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Highlights

- Cadmium sorption to plasma membrane vesicles is impeded by Cu.
- Both permeation and association of Cd to plasma membrane vesicles occurred without energy source.
- Association of Cu to plasma membrane vesicles is quicker than Cd sorption.

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Title:

Cadmium sorption to plasma membrane isolated from barley roots is impeded by copper association onto membranes

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Abbreviations:
ATP, adenosine 5'-triphosphate; EDTA, ethylenediamine-*N,N,N',N'*-tetraacetic acid, disodium salt, dehydrate; MES, 2-morpholinoethanesulfonic acid monohydrate; MOPS, 3-(*N*-morpholino) propanesulfonic acid; NADH, nicotinamide adenine dinucleotide; PM, plasma membrane; UDP, uridine 5'-diphosphate.

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Abstract

The present study was designed to examine the effect of copper (Cu) on sorption of cadmium (Cd) to plasma membrane (PM) preparations as one of the models of competition between metals on root PM. Plasma membrane preparations were obtained from roots of barley (*Hordeum vulgare* L. cv. Minorimugi) and 50 μM $CdSO_4$ with or without 50 μM $CuSO_4$ were added to the PM suspensions. The sorption of Cd to PM vesicles increased with time within 15 min while Cu sorption to the PM occurred instantaneously. The sorption of Cd to PM vesicles was inactivated immediately after the addition of Cu into the reaction mixture. Results indicate that Cu association to PM vesicles occurs quicker than Cd, and, as a result, impedes the access of Cd to PM vesicles. The present study suggests that the competition between Cd and other minerals at root PM of plants can be demonstrated by employing isolated PM preparations. We consider that the difference in the capacity among some minerals for impeding Cd sorption to PM may also be characterized by investigating the interaction between Cd and other minerals on the PM.

Key words:

barley, cadmium, copper, plasma membrane, root, sorption.

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1. Introduction

Several types of mineral sorption experiments employing PM preparations of plant tissue have been conducted [1-4]. Isolated PM preparations consist of the lipid bilayer with PM localized proteins, such as ATPase or transporters, and form into vesicles [1,5]. Sorption of minerals to PM preparations can be determined by measuring the changes in the mineral content of membrane preparations. It is also known that minerals associate onto the surface of the PM or permeate across the PM, when isolated PM is exposed to minerals in solution. For example, iron (Fe) association onto PM vesicles isolated from maize roots [2] and proton/copper (Cu) and proton/cadmium (Cd) antiport across PM vesicles isolated from cucumber roots were reported [4].

In the most of mineral sorption experiments, PM preparations are usually incubated with a single targeted mineral under various conditions [1-4], while combined applications of two or more targeted minerals are few. We suppose that isolated PM vesicles are useful for characterization of the competition among some minerals in their sorption to the PM. In the present study, we focused on the competition between Cd and other minerals at the PM of plant roots.

We chose Cd for technical and biological reasons. Technically, Cd is easy to detect without contamination in experiments from the environment. Biologically, Cd, one of the most toxic trace elements, is easily taken up by plants [6,7] and the concentration of Cd in plants is affected by the change in the amount of a certain mineral in the rhizosphere [8-12]. A case in point for involving the competition between Cd and a certain mineral is transport of various metals across membranes via ZIP family [13-15]. While, there is a report suggesting that the absorption of Cd by maize root cells is a non-specific process [16]; it is possible that a non-specific process for Cd transport across root PM is applicable for all plants because Cd uptake by plants is affected by

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not only Zn and Fe, but also Ca, Mn, and Mg [8-11,17]. In addition, it is well known that many proteins can mediate Cd transport [13-15,18-21]. Thus, we consider that competition between Cd and a variety of minerals on isolated PM vesicles would be observed.

Based on the information described above, the present study targeted the competition between Cd and Cu sorption to PM vesicles isolated from barley roots. Copper was chosen as a model of the competitor for Cd, because it is known that: 1) roots of plants are the sites of preferential Cu accumulation [22]; 2) the affinity of Cu to the PM of plants is higher than that of Zn [3]; 3) Cd absorption by excised barley roots is decreased by the existence of Cu in solutions [23]; and 4) Cd uptake by maize and wheat is decreased when Cu concentration increases in the medium [24]. In addition, we preliminarily confirmed that Cd uptake by barley plants was decreased when Cu concentration increased in the medium (data not shown). Therefore, the present study was conducted to investigate the inhibitory effect of Cu on Cd sorption to PM preparations and to characterize the sorption (association/permeation) of these metals to PM preparations. Furthermore, we suppose that not only active transport but also passive permeation of Cd across PM should be also taken into account as one of the non-specific processes because non-specific process mediated Cd absorption in plants is suggested [16]. Studies related to energy currency and Cd transport across PM or the effect of Cd on PM ATPase have been well documented [25-28], while there is little information concerning passive Cd transport across root PM. Thus, we attempted to indicate passive Cd permeation across PM.

2. Materials and Methods

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112 *2.1. Preparation of seedlings*

113

114 Seedlings of barley (*Hordeum vulgare* L. cv. Minorimugi) were grown
115 hydroponically according to Kudo *et al.* [29] with a slight modification. Briefly,
116 germinated seeds of barley were grown for 6 days on 1 mM $CaCl_2$, and transferred to
117 1/5-strength modified Hoagland and Arnon No.2 medium [30] in 15-L buckets and
118 grown for 7 days in an artificially lighted growth cabinet Koitotron (KG-206HL, Koito
119 Industries ltd., Tokyo, Japan) with a day (17 °C, 14 h, 280 μmol m^{-2} s^{-1}) and night
120 (10 °C, 10 h) regime controlled by digital program controller (KP1000; Chino corp.,
121 Tokyo, Japan). Then, the seedlings were transplanted to 1/2-strength modified Hoagland
122 and Arnon No.2 medium [30] with continuous aeration in 15-L buckets, and were grown
123 for an additional 16 days. The medium was renewed every 4 days.

