Structural Effect on Chelation Selectivity of Alkaline Earth Metal Ions with Aminopolycarboxylate-Type Chelators

Takahide KIMURA, Tetsushi MARUYAMA,† Mutsuo OKAMURA,†† Takashi SUGIYAMA,† Takashi ANDO, and Atsuyoshi OHNO*,†

Department of Chemistry, Shiga University of Medical Science, Ohtsu, Shiga 520-21
† Institute for Chemical Research, Kyoto University, Uji, Kyoto 611
†† Department of Chemistry, College of General Education, Niigata University, Niigata 950-21

(Received January 26, 1994)

Two derivatives of 1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (**H$_4$bapta**), in which the distance between the chelating functions is changed, have been synthesized. The structure–interaction relationships of their complexation behavior with alkaline earth metal ions are discussed from the viewpoint of their acidity constants and stability constants of their chelate-complexes.

Structure–interaction relationships on chelation of metal ions by polydentate ligands have been noted in studies of highly selective chelators. Many chelators in this category, especially polyamine polycarboxylate-type chelators, exert interesting patterns in selectivity for binding a series of alkaline earth metal ions from Mg^{2+} to Ba^{2+}. Namely, interest has been focused on Ca^{2+} from the viewpoint of biological activities of this ion such as muscle contraction, neurotransmitter release, hormonal response, blood clotting, etc. Calcium-binding proteins, such as troponin C[1,2] and calmodulin,[3] that exist in biological cells bind Ca^{2+} with large affinity (K_d^{Ca}: dissociation constants of Ca^{2+}-protein complex $\approx 10^{-7}$ mol dm^{-3}) and excellent selectivity for Ca^{2+} over Mg^{2+} and other alkaline earth metal ions ($>10^4$ difference in stability constant). High affinity of these proteins for Ca^{2+} has been accounted for by their structures at the binding site: four carboxylate groups with net charges of -4 are set at appropriate positions to chelate the metal ion.

Six out of eight donor atoms in 3,6-dioxaoctane-1,8-diamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (**H$_4$egta**), a representative polyamine polycarboxylate-type chelator, are oxygen atoms that hold neutral or anionic charges on them depending on pH of the aqueous solution. The net charges which this chelator can hold are exactly the same as those on calcium-binding proteins mentioned above. The analogy has brought out **H$_4$egta** as a useful model for the Ca^{2+} binding site of proteins and this chelator has been studied extensively. Based on X-ray crystallographic studies, factors that govern the stability of **egta**–Ca^{2+} complex has been discussed.[4,5]

In 1980, Tsien synthesized 1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (**H$_4$bapta**), an **H$_4$egta**-like chelator in which 1,2-disubstituted benzene rings hold amino nitrogens in the vicinity of ethereal oxygens (Chart 1).[6] In addition, **H$_4$bapta** has some novel properties, as follows: i) **H$_4$bapta** not only exerts as high selectivity for Ca^{2+} over Mg^{2+} ($Ca^{2+}/Mg^{2+} = 10^{5.2}$) as **H$_4$egta** does ($Ca^{2+}/Mg^{2+} = 10^{5.8}$)[6] at physiological pH region but also exerts faster rate for complexation with Ca^{2+} than **H$_4$egta**.[6] ii) Because of the existence of two phenyl groups in the molecule, **H$_4$bapta** exerts appropriate absorption maximum in its UV spectrum. Thus, chelating and free states of **H$_4$bapta** can be followed quite conveniently by UV spectroscopy, which makes it easy to study the chelation chemistry. The equilibria in chelation[6—10] and an X-ray crystal structure[11] have been reported.

It is interesting and meaningful to study structure–interaction relationships on selective chelation toward metal ions. In order to elucidate the relationship unequivocally, systematic variation in structure of the chelator is required. There are, however, only scattered results in reports so far published.[12,13] We selected **H$_4$bapta** as the suitable chelator for systematic variation in structure. Since **H$_4$bapta** has two phenyl moieties, modification of structure can be carried out more easily than that in **H$_4$egta** both in introducing certain functional group to a phenyl ring and in changing the length of methylene linkage between two ethereal oxygen atoms. One attempt to modify the chelator is to substitute an electron-withdrawing or -donating group on the molecule to introduce a variation in pK_a of anilinium protons.[6] The other is to introduce a hard core to the molecule by substituting a cyclopentyl, benzofuranyl or quinolinyl moiety for the phenyl rings.[6] No attempts of changing the structure of the carbon linkage between two phenoxy moieties in order to construct the most suitable structure for particular metal ion have been reported. We have worked on this topic for several years; the results based on modification of **H$_4$bapta** will be reported in this paper.

