soc.*, **97**, 3827 (1975).

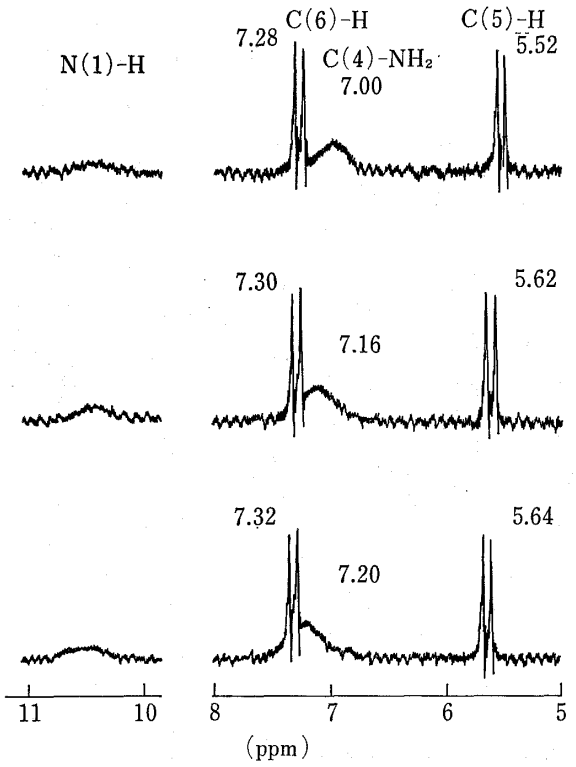


Fig. 4. Proton Magnetic Resonance Spectra in DMSO-d_6

Upper, cytosine monohydrate; center, 2:1 cytosine-$CaCl_2$; lower, 1:1 cytosine-$CaCl_2$.

field shifts of the ring proton in the cytosine–$CaCl_2$ complexes were also observed in D_2O solution, as shown in Table V.

To distinguish between the lower-field shift on metal binding and that on interaction with the anion, the effect of Cl^- on the chemical shifts of cytosine was examined in DMSO and D_2O solutions. On addition of NaCl to 0.1 M cytosine–DMSO solution (to saturation), the NH_2 signal was shifted 8 Hz to lower-field, suggesting the interaction of Cl^- with NH_2. However, the ring proton resonances were hardly affected by NaCl in DMSO or D_2O (0.4 M NaCl+0.1 M cytosine). Thus, the lower-field shift of the NH_2 signal of cytosine–$CaCl_2$ complexes indicates that the amino proton may take part in $NH_2\cdots Cl$ hydrogen bonding.[6c,18]

In the spectra of cytosine–metal complexes, the extent of the shift of the NH_2 signal varied with the kind of metal, as shown in Table IV. Moreover, the shift for cytosinium chloride was greater than those for the cytosine–metal complexes. These results indicate that a lower- field shift of the NH_2 signal of cytosine is caused not only by interaction with the anion[7] but also by the binding of a positively charged atom to the N(3) position of the cytosine ring. As shown in Table V, the ring proton resonances of cytosine unaffected by Cl^- were shifted

Table IV. Lower-Field Shifts (in Hz at 100 MHz) of Cytosinium Chloride and Cytosine–Metal Complexes from Cytosine in DMSO Solution

Samples	C(5)–H	C(6)–H	C(4)–NH_2
Cytosinium chloride	+51	+48	+172, +278
Cytosine–Hg(II)Cl	+31	+26	+62
Cytosine–$CdCl_2$	+10	+ 8	+50
Cytosine–Zn(OH)Cl[a]	+ 5	+ 6	+20
Cytosine–$ZnCl_2$[a]	+ 6	+10	+40

a) From ref. 1.

Table V. Chemical Shifts of Cytosine and Cytosine–$CaCl_2$ Complexes from DSS in D_2O

Samples	C(5)–H (ppm)	C(6)–H (ppm)
Cytosine	5.94	7.5
Cytosine–$CaCl_2$ (2/1)	5.97	7.52
Cytosine–$CaCl_2$ (1/1)	5.99	7.52

to lower field on the binding of a metal or proton to the N(3) site.[17,19] In the spectra of cytosine–$CaCl_2$ complexes, hindered rotation of the amino group[6c,17,19] was not observed, and the chemical shifts of C(5)-H and C(6)-H were not comparable to those of cytosinium chloride. Therefore, the lower-field shift of the ring proton in the cytosine–$CaCl_2$ complexes suggests the coordination of Ca with the N(3) position of the cytosine ring.

In the spectra of the cytosine–metal complexes in TFA, no variation of the proton resonances was observed when compared with those of cytosine, indicating decomposition of the complexes in strong acid.

Conclusion

In the IR spectra of many cytosine–metal complexes, metal coordination with the N(3) and C(2)=O sites of cytosine leads to the appearance of skeletal deformation bands of cytosine coordinated by the metal near 400, 610, and 710 cm^{-1}. In the PMR spectra of these complexes, the binding of a diamagnetic metal to the N(3) site causes the lower-field shift of ring proton resonances of cytosine.

On the basis of elemental and spectral analyses, it was found that calcium forms cytosine–$CaCl_2$ (2/1) and (1/1) complexes with cytosine in 70% ethanol and water, respectively, and that calcium is coordinated with the N(3) and C(2)=O sites of cytosine.[20]

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20) The X-ray structure analysis of cytosine–$CaCl_2$ (1/1) complex has been carried out by Tomita *et al.* The details will be reported elsewhere.