124

125 *2.2. Isolation of plasma membrane of barley roots*

126

127 Plasma membrane was isolated from root tissue (50 g fresh weight) of barley
128 plants using an aqueous two-phase partition method [5]. The concentration of both
129 polyethylene glycol 3350 and dextran T500 in the two-phase system was modified to
130 6.0% (w/w). After the partition, the upper phase was collected and diluted to 5-6 fold
131 with a buffer solution consisting of 0.3 M sorbitol and 10 mM MOPS-KOH (pH 7.3),
132 and centrifuged at 303,000 *g* for 50 min. The pellet was suspended in a buffer solution
133 consisting of 0.3 M sorbitol and 10 mM MES-KOH (pH 6.0) and again centrifuged. The
134 amount of isolated PM was calculated as protein content determined by the Bradford
135 method [31]. In the present study, PM preparation obtained from 50 g (fresh weight) of

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root tissue was equivalent to 500-800 µg protein. The purity of PM preparations was estimated on the base of marker enzyme activities (vanadate-sensitive ATPase for the PM, nitrate-sensitive ATPase for the tonoplast, Triton X-100-stimulated UDPase for Golgi bodies, cytochrome *c* oxidase for mitochondria, and NADH cytochrome *c* reductase for endoplasmic reticulum) [5,32]. Only vanadate-sensitive ATPase activity (88% inhibited by vanadate) was detected in the PM fraction, suggesting little contamination of other organelles. The PM preparations were frozen with liquid nitrogen and kept at −80 ºC until use except for enzyme assay.

2.3. Application and determination of Cu and Cd to PM vesicles isolated from barley roots and element analysis

Aliquots of PM preparations (20-30 µg protein) were suspended in a buffer solution consisting of 0.3 M sorbitol and 10 mM MES-KOH (pH 6.0) (final volume 3 ml), and incubated for 5 min at 17ºC. Then, treatment with $CdSO_4$ and $CuSO_4$ were conducted as described in the following paragraphs. The final concentration of both metals was 50 µM, because our preliminary experiments confirmed that this concentration was expedient to observe significant sorption of these metals to PM vesicles. After incubation, the mixtures were filtered through a nitrocellulose filter (ADVANTEC, polymer, cellulose nitrate; pore size, 0.45 µm; diameter, 25 mm) with suction. The nitrocellulose filters holding PM vesicles were digested with nitric acid (Kanto Chemical Co., Tokyo, Japan) at 140°C. The amounts of Cd and Cu in digested solutions were analyzed by flameless atomic absorption spectroscopy (Atomic Absorption Spectrophotometer 180-30 equipped with a Graphite Atomizer GA-2B, Hitachi Ltd., Tokyo, Japan). The contents of Cd and Cu of PM vesicles were calculated

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as nmol metal mg^{-1} protein.

2.4. Various treatments of PM vesicles in the presence of Cd and/or Cu

2.4.1. Time course of the sorption of Cd and Cu

Cadmium with or without Cu was added into PM preparations. After being mixed well, the mixtures were incubated for 0, 5, 10, or 15 min at 17°C. For 0 min treatment, the mixtures were filtered immediately after the mixing.

2.4.2. Application of Cd and Cu to PM vesicles in permuted order

Plasma membrane vesicles were exposed to Cd for 20 min at 17°C in combination with Cu in different ways: 1) Cu was added and incubated for 10 min, and then Cd was added (pre-application of Cu); 2) a mixed solution of Cd and Cu was added (co-application of Cu with Cd); and 3) Cd was added and incubated for 10 min, and then Cu was added (post-application of Cu). Plasma membrane vesicles incubated with Cd for 20 min without Cu were taken as control. After incubation, the mixtures were filtered through a nitrocellulose filter as described above.

2.4.3. Treatment of PM vesicles with EDTA after the incubation with Cd and/or Cu

Plasma membrane vesicles were incubated with Cd and/or Cu for 10 min at 17°C, and then filtered through a nitrocellulose filter. This nitrocellulose filter holding PM was referred to as ‘filter A’. For the treatment of EDTA, the filter A was soaked in a

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buffer solution consisting of 0.3 M sorbitol, 10 mM MES-KOH (pH 6.0), and 0 or 50 µM EDTA (Dojindo Laboratories, Kumamoto, Japan) and incubated for 10 min at 17ºC. After the incubation, the filter A was collected and the solution in which the filter A had been soaked was filtered through another nitrocellulose filter, 'filter B'. Then, the filters A and B together were digested with nitric acid as described above. Samples incubated with Cd or Cu without EDTA treatment were taken as control in each experiment.

2.4.4. Application of osmotic shock to PM vesicles applied with Cd

Plasma membrane vesicles were incubated with Cd and filtered as described in the previous paragraph, and the buffer solutions were displaced with a hypotonic solution consisting of 10 mM MES-KOH (pH 6.0). Plasma membrane vesicles treated with an isotonic solution consisting of 0.3 M sorbitol and 10 mM MES-KOH (pH 6.0) were taken as control.

2.4.5. Treatment of PM vesicles with Triton X-100 after the incubation with Cd

Plasma membrane vesicles were incubated with Cd 10 min at 17°C. Triton X-100 (Nacalai Tesque, Inc., Kyoto, Japan) was then added into the reaction mixtures and incubated for 5 min. The final Triton X-100 concentrations were 0 (control), 0.01, 0.02, 0.03, 0.04, and 0.05% (v/v).

In parallel, ATPase activity of Triton X-100 treated PM vesicles were determined as an indicator of permeability or 'leakiness' of PM vesicles [33] in the presence of Triton X-100 according to the method of Uemura and Yoshida [5].