Results

Preparation of Materials. 1,3-Bis(*o*-aminophenoxy)propane-*N*,*N*,*N*′,*N*′-tetraacetic acid (**H$_4$bappta**) and 1,4-bis(*o*-aminophenoxy)butane-*N*,*N*,*N*′,*N*′-tetraacetic acid (**H$_4$bapbta**) have been synthesized following the procedure reported by Tsien.[6] The nitro groups, however, were reduced by powdery tin/concd HCl instead of H_2/Pd–C. In a relatively large scale preparation, reduction of nitro group by the former procedure proceeds more effectively and affords the correspond-

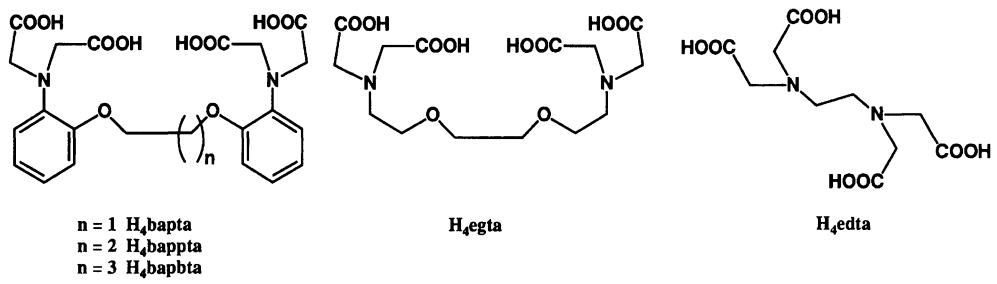


Chart 1.

ing amino compounds in higher yield and purity. The synthetic procedures are summarized in Scheme 1.

Determination of Acidity Constants. All equilibrium constants employed in this report are defined in Table 1. It is generally considered that these polyamine-*N*-polycarboxylate type chelators exist as zwitterionic forms in aqueous solutions; thus the lower two pK_{ai}s can be defined as the acidity constants of two of four carboxyl groups and the higher two pK_{ai}s as those of two anilinium ions. Acidities of these compounds have been determined by the conventional titration method employing the value of 0.83 as the activity coefficient of proton (γ_H).[15] Table 2 summarizes all the pK_{ai}s of **H4bappta** and **H4bapbta** together with those reported for others.

In order to confirm the reliability in comparing the measured pK_{ai}s with those reported for other chelators, those of **H4bapta** were measured by us simultaneously and compared with those reported.[6,22,23] Two independent measurements on **H4bapta** under the same titration conditions showed reasonable agreement in equal pK_{a3} and pK_{a4}, whereas small deviations from the constants which have been reported already by Yuchi et. al. were observed in pK_{a1} and pK_{a2}. Thus the values of pK_{a3} and pK_{a4} for **H4bappta** and **H4bapbta** may safely be used for discussion. Because of insolubility of **H4bappta** and **H4bapbta**, the accuracy of the latter pK_{ai}s are unreliable.

Determination of Stability Constants. Potentiometric titration was employed for the determination. For all titrations, the concentration of metal ion was kept 10-fold excess over that of the chelator. In order to elucidate the value of K_{M2L} accurately, however, it was necessary to run an additional titration with a solution containing equimolar amounts of a metal ion and a chelator.

The titration spectra were analyzed according to Schwarzenbach's method[17,18] and all four thus elucidated possible equilibrium constants for newly synthesized compounds, **H4bappta** and **H4bapbta**, are listed in Table 3. Titration spectra in the absence and presence (10 equivs in concentration) of alkaline earth metal ions are illustrated in Figs. 1 and 2.

Table 1. Definitions of Acidity and Stability Constants

Equilibrium	Constant
$H_4L \rightleftharpoons H^+ + H_3L^-$	K_{a1}
$H_3L^- \rightleftharpoons H^+ + H_2L^{2-}$	K_{a2}
$H_2L^{2-} \rightleftharpoons H^+ + HL^{3-}$	K_{a3}
$HL^{3-} \rightleftharpoons H^+ + L^{4-}$	K_{a4}
$H^+ + ML^{2-} \rightleftharpoons MHL^-$	K_{MLH}
$M^{2+} + L^{4-} \rightleftharpoons ML^{2-}$	K_{ML}
$M^{2+} + ML^{2-} \rightleftharpoons M_2L$	K_{M2L}
$M^{2+} + HL^{3-} \rightleftharpoons MHL^-$	K_{MHL}

Discussion

Acidity of Functional Groups. First of all, it appears in Table 2 that aliphatic nitrogens are much stronger bases than aromatic ones (pK_{a3}s and pK_{a4}s), which is a generally observed result for aliphatic and aromatic amines.

The difference in pK_{ai}s of two nitrogen atoms ($\delta_{3,4}^{\text{chelator}} = pK_{a4} - pK_{a3}$) in ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (**H4edta**) is much larger than those in other chelators ($\delta_{3,4}^{\text{edta}} = 4.06$). This observation is understandable on the basis of their structures: two nitrogens in **H4edta** are connected to each other by a relatively short chain of two methylene groups, whereas those in

