Note

Complexing of cycloinulo-oligosaccharides with metal ions

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It is well known that carbohydrates, polyols[1], and crown ethers[2] form stable complexes with metal ions in aqueous solution. Recently, cycloinulo-oligosaccharides (cyclofructans) have been prepared from inulin by the action of cycloinulo-oligosaccharide fructanotransferase of *Bacillus circulans* OKUMZ 31B[3]. The fine structure of cycloinulohexaose was established by X-ray crystallographic analysis[4,5]. Its molecule has the characteristic 18-crown-6 skeleton and six fructofuranose residues which are linked to the crown ring in a spiro arrangement. It can be predicted from the molecular structure that there should be some interactions between cyclofructans and metal ions.

The present report provides evidence that the sugars act as ligands in complex formation with the Ba^{2+} ion and other metal ions. A comprehensive survey of the complex formation between cyclofructans and metal ions was carried out by thin-layer ligand-exchange chromatography[6], and stoichiometric behaviour of complex formation with the Ba^{2+} ion was detected by using a conductometric technique with an electrode.

Cycloinulohexaose was prepared from the enzyme digest of inulin by the methods described previously[3]. Cycloinuloheptaose and cycloinulo-octaose were isolated from the same enzyme digest by chromatographic methods[7]. POLY-GRAM IONEX-25 SA-Na sheets (Macherey-Nagel GmbH, Duren) were converted into various metal-cation forms as described by Briggs et al.[6]. Cyclofructans (1 μL of aq 1.0% solution) were spotted and the chromatograms developed (1–1.5 h) with distilled water or aq 50% MeOH (v/v). Sugar detection was carried out by treatment of the dried sheets with ethanolic 5% H_2SO_4 at 100°C.

Tables I and II show the observed R_f values of cyclofructans on IONEX-25 SA sheet in the various metal-ion forms, developed with distilled water and/or aq

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TABLE I

R_f values in TLC and percentages of complexed cycloinulohexaose

Cation (Cl salt)	H$_2$O			50% Methanol		
	R_f	K [a]	(%)	R_f	K	(%)
H$^+$	> 0.99	0	0	0.96	0	0
Li$^+$	0.99	0.01	1.0	0.96		
Na$^+$	> 0.99			0.86	0.11	10.4
K$^+$	0.98	0.02	2.0	0.44	1.18	54.2
Rb$^+$	0.96	0.04	4.0	0.40	1.40	58.3
Cs$^+$	0.98	0.02	2.0	0.76	0.26	20.8
Cu^{2+}	> 0.99			0.88	0.09	8.3
Ag$^+$ [b]	0.94	0.06	6.0	0.36	1.66	62.5
				−0.74	−0.30	−22.9
Mg^{2+}	> 0.99			0.94	0.02	2.1
Ca^{2+}	> 0.99			0.96		
Sr^{2+}	0.98	0.02	2.0	0.93	0.03	3.1
Ba^{2+} [c]	0.67	0.49	33.0	0.17	4.65	82.3
Zn^{2+}	0.98	0.02	2.0	0.95	0.01	1.0
Cd^{2+}	0.98	0.02	2.0	0.92	0.04	4.2
Al^{3+}	> 0.99			0.94	0.02	2.1
La^{3+}	0.98	0.02	2.0	0.87	0.10	9.4
Pb^{2+}	0.95	0.05	5.0	0.23	3.17	76.0
Cr^{3+}	0.97	0.03	3.0	0.86	0.12	10.4
Fe^{2+}	0.96	0.04	4.0	0.88	0.09	8.3
Fe^{3+}	0.99	0.01	1.0	0.88	0.09	8.3
Co^{2+}	> 0.99			0.87	0.10	9.4
Ni^{2+}	> 0.99			0.93	0.03	3.1

[a] Complexation coefficient. [b] AgNO$_3$, strong tailing. [c] Ba(OAc)$_2$.

50% MeOH. The percentages of complexed ligand were calculated by the methods of Briggs et al.[6].