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211 *2.5 Statistical analysis*

212

213 The experiments employing PM vesicles were conducted in triplicates. All the data were
214 subjected to an ANOVA [34] using the computer 'NEC SX-9/8B' in the Tsukuba Office,
215 Agriculture, Forestry and Fisheries Research Council Secretariat, Japan. Differences
216 between means were evaluated by using the Ryan-Einot-Gabriel-Welsch multiple range
217 test ($P < 0.05$).

218

219

220 **3. Results and discussion**

221 Highly purified PM preparations were successfully obtained in the present
222 study (see section 2.2.). The latency of ATPase activity of PM preparations (Fig. 5b)
223 shows that the orientation of PM vesicles was primarily right-side-out [35,36].

224 The sorption of Cd to PM vesicles increased with incubation time over the
225 range of 0 to 15 min (Fig. 1a). Even at 0 min incubation (*i.e.*, PM vesicles were filtered
226 immediately after Cd was added into the reaction mixture), Cd was detected in PM
227 suspension. However, the initial level of Cd sorption to PM vesicles was lowered when
228 Cu was added into the PM suspension simultaneously with Cd addition (Fig. 1a).
229 Further, Cd sorption to PM vesicles did not increase with time when Cu was in the
230 reaction mixture (Fig. 1a), indicating that Cd sorption to PM vesicles was lowered in the
231 presence of Cu in PM suspension. When Cd and Cu were simultaneously added, the
232 level of Cu sorption to PM vesicles was higher than that of Cd, and Cu sorption to PM
233 vesicles occurred instantaneously (Fig. 1b). This high and quick sorption of Cu to PM
234 vesicles was considered to be due to the high sorption affinity of Cu to the PM [3].
235 These results suggest that Cu sorption to PM vesicles occurs predominantly to Cd, and

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high Cu sorption to PM vesicles inactivates Cd sorption when both Cd and Cu are present in the suspension of PM vesicles.

The effect of Cu on Cd sorption to PM vesicles was further examined with different approaches, *i.e.*, the procedures of the pre-, co-, and post-application of Cu (see section 2.3.2.), in order to determine how Cu decreased Cd sorption to the PM. We found that Cd sorption to PM vesicles was lowered to 17%, 26%, and 64% of control by the pre-, co-, and post-application of Cu, respectively (Fig. 2a). In addition, there was no significant difference in Cu sorption to PM vesicles among the three procedures (Fig. 2b).

In the procedure of the pre-application of Cu, Cu sorbed to PM vesicles before Cd was added into PM suspension, and thus, Cd sorption did not increase. The condition of co-application of Cd with Cu was the same as the experiment of the time course shown in Fig. 1. Under this condition, Cu sorbed to PM vesicles predominantly to Cd and inactivated the Cd sorption.

In the case of post-application of Cu, Cd sorption to PM vesicles occurred before Cu was added into PM suspension. Hence, Cd sorption to PM vesicles was higher than that in the pre- and co-applications. It is noteworthy that Cd sorption to PM vesicles was lower than that of control in this procedure (Fig. 2a). As shown in Fig. 1, Cd sorption linearly increased with incubation time until 15 min, and Cu sorption was saturated soon after Cu addition. Based on these results, we consider that under the condition of post-application of Cu, Cd sorption to PM vesicles is linearly increased until Cu is added into the suspension, and after Cu addition Cu sorbs to PM vesicles and inactivates Cd sorption to PM immediately.

Alternatively, it is possible that Cd is displaced by Cu on PM vesicles. If Cd was displaced by Cu, Cd sorption would decrease to the same levels regardless of the

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261 order of Cu addition. However, this is not the case because Cd sorption to PM vesicles
262 was different among three procedures. These results suggest that the competition of Cd
263 and Cu at PM vesicles is not antagonistic under the current conditions and that the
264 sorption of Cu to PM impedes the sorption of Cd to the PM, *i.e.* Cu can act as a barrier
265 for impeding Cd sorption to root PM.

266 Next, some membrane perturbation experiments were conducted to estimate
267 the distribution of metals between membrane surface (associated onto the surface of
268 membrane vesicles) and vesicle interior (located inside membrane vesicles). We
269 attempted to apply EDTA, osmotic shock, and Triton X-100 to the PM vesicles
270 incubated with Cd and/or Cu.

271 When PM vesicles were treated with EDTA after the sole incubation Cd or Cu,
272 the retention of Cd in PM vesicles was decreased to about a half of control (Fig. 3a),
273 while that of Cu was decreased to about one-fifth of control (Fig. 3b). The decrease of
274 retention of Cd as well as Cu by the treatment of EDTA (Fig. 3) suggests the association
275 of these metals onto PM vesicles after the incubation. When PM vesicles were treated
276 with EDTA after being incubated with both Cd and Cu simultaneously, retention of Cd
277 was not altered significantly (Fig. 3a) but the retention of Cu was remarkably decreased
278 (Fig. 3b). This result suggests that only a small amount of Cd is associated onto the
279 outer-surface of PM vesicles, although the level of Cu association is about four-fifths of
280 sorbed Cu to PM vesicles. Therefore, we consider that Cu association onto the PM
281 inhibits Cd association considerably when PM vesicles are incubated with Cd and Cu
282 simultaneously.

283 Since the Cu retention in PM vesicles was remarkably decreased by EDTA
284 (Fig. 3b), we consider that Cu sorption to PM vesicles is mainly due to association.
285 Association of both metals to PM was indicated as above, but, in the case of Cd we

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286 consider that permeation into PM vesicles also occurred, because, Cd was still retained
287 in PM vesicles (about half of control) after the incubation of Cd treated PM vesicles
288 with EDTA (Fig. 3a). The affinity of EDTA with Cd and Cu [37] might explain the
289 difference in the amount of the retention of Cd or Cu with PM vesicles after the
290 treatment with EDTA (Fig. 3a and b). Another possibility is that the Cd retention in PM
291 vesicles after the treatment with EDTA may show the permeation of Cd into PM
292 vesicles.