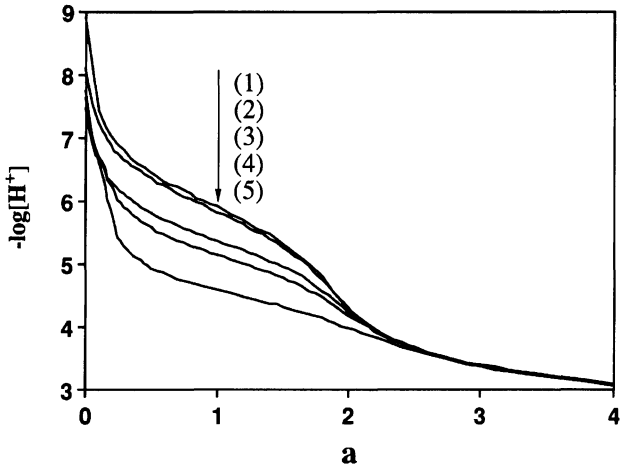
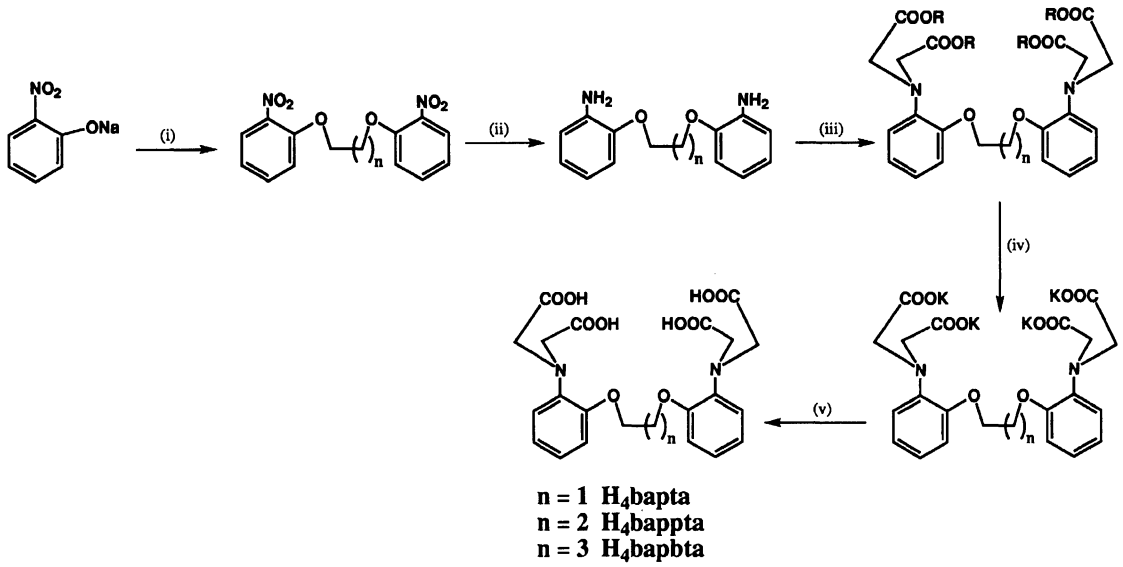


Fig. 1. Titration curves of **K4bappta** in the absence and presence of the alkaline earth metal ions. a = [HCl]/[chelator] (1) No metal ion, (2) Mg^{2+}, (3) Sr^{2+}, (4) Ba^{2+}, and (5) Ca^{2+} ($[M^{2+}] > 10[\mathbf{K_4bappta}]$). All titrations were performed at 25.0±0.2°C, I=0.1 mol dm^{-3} (KNO_3).



(i) $Br(CH_2)_nBr$, DMF, 100 °C; (ii) Sn, conc.HCl, EtOH, reflux; (iii) 1,8-bis(diethylamino)naphthalene, $BrCH_2CO_2R$ (R = Me or Et), acetonitrile, reflux; (iv) KOH, $EtOH/H_2O$, 60 °C; (v) Dowex 50wX8, H_2O

Scheme 1.

Table 2. Acidity Constants[a] of Several Chelators

Chelator	pK_{a1}	pK_{a2}	pK_{a3}	pK_{a4}
edta[b]	1.99	2.68	6.11	10.17
egta[c]	2.00± 0.00	2.66± 0.02	8.85± 0.01	9.47± 0.02
bapta[d]	2.67	3.48	5.44	5.95
bapta[e]	—	—	5.47± 0.1	6.36± 0.1
bapta	2.31± 0.01	3.12±0.02	5.29± 0.01	5.92± 0.00
bappta	—[f]	2.82± 0.02	5.40± 0.01	6.37± 0.00
bapbta	—[f]	2.93± 0.03	5.58± 0.01	6.51± 0.00

a) All the constants are defined as follows; $pK_{an} = -\log K_{an}$ (K_{an}/mol dm^{-3}). b) Constants from Martell and Smith[14] (I=0.1 mol dm^{-3}, 25 °C). c) Constants from Martell and Smith[14] (I=0.1 mol dm^{-3}, 20 °C). d) Constants from Yuchi et al.[22] (I=0.1 mol dm^{-3} (KNO_3), 25 °C). e) Constants from Tsein et al.[6] (I=0.1 mol dm^{-3} (KCl), 20 °C). f) No appropriate value was obtained within confidential accuracy.

