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Interactions of Cadmium with Yeast Mannans

YASUO ODA,*,[a] SEIJI ICHIDA,[a] SHIGERU AONUMA[a] and TAKESHI SHIBAHARA[b]

Faculty of Pharmaceutical Sciences, Kinki University,[a] Kowakae, Higashi-Osaka 577, Japan and Fukuzaki Factory, Senju Pharmaceutical Co., Ltd.,[b] Fukuzaki-cho, Hyogo 677–22, Japan

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The interactions of cadmium (Cd) ions with mannans from six species of yeast were studied using equilibrium dialysis. Among the six mannans examined, the binding capacity of the mannan of *S. carlsbergensis* was the greatest, at about 146 μmol/g mannan. For the other mannans, the amounts of bound metal ions ranged from 20—46 μmol/g mannan. However, the dissociation constants of Cd-binding to each mannan were very similar, at about 0.5×10^{-3} M. Scatchard plot analysis indicated that the mannans had a single type of binding sites.

Pronase digestion of the mannans decreased their total nitrogen, accompanied with significant losses of Cd-binding. These results suggest that the carbohydrate moieties of the mannans were not positively involved in the Cd-binding of the yeast cell walls.

Keywords——cadmium; yeast mannan; binding study; equilibrium dialysis

Introduction

The toxicity of cadmium (Cd) is well known and the metal binds to various cellular components. Many studies on the interactions of cadmium ion with intracellular materials such as metallothionein and other cadmium-binding proteins in yeasts have been reported.[1] However, there is little information concerning the interaction of cadmium ion with the cell surface or cell walls of yeasts, although cadmium distribution to the cell surface or cell wall of yeasts has been observed.[2]

One important outer component of the yeast cell wall is mannan,[3] a polysaccharide, which is composed mainly of D-mannose and usually contains a small amount of proteins.

In the present study, we examined the interaction of cadmium with various yeast mannans.

Experimental

Equilibrium Dialysis——Equilibrium dialysis was performed in acrylic plastic cells composed of three chambers (Sanko Co., Tokyo) in 50 mM Tris–HCl buffer, pH 7.0, at 4 °C. Dialysis tubing (Union Carbide Corp., New York) was boiled in 1% $NaHCO_3$–0.01% ethylenediaminetetraacetic acid (EDTA) and washed exhaustively with distilled water before use. Cadmium 109 (specific activity, >50 μCi/μg Cd) was purchased from Amersham (Buckinghamshire). Aliquots (500 μl) of cadmium solutions containing the appropriate isotopically labelled metal and the same volume of mannan solution were placed in the outer chambers and the inner chamber, respectively. A 2-mm glass bead was placed in each outer chamber and the cells were gently shaken for 24 h to establish equilibrium. Then, 20 μl aliquots were removed from each chamber and mixed with 5 ml of an aqueous counting scintillant (ACS II, Amersham). The radioactivity in each sample was determined with a Packard Tri-Carb liquid scintillation counter. Binding experiments were carried out in triplicate at eight different cadmium concentrations ranging from 1×10^{-4} to 8×10^{-3} M; the concentration of mannan solution was 4 mg/ml.

In inhibitory experiments, solutions containing cadmium (1×10^{-4} M) and other metals (1×10^{-4}—1×10^{-3} M)

and mannan solutions (4 mg/ml) were added to the outer and inner chambers, respectively, and equilibrium dialysis was carried out for 24 h as described above.

In replacement experiments, inhibitory divalent metals were added to the outer chambers after equilibrium dialyses had been performed for 24 h to attain equilibrium in the cadmium–mannan binding, and the dialyses were continued for a further 24 h.

Preparation of Mannans——Mannans were prepared from the following yeasts: *Saccharomyces cerevisiae* (IFO 1234), *S. carlsbergensis* (IFO 0565), *S. sake* (IFO 0309), *Candida albicans* (IFO 1385), *C. krusei* (IFO 0011), and *C. tropicalis* (IFO 0006). These organisms were obtained from the Institute for Fermentation (Osaka). Each organism was grown for 3—4 d at 30 °C with shaking on the following medium: glucose (20 g), yeast extract (5 g), Bactopeptone (10 g) and water to 1 l (pH 5.4). Cells were harvested by centrifugation and washed with saline three times.

Mannans were isolated by extraction with citrate buffer, pH 7.0, followed by copper complex formation.[4] The total nitrogen of the mannans was determined according to the method of Long and Staples.[5] Optical rotation was determined with a Union PM-101 polarimeter.

Pronase Digestion of Mannans——The mannans of *S. carlsbergensis* (50 mg) and *S. cerevisiae* (50 mg) were digested with pronase (Calbiochem., San Diego) in 50 mM sodium borate buffer, pH 7.9, containing 1 mM $CaCl_2$ at 37 °C. Pronase was added initially in an amount equal to 2% of the weight of the mannans, and again after 48 and 72 h in amounts equal to 1% of the weight of the mannans. After incubation for a total period of 96 h, the digests were concentrated by evaporation and applied to a concanavalin A-Sepharose 4B column (1.5 × 22 cm) at 4 °C. The column was exhaustively washed with 50 mM Tris–HCl buffer, pH 7.0, and the same buffer containing 1 M NaCl, and was then eluted with the same buffer containing 0.2 M glucose (200 ml). The fractions eluted with 0.2 M glucose were pooled and lyophilized after dialysis against distilled water.

Preparation of Affinity Resin of Concanavalin A-Sepharose 4B——Concanavalin A (500 mg; Seikagaku Kogyo, Tokyo) was coupled to 50 ml aliquots of Sepharose 4B (Pharmacia, Uppsala) which had been activated with cyanogen bromide.[6] From the amount of unbound concanavalin A recovered, it was estimated that about 9 mg of concanavalin A was bound per ml of settled gel.