293 Osmotic shock decreased Cd retention with PM vesicles to about half that of
294 control (Fig. 4). It was presumed that some proportion of PM vesicles might be
295 fragmented by osmotic shock and thus not be collected on the nitrocellulose filter.
296 However, we confirmed in a separate experiment that protein content in the filtrate did
297 not increase after the application of osmotic shock (data not shown), suggesting that
298 there was no significant loss of PM vesicles on the filter. Therefore, the decreased
299 retention of Cd with PM vesicles after the application of osmotic shock (Fig. 4) is due to
300 the release of Cd from the capsular space of PM vesicle between short-time-burst and
301 re-formation of PM vesicles, suggesting that Cd is permeated into the PM vesicles after
302 the incubation.

303 Next, PM preparations incubated with Cd were treated with 0.01-0.05% (v/v)
304 of Triton X-100. We found a remarkable decrease of retention of Cd at 0.02% of Triton
305 X-100 in the reaction mixture (Fig. 5a). It is well known that detergents such as Triton
306 X-100 induce micellar solubilisation of membrane vesicles in a
307 concentration-dependent manner [38], and are often used to increase the permeability of
308 membranes. We confirmed that the stimulation of latent ATPase activity, considered as
309 an indicator for permeability of PM vesicles [32,33,35], occurred at 0.02% of Triton
310 X-100 in the reaction mixture (Fig. 5b). This result suggests that the sealedness of PM

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311 vesicles in the present study is lost at 0.02% of Triton X-100. Gries and Wagner [39]
312 reported that retention of metal elements located into or associated onto membrane
313 vesicles was steeply or gradually decreased respectively by increasing the Triton X-100
314 concentration in the reaction mixture. Therefore, we consider that the remarkable
315 decrease in Cd retention with PM vesicles at 0.02% of Triton X-100 (Fig. 5a) is likely
316 due to Cd leakage from the capsular space of PM vesicles, again suggesting that Cd is
317 located (or permeated) in the capsular space of PM vesicles after the incubation.
318 Based on these results (Figs. 3a, 4, and 5), we consider that both the
319 association and the permeation of Cd to PM occurred when PM vesicles were incubated
320 with Cd. We speculate that about a half of Cd that sorbs to PM vesicles is due to Cd
321 permeation into PM vesicles. It is noteworthy that the results in the present study (Figs.
322 3a, 4, and 5) suggested the occurrence of permeation of Cd into capsular space of PM in
323 the absence of energy source (*e.g.* ATP or secondary driving force) Thus, the Cd
324 sorption observed in the present study is an energy-independent sorption of Cd to PM
325 vesicles, *i.e.*, passive permeation and chemical association. We infer that Cd permeates
326 through a kind of passive pathway such as channels which are permeable to a wide
327 variety of cations similar to *rca* channel observed in wheat root [40-42]. The fact that
328 Cd permeates through PM without an artificial driving force might suggest that Cd
329 could enter into root cells even when active ion transport does not work. This might also
330 support the plant uptake of Cd into root cells in a non-specific process [16].
331 The sorption of Cu was not affected by Cd (Fig. 3b). This is probably because
332 Cu sorption is quick (Fig. 1b) and the affinity of Cu with PM is high [3]. Combined
333 with membrane perturbation experiments (Figs. 3, 4, and 5), we propose that Cu
334 association onto the metal sorption site on the PM vesicles occurs quicker than Cd, and
335 inactivates both the association and permeation of Cd by impeding the access of Cd to

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PM vesicles. Although we attempted to determine Cd permeation into PM vesicles in the presence of Cu, the level of Cd sorption to PM vesicles in the presence of Cu was too low to observe the effect of osmotic shock or Triton X-100 on retention of Cd with PM (data not shown). We hypothesize that it may be possible that Cd permeation as well as association to PM vesicles might be decreased by Cu because of the impeded access of Cd to PM in the presence of Cu.

In conclusion, Cu associated to PM vesicles impeded the Cd sorption. It is possible that the Cu sorbed to the PM forms a thin layer around PM vesicles, and impedes the access of Cd to the PM. If it occurs *in situ*, it might contribute to a decrease in Cd transport across root PM. The present study suggests that the competition between Cd and other minerals at root PM can be directly proved by employing isolated root PM of plants. The difference in the capacity among some minerals for impeding Cd sorption to PM may be also characterized by investigating the interaction between Cd and other minerals on the PM.

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Figures

Fig. 1 Sorption of (a) cadmium (Cd) and (b) copper (Cu) to plasma membrane (PM) vesicles isolated from barley roots. The suspension of PM vesicles (20-30 µg protein) was applied with 50 µmol L^{-1} $CdSO_4$ with or without 50 µmol L^{-1} $CuSO_4$ (final volume 3 mL) for 0, 5, 10, or 15 min at 17ºC. The closed symbol (■) is for PM vesicles incubated with single application of Cd. The open symbols (□ and ○) are for PM vesicles incubated with combined application of Cd and Cu. Each value is the means ± SD (n = 3). Different letters at the top of each symbol indicate significant differences ($p < 0.05$) according to the Ryan-Einot-Gabriel-Welsch multiple range test.