H_4egta ($\delta_{3,4}^{egta}$=0.62) and other chelators ($\delta_{3,4}^{bapta}$=0.63, $\delta_{3,4}^{bappta}$=0.97, and $\delta_{3,4}^{bapbta}$=0.93) are separated by many bonds. This large value of $\delta_{3,4}^{edta}$ can be interpreted that the first protonation on one nitrogen atom (pK_{a4}) in **H_4edta** prevents the second protonation on the other nitrogen atom (pK_{a3}). In other words, these two nitrogens in **H_4edta** cooperatively stabilize the state of initial protonation by bridging a proton between two nitrogen atoms; the system may be represented by $N^{\delta+}\cdots H\cdots N^{\delta+}$. Whereas, smaller $\delta_{3,4}$ values (from 0.6 to 1.0) in three **H_4bapta** derivatives including **H_4egta** than $\delta_{3,4}^{edta}$ implies more or less independent protonation on both of equivalent two nitrogen atoms which are separated in long distances. It is reported that the difference in pK_a of intramolecular but infinitely separated two ammonium ions is statistically about 0.6.[19] When the difference is inspected more carefully, however, it is recognized that the differences in **H_4bappta** and **H_4bapbta** are obviously larger than those in **H_4egta** and **H_4bapta**. This observation of reversing effect cannot be accounted for by the through-space electronic effect of an anilinium ion only.

Although there is no evidence at present, we suspect that a carboxylate group on the other nitrogen is involved for the first protonation, resulting in difficulty in protonation on this second nitrogen. This sort of participation by a carboxylate anion becomes easier as the chain becomes longer and the structural flexibility becomes larger.

Unfortunately, in both **H_4bappta** and **H_4bapbta**, no appropriate values of pK_{a1} and pK_{a2} were obtained within confidential accuracy because of the low solubility of tri- and tetra-protonated species of **bapta**-derivatives. All the titrations in our experiments were car-

Table 3. Equilibrium Constants[a] of Several Chelators

Chelator		log K			
		Mg^{2+}	Ca^{2+}	Sr^{2+}	Ba^{2+}
edta[b]					
	log K_{ML}	8.83	10.61	8.68	7.80
egta[b]					
	log K_{ML}	5.28	10.86	8.43	8.40
	log K_{MHL}[c]	3.37	5.33	4.37	4.26
bapta[d]					
	log K_{ML}	1.77[e]	6.97[e]	5.28	5.78
	log K_{M2L}	0.3[e]	—[e,f]	—[f]	—[f]
	log K_{MHL}	—[g]	—[g]	1.48	3.21
	log K_{MLH}	—[g]	—[g]	2.12	3.35
bappta					
	log K_{ML}	1.82	4.68	2.92	3.48
	log K_{M2L}	2.12	—[f]	1.77	—[f]
	log K_{MHL}	0.16	2.56	1.91	2.18
	log K_{MLH}	4.71	4.25	5.37	5.08
bapbta					
	log K_{ML}	1.22	3.30	2.46	2.72
	log K_{M2L}	2.60	1.80	2.21	2.07
	log K_{MHL}	0.90	2.41	1.90	2.20
	log K_{MLH}	6.18	5.62	5.96	6.00
Calmodulin[h]		3.0	6.0—7.2		

a) All equilibrium constants are defined in Table 1. b) Constants from Martell and Smith[14] (I=0.1 mol dm^{-3}, 20 °C). c) Constants from Anderegg.[13] d) Present results. e) Constants from Tsien[6] (I=0.1 mol dm^{-3}, 22 °C). f) The value is negligibly small. g) Not determined. h) Constants from Refs. 24 and 25.

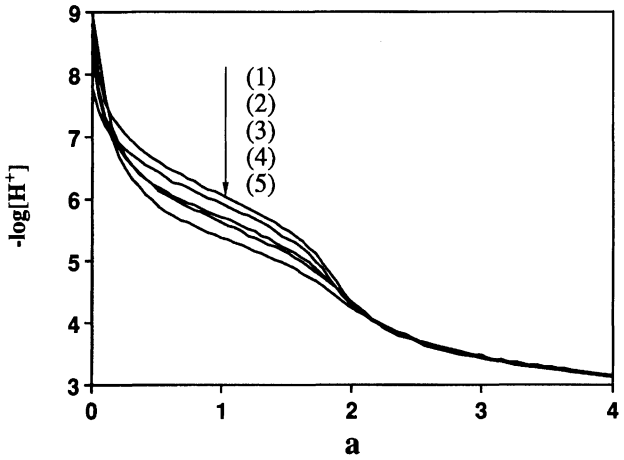


Fig. 2. Titration curves of **K_4bapbta** in the absence and presence of the alkaline earth metal ions. a= [HCl]/[chelator] (1) No metal ion, (2) Mg^{2+}, (3) Sr^{2+}, (4) Ba^{2+}, and (5) Ca^{2+} ([M^{2+}] > 10[**K_4bapbta**]). All titrations were performed at 25.0±0.2°C, I=0.1 mol dm^{-3} (KNO_3).

ried out in solutions initially containing 10^{-3} mol dm^{-3} of tetra-ionic form of a chelator which is higher than the solubility of **H_4bapta**, ca. 10^{-4} mol dm^{-3} (at $-\log[H^+]<2.6$).[22,23] **H_4bappta** and **H_4bapbta** might be less soluble than **H_4bapta**, because the formers have longer alkyl chain, or a hydrophobic moiety than the latter. It should be noted that the values for pK_{a1} and pK_{a2} are not important, fortunately, in determining the stability constants, because the complexation with alkaline earth metal ions does not usually occur at low pH region.