In Table II, cycloinuloheptaose had the maximum R_f value of 0.94 in the H$^+$ form of the layer, i.e., $\xi = 1/0.94 = 1.06$; in the case of cycloinulo-octaose, the

TABLE II

R_f values in TLC and percentages of complexed cycloinuloheptaose and cycloinulo-octaose in the aqueous methanol system (50% MeOH v/v)

Cation (Cl salt)	Cycloinuloheptaose			Cycloinulo-octaose		
	R_f	K [a]	(%)	R_f	K	(%)
H$^+$	0.94	0	0	0.94	0.03	2.9
Li$^+$	0.94	0	0	0.97	0	0
K$^+$	0.85	0.11	9.9	0.93	0.04	3.8
Rb$^+$	0.86	0.09	8.3	0.89	0.09	8.3
Cs$^+$	0.82	0.15	13.0	0.89	0.09	8.3
Ag$^+$ [b]	0.51	0.84	45.6	0.73	0.33	24.8
	−0.67	−0.40	−28.6	−0.89	−0.09	−8.3
Ba^{2+} [c]	0.89	0.06	5.7	0.96	0.01	1.0
La^{3+}	0.94	0	0	0.89	0.09	8.3
Pb^{2+}	0.93	0.01	1.0	0.93	0.04	3.8

[a] Complexation coefficient. [b] AgNO$_3$, strong tailing. [c] Ba(OAc)$_2$.

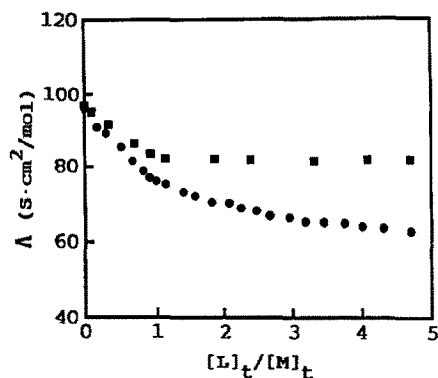Fig. 1. Λ vs. $[L]_t/[M]_t$ curves for cycloinulohexaose and 18-crown-6–barium acetate systems in aqueous 50% methanol solution at 24°C: ●, cycloinulohexaose; ■, 18-crown-6.

maximum R_f value was observed in the Li^+ form of the layer; $R_f = 0.97$, i.e., $\xi = 1/0.97 = 1.03$.

Table I shows that complex formation of cycloinulohexaose with the barium ion is very selective (33.0%) in the aq system, other divalent alkaline-earth metal ions, alkali metal ions, and other metal ions giving much lower percentages of complexing. The higher percentage is explained by greater electrostatic bond energy for barium ion, whose radius best matches that of the 18-crown-6 cavity of the ligand molecule. However, in the aq MeOH system, other metal ions such as K^+, Rb^+, Cs^+, Ag^+, and Pb^{2+} ions have considererable interactions with cycloinulohexaose. In the aq MeOH system, the percentage of complexing with the Ba^{2+} ion also increased to 82.3%. Presumably, the molecular conformation of cycloinulohexaose makes a difference suitable for complex formation in the aq MeOH system, and the energy of the cation desolvation is decreased in the solvent of lower dielectric constant. In the cases of cyclofructans which have higher dp, the ability to form complexes decreases in the order cyclohexaose > cycloheptaose > cyclooctaose (Table II). In the case of cycloinuloheptaose, the much larger Cs^+ ion is the best complexer. The complex formation of cyclofructans is seen to be dependent on the size of the crown ring moiety of the molecule.

The stoichiometry of the complexation reaction of cycloinulohexaose with the Ba^{2+} ion was estimated by a conductometric method. The conductance measurements were performed on a Shimadzu Kohlrausch's bridge apparatus, model K-2, at $24.00 \pm 0.15°C$. A cell was used with cell constant of 0.403 cm^{-1}. An aq 50% MeOH (v/v) solution of barium acetate (2 mM, 60 mL) was placed in the cell (volume 80 mL) and the resistance of the solution was measured. A step-by-step increase in the cycloinulohexaose concentration was effected by addition of the compound to the solution. After each addition of the sugar, the solution was stirred for 30 min, and the resistance of the solution was measured.

The molar conductivity, Λ vs. $[L]_t/[M]_t$ plot in aq 50% MeOH at 24°C is given in Fig. 1, where $[L]_t$ and $[M]_t$ are the total concentration of the cycloinulohexaose

and the Ba^{2+} ion, respectively. Fig. 1 also includes a Λ vs. $[L]_t/[M]_t$ plot for 18-crown-6, a typical example of complex formation between Ba^{2+} ion and a ligand molecule. In the case of cycloinulohexaose, the Λ vs. $[L]_t/[M]_t$ plot shows a decrease of Λ with an increase in the cycloinulohexaose concentration, and the slope of the curve changes at the point where $[L]_t/[M]_t$ is ∼ 1, and is followed by a gentle slope. These results suggest that the cycloinulohexaose forms a 1 : 1 complex with Ba^{2+} ion, and an excess of cation will also complex weakly with other sites from the 18-crown-6 moiety in the molecule.

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