Fig. 2 Sorption of (a) cadmium (Cd) and (b) copper (Cu) to plasma membrane (PM) vesicles isolated from barley roots. The suspension of PM vesicles (20-30 µg protein) was incubated with 50 µmol L^{-1} $CuSO_4$ and 50 µmol L^{-1} $CdSO_4$ (final volume 3 mL) in the following ways: 1) Cd was added to the suspension (control; no application of Cu); 2) Cu was added to the suspension and incubated for 10 min before Cd addition (pre-application of Cu); 3) Cu and Cd was simultaneously added to the suspension as a mixed solution (co-application of Cu with Cd); and 4) Cu was added to the suspension at 10 min after Cd addition and incubated for additional 10 min (post-application of Cu). Reaction mixture was incubated for 20 min at 17 °C after the addition of Cd to the suspension in all 4 treatments. Each value is the means ± SD (n = 3). Different letters at the top of each bar indicate significant differences ($p < 0.05$) according to the Ryan-Einot-Gabriel-Welsch multiple range test. ND = not detected.

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Fig. 3 Effect of EDTA on sorption of (a) cadmium (Cd) and (b) copper (Cu) to plasma membrane (PM) vesicles isolated from barley roots. The suspension of PM vesicles (20-30 μg protein) was incubated with 50 μmol L^{-1} $CdSO_4$ and/or 50 μmol L^{-1} $CuSO_4$ (final volume 3 mL) for 10 min at 17ºC. The reaction mixture was filtered through a nitrocellulose filter, and the filter holding PM vesicles was soaked in 30 mL of 0 or 50 μmol L^{-1} EDTA and incubated for 10 min at 17ºC. After the incubation, the filter was collected and the solution in which the filter had been soaked was filtered through another nitrocellulose filter. Then, these two filters were analyzed together. Each value is the mean of the percent of control ± SD (n = 3). Different letters at the top of each bar indicate significant differences ($p < 0.05$) according to the Ryan-Einot-Gabriel-Welsch multiple range test.

Fig. 4 Effect of osmotic shock on cadmium (Cd) sorption to PM vesicles. The suspension of PM vesicles (20-30 μg protein) was incubated with 50 μmol L^{-1} $CdSO_4$ (final volume 3 mL) for 10 min at 17ºC. The reaction mixture was filtered through a nitrocellulose filter, and the filter holding PM vesicles was soaked in 30 mL of a suspending buffer (isotonic solution) consisting of 0.3 mol L^{-1} sorbitol and 10 mmol L^{-1} MES-KOH (pH 6.0) (control) or a hypotonic solution consisting of 10 mmol L^{-1} MES-KOH (pH 6.0). After the incubation, the filter was collected and the solution in which the filter had been soaked was filtered through another nitrocellulose filter. Then, these two filters were analyzed together. Different letters at the top of each bar indicate significant differences ($p < 0.05$) according to the Ryan-Einot-Gabriel-Welsch multiple range test.

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536 **Fig. 5** Permeability of plasma membrane (PM) vesicles as affected by Triton X-100
537 treatment. (a) Retention of cadmium (Cd) with plasma membrane (PM) vesicles after an
538 incubation with Triton X-100. The suspension of PM vesicles (20-30 μg protein) was
539 incubated with 50 μmol L^{-1} of $CdSO_4$ (final volume 3 mL) for 10 min at 17ºC. Then,
540 0−0.05% (v/v) of Triton X-100 was added and incubated for an additional 5 min and
541 filtered through a nitrocellulose filter. The content of Cd on the filter was measured by
542 flameless atomic absorption spectroscopy. (b) Triton X-100 concentration dependent
543 ATPase activity. Plasma membrane ATPase activity was measured in the presence of
544 0−0.05% (v/v) of Triton X-100.
545 Each value is the mean of the percent of control ± SD (n = 3). Different letters at the
546 top of each bar indicate significant differences ($P < 0.05$) according to the
547 Ryan-Einot-Gabriel-Welsch multiple range test.

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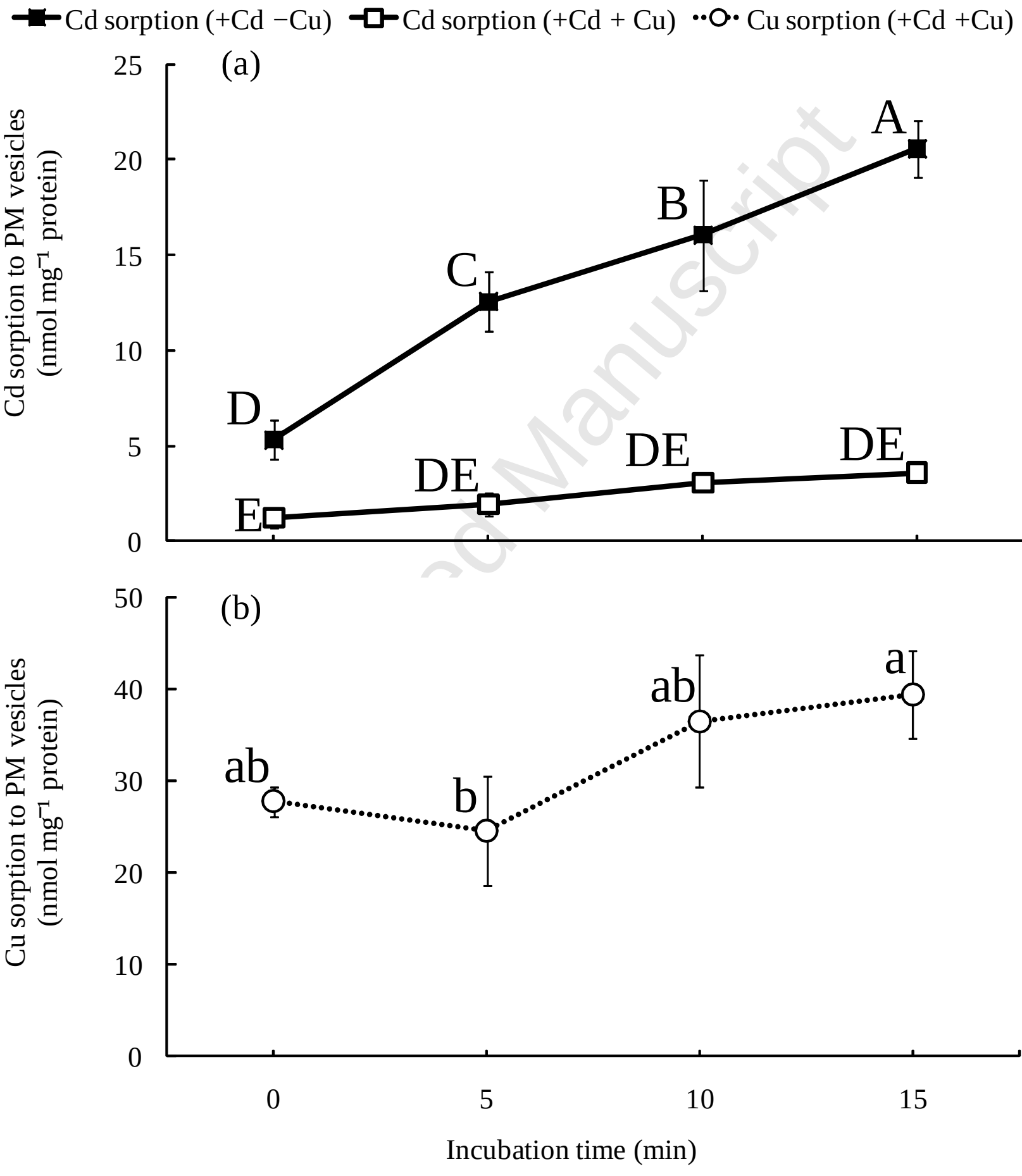