Stability of the Complex. Figure 1 demonstrates that the order of affinity of alkaline earth metal ions toward **bappta^{4-}** is $Ca^{2+} > Ba^{2+} > Sr^{2+} \gg Mg^{2+}$ when excess metal ions exist in the system. The relationship also holds with **bapbta^{4-}** (Fig. 2). However, detailed inspection with the aid of Table 3 reveals that this order is valid only for the formation of 1 : 1 (ML) complexes. For the formation of 2 : 1 (M_2L) complexes, on the other hand, the order changes to $Mg^{2+} \gg Sr^{2+} > Ba^{2+} > Ca^{2+}$: The order is reversed and the position of Mg^{2+} changes dramatically. When a chelator forms a complex with a cation, it is quite conceivable that all of four carboxylate groups and two nitrogen atoms participate in holding the metal ion, which is much larger than a proton (*vic*-**ML^{2-}**) (Chart 2). In comparison with **bapta^{4-}**, however, **bappta^{4-}** and **bapbta^{4-}** are anions that prefer to catch a cation by the geminal-type mode (*gem*-**ML^{2-}**) because of their longer linkage between two ethereal oxygen atoms. Thus, the smallest Mg^{2+} ion is most favorable for a M_2L-complex with **bappta^{4-}** or **bapbta^{4-}**, whereas Mg^{2+} ion is too small and entropically least favored for the formation of a ML-complex with these chelators in a vicinal-type mode. It should

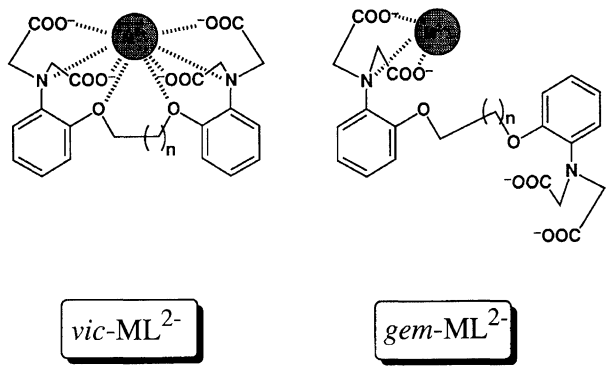


Chart 2.

also be noted that the smallest ion has the largest charge density.

During spectrophotometric measurement of stability constants for **bapta**$^{4-}$, it was recognized that the spectral shift on complexation with Mg^{2+} is much less than that with Ca^{2+} and about twice as much Mg^{2+} as that of Ca^{2+} is required to cause the shift to a similar extent. This observation indicates sequential binding of Mg^{2+} to each end of the chelator (*gem*-**ML**$^{2-}$) in contrast to competitive binding of Ca^{2+} to the central part of the anionic cavity (*vic*-**ML**$^{2-}$). The preference in forming an M_2L complex with Mg^{2+} over other metal ions is demonstrated by their log K_{M2L} values.

Table 3 lists logarithmic stability constants of ML- and M_2L-type complexes. For three cations: Ca^{2+}, Sr^{2+}, and Ba^{2+}, the stability constants for ML-type complexes decrease appreciably with the increase in number of methylene groups, whereas they are little affected for Mg^{2+}. **Bapta**$^{4-}$ prefers to form a complex of vicinal-type even for proton. This might be true for larger alkaline earth metal ions. On the other hand, this tendency disappears gradually with the increase in number of the methylene group. In the case of **bappta**$^{4-}$ or **bapbta**$^{4-}$, non-negligible contribution of M_2L-type complexes are observed with simultaneous formation of the geminal-type ML complexes.

The validity of discussion is also supported by K_{MLH}, acidity of the ML-complex. The value is the largest with Mg^{2+} and the smallest with Ca^{2+} in **M(bappta)**$^{2-}$. Since Mg^{2+} ion is bound by **bappta**$^{4-}$ sequentially, or at one corner of the chelator by the aid of a tridentate ligation, the other nitrogen at the other end of the chelator is free to bind a proton, whereas since the chelator holds Ca^{2+} ion in its central position, two nitrogen atoms in this chelator participate to chelation equally at the sacrifice of its affinity toward a proton, or acidity. Similar discussion is also valid for **bapbta**$^{4-}$.

The Ca^{2+}/Mg^{2+} selectivity ratios for ML-type complexes are 1.79, 5.58, 5.20, 2.86, and 2.08 for **edta**$^{4-}$, **egta**$^{4-}$, **bapta**$^{4-}$, **bappta**$^{4-}$, and **bapbta**$^{4-}$, respectively. Entropically most favorable **egta**$^{4-}$ in formation of a *vic*-ML-type complex exerts the largest value and **edta**$^{4-}$ which has the smallest anionic cavity exerts the smallest ratio. Compensation of entropic and *gem*-preference effects results in similar selectivity for **bappta**$^{4-}$ and **bapbta**$^{4-}$. The smaller Ca^{2+}/Mg^{2+} selectivity ratios for **bappta**$^{4-}$ and **bapbta**$^{4-}$ than **egta**$^{4-}$ and **bapta**$^{4-}$ suggest that high affinity for Ca^{2+} is attributed to suitable molecular length and favorable configuration of the ligating groups.