Results and Discussion

In our system, equilibrium was attained within 10 h. Scatchard plots of the binding data of cadmium ions with the yeast mannans are shown in Fig. 1. Among the six mannans examined, the binding capacity of the mannan of *S. carlsbergensis* was the highest, at 146.3 μmol/g mannan. This value is higher than that of the typical protein, bovine serum albumin, reported by Verma *et al.*[7] (*ca.* 54 μmol/g protein was reported for bovine serum albumin). In the other mannans, the amounts of metals bound ranged from 20 to 46 μmol/g mannan. However, all the Scatchard plots for the cadmium–mannan interactions showed a single slope, and the dissociation constants estimated from the slopes of the lines were very

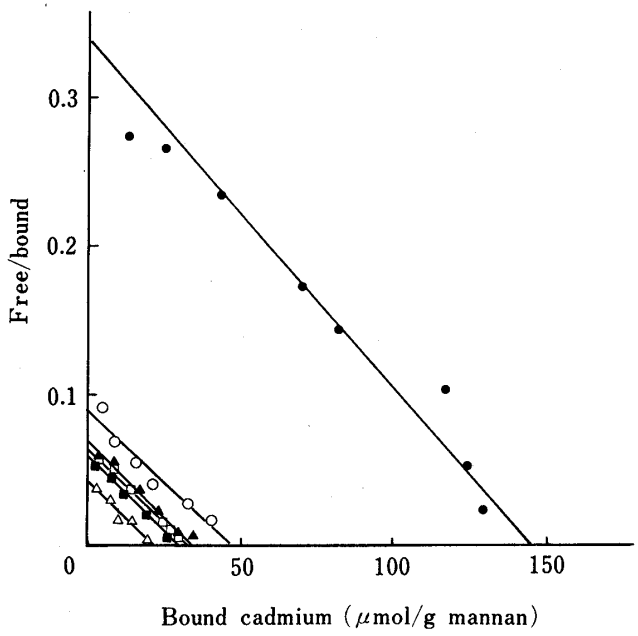

Fig. 1. Scatchard Plot of the Binding of Cadmium to Yeast Mannans

Each point represents the average of two separate experiments. ●, *S. carlsbergensis*; ○, *S. cerevisiae*; □, *S. sake*; △, *C. albicans*; ▲, *C. tropicalis*; ■, *C. utilis*.

Mannans	Maximum binding[a] (μmol/g mannan)	KD[b] (M)
S. carlsbergensis	146.3	0.47×10^{-3}
S. cerevisiae	45.9	0.53×10^{-3}
S. sake	30.0	0.52×10^{-3}
C. albicans	20.5	0.49×10^{-3}
C. tropicalis	35.0	0.50×10^{-3}
C. utilis	28.5	0.50×10^{-3}

a) The maximum binding was estimated from the intercept of the line on the abscissa. *b*) KD was estimated from the slope of the line.

TABLE I. Effects of Various Divalent Ions on Cadmium Binding to *S. carlsbergensis* Mannan

Divalent ion	Concentration (mM)	Binding of cadmium (%)
None		100.0
Mg^{2+}	1.000	64.0
Ca^{2+}	1.000	57.2
Mn^{2+}	1.000	36.2
Hg^{2+}	1.000	91.1
Co^{2+}	1.000	68.0
Ni^{2+}	1.000	63.2
Zn^{2+}	1.000	46.8
Cu^{2+}	1.000	18.3
Cr^{2+}	1.000	0
	0.500	27.5
	0.250	30.0
	0.125	59.1

TABLE II. Chemical Properties of Yeast Mannans

Mannans	Total nitrogen (%)[a]	$[\alpha]^{20}$ [b]
S. carlsbergensis	1.32	+67.0°
S. cerevisiae	1.28	+80.0°
S. sake	1.44	+75.0°
C. albicans	0.84	+62.0°
C. tropicalis	1.12	+29.0°
C. utilis	1.12	+82.0°
Pronase-digested		
S. carlsbergensis	0.92	+69.0°
S. cerevisiae	1.04	+82.0°

a) Determined by the method of Long and Staples.[4] *b*) In water; $c = 1.0$.

similar, at about 0.5×10^{-3} M. These results suggest that the mannans had a single type of binding sites, and the binding sites appeared to be common to the mannans.

The inhibitory effects of various divalent metal ions on the binding of cadmium ions to the mannans of *S. carlsbergensis* were examined. The results are summarized in Table I. Chromic ion was the most potent inhibitor, followed by cupric ion. Mercury ion exhibited little inhibition, although in the cadmium–bovine serum albumin interaction, mercury ion has been reported to be a potent inhibitor besides cupric ion.[7] These findings suggest that the cadmium binding sites were different from those of bovine serum albumin. Similar results were obtained in the replacement experiments. The chemical properties of the mannans prepared in this study are shown in Table II. The mannans contained 0.8—1.5% nitrogen, corresponding to about 5.0—9.4% protein. To examine whether cadmium ions preferentially bound to carbohydrate moiety or protein moiety in the mannans, the mannans of *S. carlsbergensis* and *S. cerevisiae* were digested with pronase. The total nitrogen of these mannans was significantly decreased by the pronase digestion (Table II). Furthermore, this treatment markedly reduced the Cd-binding capacities of these mannans: the maximum binding of the pronase-digested mannans was below 10 μmol/g mannan (data not shown). These findings indicate that the large binding capacity of the mannan prepared from *S. carlsbergensis* was due to its protein moiety, while the carbohydrate moieties of the mannans

appeared not to be positively involved in the cadmium-binding of the yeast cell walls.

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