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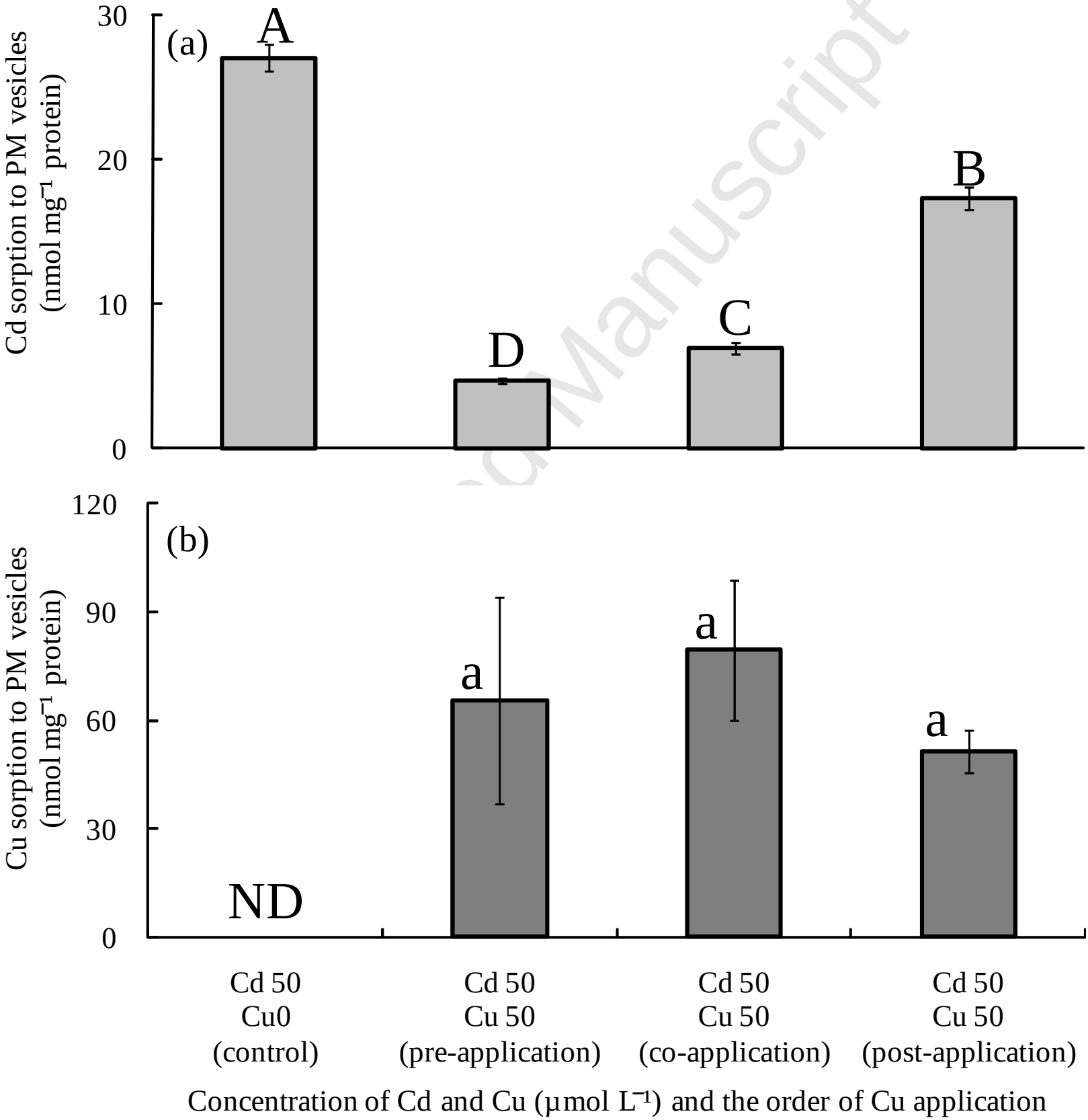