In general, the increase in size of chelating ring leads to the decrease in stability. The phenomenon has been accounted for by an entropic effect associated with the freezing of freedom of bond rotation.[16] Hancock and Martell, however, proposed another interpretation for this phenomenon based on molecular mechanics:[20,21] A decrease in stability is not due to the entropy effect but due to the enthalpy effect associated with unfavorable steric strain of the chelating ring. Their results reveal that the increase in size of chelating ring leads to greater destabilization with larger metal ions than with smaller ones.

When this idea is employed for understanding the present result, greater decrease in stability constant for Ca^{2+} ion than for Mg^{2+} ion on going from **bapta**$^{4-}$ to **bapbta**$^{4-}$ is a reasonable consequence. The present systems will be subjected to thermodynamic measurements. Whichever the major contribution is, the net effect has to be interpreted in terms of *geminal*- vs. *vicinal*-type and M_2L- vs. ML-type chelation simultaneously. Neither can predict the tendency independently.

It is interesting to note that the Ca^{2+}/Mg^{2+} selectivity of **bappta**$^{4-}$ resembles that of Ca^{2+}-transporting protein, calmodulin,[24,25] the value for which is also listed in Table 3.

Finally, it should be pointed out that the difference in K_{M2L} and K_{ML} for Mg^{2+} and Ca^{2+} ions may be applied to construct an active transportation system of Mg^{2+} ion coupled with passive transportation of Ca^{2+} ion. The research is in progress in our laboratory and the result will be reported elsewhere.

Experimental

Instruments. ^{1}H NMR spectra were recorded at 200 MHz on a Varian VXR 200FT NMR spectrometer. UV spectra were recorded on a Hitachi U-3210 spectrophotometer at 25.0±0.2 °C adjusted by a Hitachi SDR-30 temperature controller. Elemental analyses were performed with a Yanaco MT-5 Elemental Analyzer. Potentiometric titration was performed with a manual-operating titration system which consists of a Horiba digital ion meter N-8, a Horiba combination pH electrode 6028-10T, an inlet for argon gas, a glass burette and 50 cm^3 of three-necked round bottomed flask.

Materials. Chemicals and solvents were used as obtained from commercial sources unless otherwise noted. Distilled water used for preparation of stock solutions of chelators was decarbonated by refluxing for 2 h and stored in plastic bottles made of polypropylene. Acetonitrile was distilled from calcium hydride immediately before the use. Nitrates were used as the source of all alkaline earth metal ions. They were dried over 2 h at room temperature under reduced pres-

sure. Standard solutions of aqueous hydrochloric acid were purchased from Nacalai Tesque Co., Ltd. **H_4bapta** was prepared according to the literature procedure.[6)]

Potentiometric Titration. All titrations were carried out under argon saturated with water at 25±0.2°C. Ionic strength was adjusted to 0.10 by 0.10 mol dm^{-3} KNO_3 for all titrations.

For preparation of sample solutions which were used in titrations for stability constant determination, alkaline earth metal nitrate salt was weighed in a 50 cm^3 three-necked flask filled with argon, and 20 cm^3 of a stock solution (1.00×10^{-3} mol dm^{-3}) of tetrapotassium salt of a chelator was added to the flask. Thus, initial concentrations of the metal ions were kept in the region of $(1.01—1.10)\times10^{-2}$ mol dm^{-3}. The flask was allowed to stand for 20 min in a thermostat prior to titration. Stability constants for complexes were determined by the conventional Schwarzenbach procedure.[17,18)]

Acidities (pK_as) of chelators defined in Table 1 were determined by analyzing the data obtained from titrations performed in the absence of metal ion using a non-linear least squares method. The titration for determining the acidity was carried out in the same procedure as described above.

Preparation of 1,3-Bis(*o*-nitrophenoxy)propane. A mixture of 11.5 g of 1,3-dibromopropane (0.06 mol), 18.4 g of sodium *o*-nitrophenolate (0.11 mol) and 400 cm^3 of dimethylformamide was placed in a 1 dm^3 three-necked round-bottomed flask equipped with a reflux condenser and stirred at room temperature under an argon atmosphere. After the materials were dissolved completely, the reaction mixture was heated to 100 °C. The reaction mixture was allowed to stand for another 24 h. Then, the solvent was removed under reduced pressure and the resulting residue was poured into 150 cm^3 of distilled water. A yellow precipitate appeared and the supernatant was separated by decantation. The precipitate was dissolved into dichloromethane and the solution was washed with an aqueous solution saturated with sodium carbonate. This organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure to obtain 11.8 g (65% yield) of 1,3-bis(*o*-nitrophenoxy)-propane as yellow solid. ^{1}H NMR ($CDCl_3$) δ=2.35 (m, 2H), 4.36 (t, 4H), 7.00 (dt, 2H), 7.14 (dd, 2H), 7.52 (dt, 2H), and 7.81 (dd, 2H).