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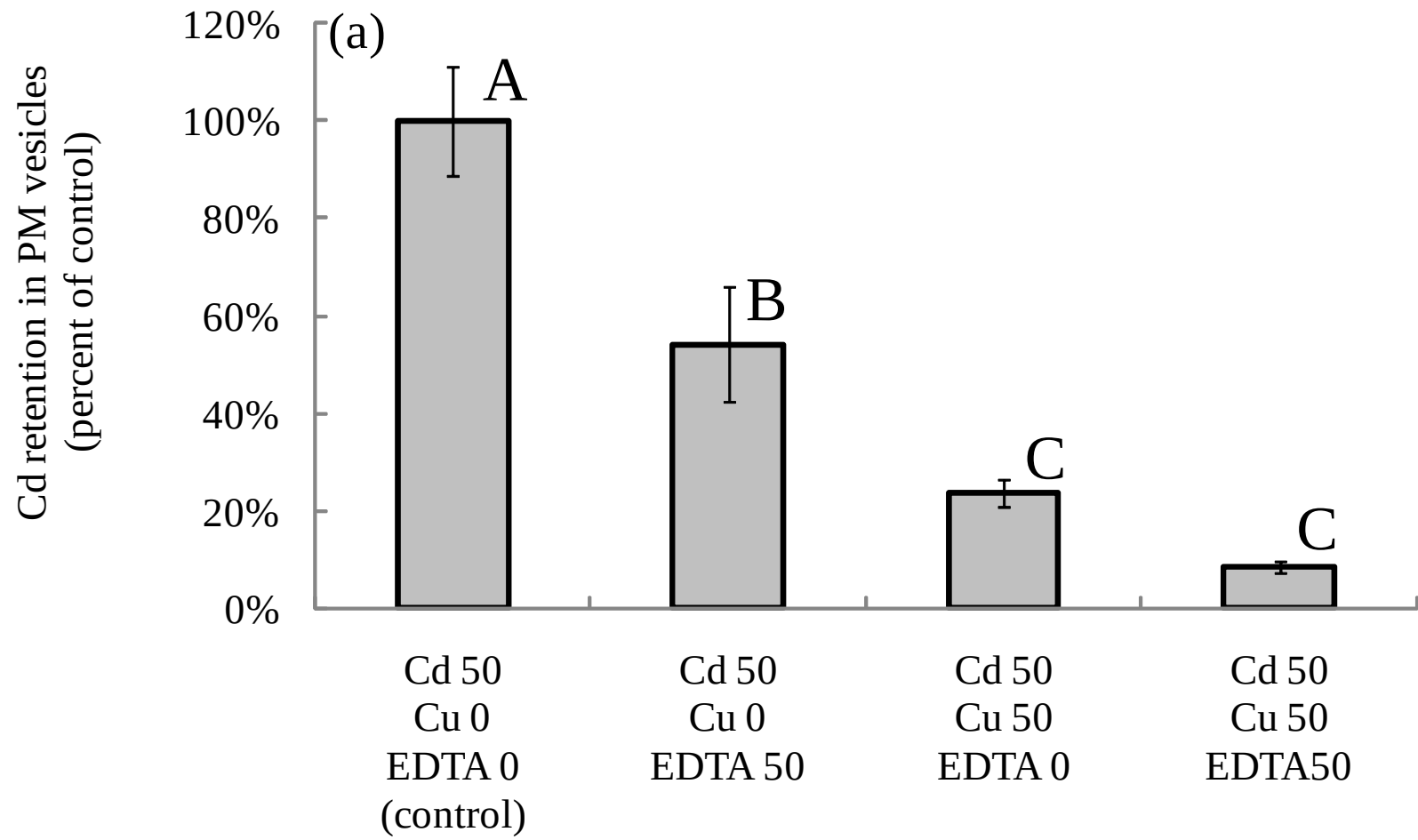