Preparation of 1,3-Bis(*o*-aminophenoxy)propane. A mixture of 6.7 g (21.0 mmol) of 1,3-bis(*o*-nitrophenoxy)-propane, 10.1 g of powdery tin, and 100 cm^3 of 99% ethanol was placed in a 500 cm^3 round-bottomed flask equipped with a dropping funnel (100 cm^3 capacity). The mixture was stirred vigorously on an ice bath, then 40 cm^3 concd HCl was added dropwise through the dropping funnel. The reaction proceeded exothermally. The reaction mixture was heated at 60 °C for 4 h, until the mixture became a clear homogeneous solution. The solution was cooled on an ice-water bath and was added 300 cm^3 (40 g) of aqueous solution of ca. 10% sodium hydroxide. A large amount of gray precipitate appeared. The precipitate was collected and washed with 400 cm^3 of ethyl acetate on a filter. The alkaline solution obtained as a filtrate was extracted with 400 cm^3 of ethyl acetate. The organic layers were combined and dried over sodium sulfate. Evaporation of the solvent and recrystallization of the residue from 99% ethanol gave 5.0 g (92% yield) of 1,3-bis(*o*-aminophenoxy)propane. ^{1}H NMR ($CDCl_3$) δ=2.33 (t, 2H), 3.81 (bs, 4H), 4.21 (t, 4H), and 6.7—6.9 (m, 8H).

Preparation of 1,3-Bis[2-[bis[(ethoxycarbonyl)-methyl]amino]phenoxy]propane. A mixture of 2.9 g (11 mmol) of 1,3-Bis(*o*-aminophenoxy)propane, 11.8 g (55 mmol) of 1,8-bis(dimethylamino)naphthalene and 30 cm^3 acetonitrile as a solvent were placed in a 100 cm^3 round-bottomed flask equipped with a Dimroth condenser and stirred vigorously at room temperature under argon atmosphere until the solution became homogeneous. Onto this solution, 7 cm^3 (63 mmol) of ethyl bromoacetate was added through a 10 cm^3 syringe. After 30 min, white precipitates appeared. The reaction mixture was allowed to stand for 4 d. The reaction mixture was then cooled to room temperature and diluted with toluene. Subsequently, the mixture was stirred vigorously for a few minutes and the white precipitates were removed by filtration on a glass filter. The filtrate was evaporated under reduced pressure to give dark brown oil as a residue. The residue was dissolved into 50 cm^3 of ethyl acetate and washed twice with 50 cm^3 of water saturated with sodium chloride. After the organic layer was dried over sodium sulfate, the solvent was evaporated under reduced pressure to give light brown oil. This residue was chromatographed on a column of silica gel (Nacalai Silica Gel 60) with a mixture of hexane and ethyl acetate as an eluent in a volume ratio of 7:3. Consequently, 1.92 g (29% yield) of 1,3-bis[2-[bis[(ethoxycarbonyl)methyl]amino]phenoxy]propane (3.2 mmol) was obtained as white crystals after recrystallization from ethanol: Mp 75.5—76.5 °C. ^{1}H NMR ($CDCl_3$) δ=1.26 (t, 12H), 2.23 (m, 2H), 4.14 (q, 8H), 4.20 (t, 4H), and 6.8—6.9 (m, 8H). Anal. Calcd for $C_{31}H_{42}N_2O_{10}$: C, 61.8; H, 7.02; N, 4.65%. Found: C, 61.05; H, 7.10; N, 4.48%.

Hydrolysis of 1,3-Bis[2-[bis[(ethoxycarbonyl)-methyl]phenoxy]propane. In a 20 cm^3 round-bottomed flask equipped with a dimroth condenser, 1.92 g of 1,3-bis-[2-[bis[(ethoxycarbonyl)methyl]amino]phenoxy]propane (3.2 mmol) and 2 dm^3 of 99% ethanol were mixed and warmed to 50 °C. Into this mixture, 3.0 cm^3 of aqueous potassium hydroxide containing 907 mg of solid potassium hydroxide (12.7 mmol) was added. The reaction temperature was kept at 50 °C for 30 min. Subsequently, the solution was concentrated under reduced pressure to give a white amorphous solid. The solid was dissolved into distilled water and extracted with dichloromethane to remove the unhydrolized ester. The aqueous solution was poured into 200 cm^3 of acetone. White precipitate appeared, which was removed by decantation. The precipitate was transferred to another 20 cm^3 round-bottomed flask as washing with acetone. Again, the acetone layer as the supernatant was removed by decantation and the precipitate was dried for 5 h at 60 °C under reduced pressure to give **K_4bappta** as white solid quantitatively. ^{1}H NMR ($CDCl_3$) δ=1.57 (t, 2H), 3.16 (s, 8H), 3.50 (t, 4H), and 6.0—6.5 (m, 8H). The free acid **H_4bappta** was easily obtained by stirring **K_4bappta** with DOWEX 50wX8 ion exchange resin in an aqueous solution. Anal. Calcd for $C_{23}H_{26}N_2O_{10}$: C, 56.3; H, 5.34; N, 5.71%. Found: C, 55.91; H, 5.69; N, 5.64%.