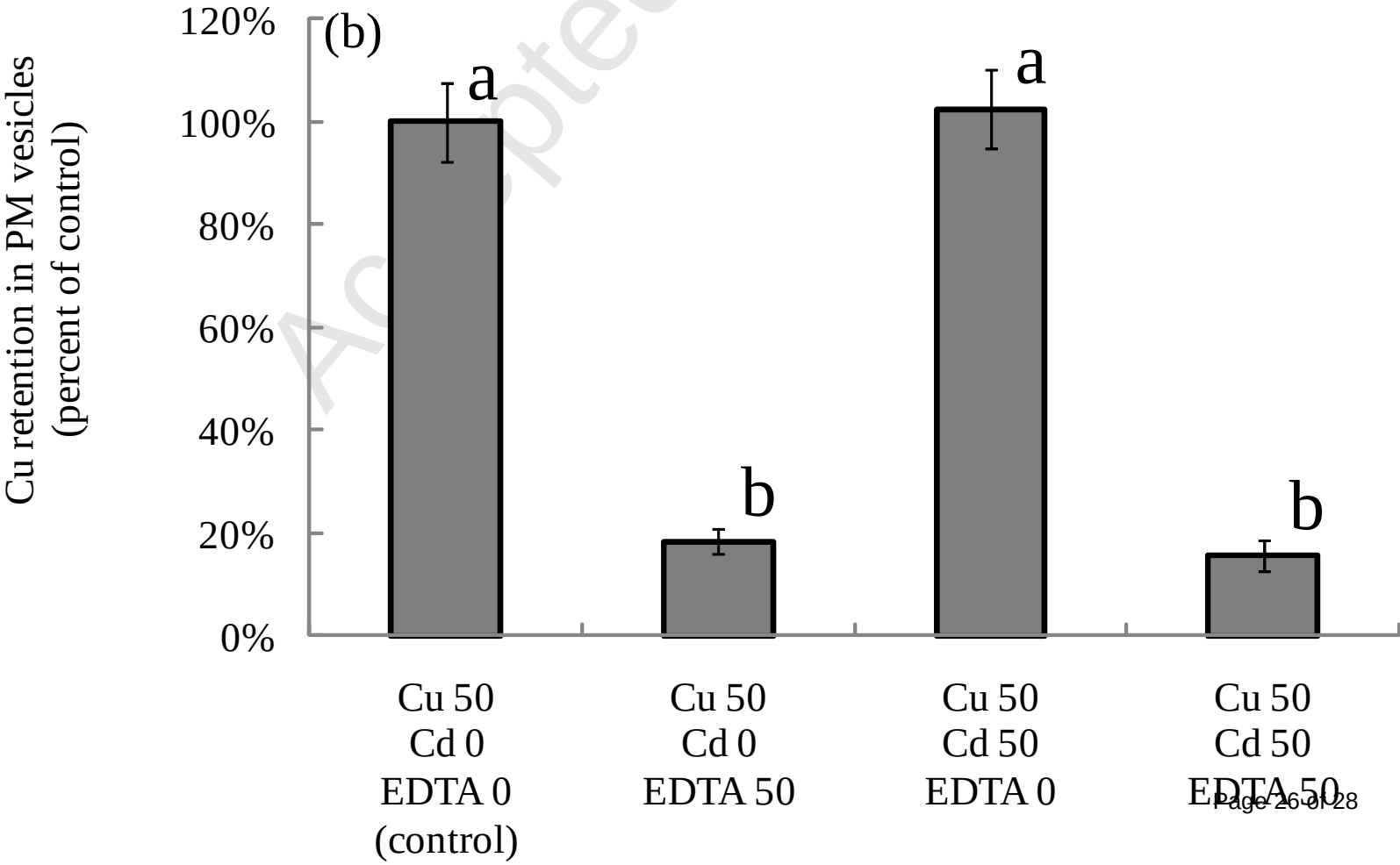

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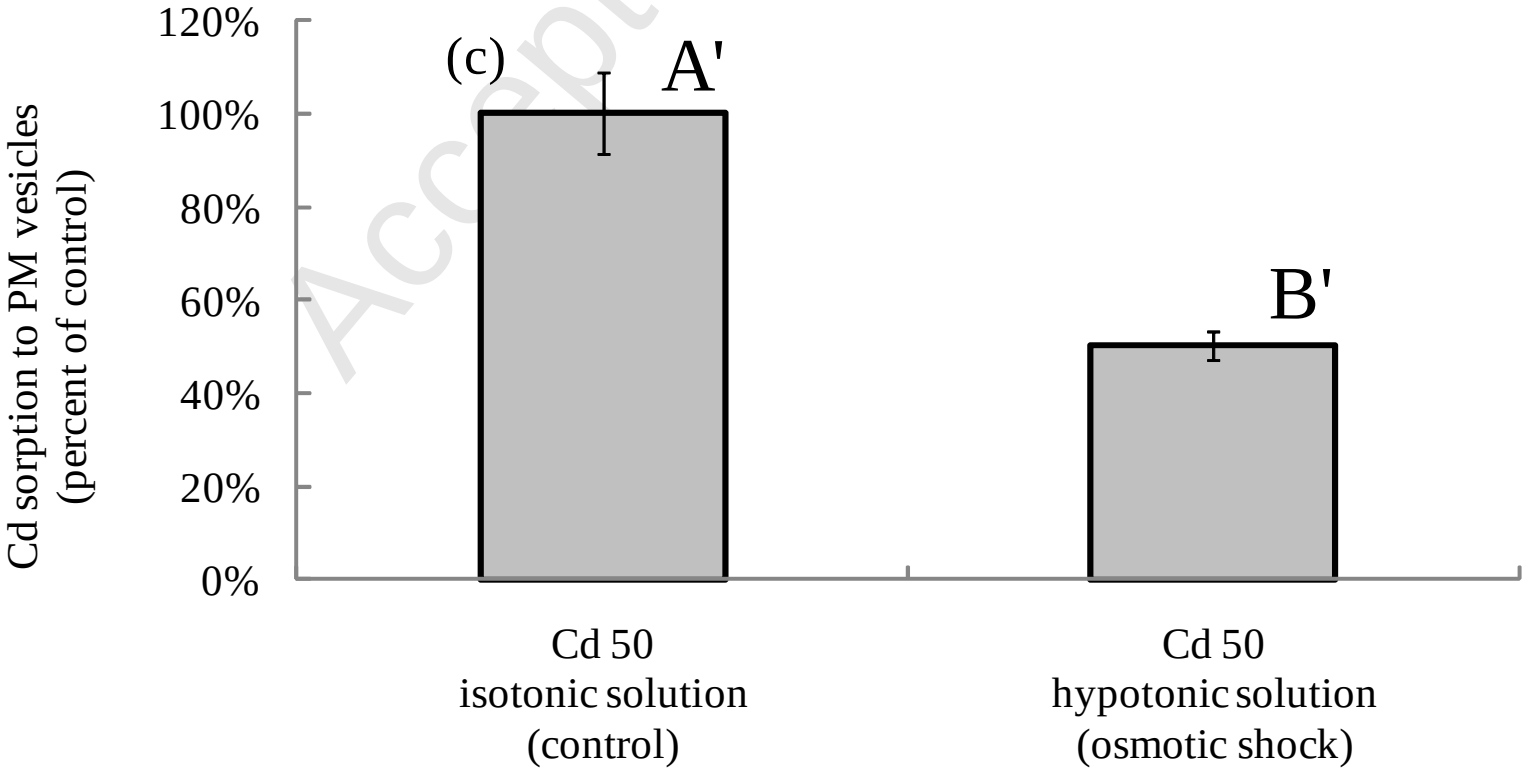

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