Preparation of H_4bapbta. The procedure for the preparation of **H_4bapbta** is identical to that of **H_4bappta**. 1,4-Bis(*o*-nitrophenoxy)butane. ^{1}H NMR ($CDCl_3$) δ=2.01 (m, 4H), 4.22 (m, 4H), 7.02 (dt, 2H), 7.11 (dd, 2H), 7.53 (dt, 2H), and 7.82 (dd, 2H). 1,4-Bis(*o*-aminophenoxy)butane. ^{1}H NMR ($CDCl_3$) δ=2.05 (m, 4H), 2.82 (bs, 4H), 4.10 (m,

4H), and 6.7—6.9 (m, 8H). Anal. Calcd for $C_{16}H_{20}N_2O_2$: C, 70.56; H, 7.40; N, 10.29%. Found: C, 70.52; H, 7.34; N, 10.17%. 1,4-Bis[2-[bis[(methoxycarbonyl)methyl]amino]-phenoxy]butane. ^{1}H NMR ($CDCl_3$) δ=1.92 (m, 4H), 3.71 (s, 12H), 4.02 (m, 4H), 4.12 (s, 8H), and 6.8—6.9 (m, 8H). Anal. Calcd for $C_{28}H_{36}N_2O_{10}$: C, 60.00; H, 6.47; N, 5.00%. Found: C, 59.67; H, 6.45; N, 4.89%. 1,4-Bis(*o*-aminophenoxy)butane-*N*,*N*,*N*′,*N*′-tetraacetic acid (**H_4bappta**) Anal. Calcd for $C_{28}H_{36}N_2O_{10}$: C, 57.14; H, 5.59; N, 5.55%. Found: C, 56.23; H, 5.88; N, 5.36%.

We thank financial support for a part of this research by the Ministry of Education, Science and Culture under the Grants-in-Aid Nos. 04403006 and 04303002.

References

1) M. Sundaralingam, R. Bergstorm, G. Strasburg, S. T. Rao, P. Roychrowdhury, M. Greaser, and B. C. Wang, *Science* (*Washington, DC*), **227**, 945 (1985).
2) O. Herzberg and M. N. G. James, *Nature* (*London*), **313**, 653 (1985).
3) Y. S. Babu, J. S. Sack, T. J. Greenhough, C. E. Bugg, A. R. Means, and W. J. Cook, *Nature* (*London*), **315**, 37 (1985).
4) C. K. Schauer and O. P. Anderson, *J. Am. Chem. Soc.*, **109**, 3646 (1987).
5) C. K. Schauer and O. P. Anderson, *Inorg. Chem.*, **27**, 3118 (1988).
6) R. Y. Tsien, *Biochemistry*, **19**, 2396 (1980).
7) G. Grynkiewicz, M. Poenie, and R. Y. Tsien, *J. Biol. Chem.*, **260**, 3440 (1985).
8) A. Minta, J. P. Y. Kao, and R. Y. Tsien, *J. Biol. Chem.*, **264**, 8171 (1989).
9) S. M. Harrison and D. M. Bers, *Biochem. Biophys. Acta*, **925**, 133 (1987).
10) B. Svensson, B. Jonsson, M. Fushiki, and S. Linse, *J. Phy. Chem.*, **96**, 3135 (1992).
11) J. T. Gerig, P. Singh, L. A. Levy, and R. E. London, *J. Inorg. Biochem.*, **31**, 113 (1987).
12) G. Schwarzenbach and H. Ackermann, *Helv. Chim. Acta*, **31**, 1029 (1948).
13) G. Anderegg, *Helv. Chim. Acta*, **47**, 1801 (1964).
14) A. E. Martell and R. M. Smith, "Critical Stability Constants," Plenum, New York (1974), Vol. I.
15) J. Kielland, *J. Am. Chem. Soc.*, **59**, 1675 (1937).
16) G. Schwarzenbach, *Helv. Chim. Acta*, **35**, 2344 (1952).
17) G. Schwarzenbach, *Helv. Chim. Acta*, **33**, 974 (1950).
18) G. Schwarzenbach and E. Freitag, *Helv. Chim. Acta*, **34**, 1492 (1951).
19) G. Bonhomme, *Bull. Soc. Chim. Fr.*, **1**, 60 (1968).
20) R. D. Hancock, G. J. McDougall, and F. Marsicano, *Inorg. Chem.*, **18**, 2847 (1979).
21) R. D. Hancock and A. E. Martell, *Chem. Rev.*, **89**, 1875 (1989).
22) A. Yuchi, M. Hirai, H. Wada, and G. Nakagawa, *Chem. Lett.*, **1990**, 773.
23) A. Yuchi, A. Tanaka, M. Hirai, T. Yasui, H. Wada, and G. Nakagawa, *Bull. Chem. Soc. Jpn.*, **66**, 773 (1993).
24) C. B. Klee and T. C. Vanaman, *Adv. Protein Chem.*, **35**, 213 (1982).
25) J. Haiech, C. B. Klee, and J. G. Demaille, *Biochemistry*, **20**, 3890 (1981).