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Effects of heavy metal ions on cell adhesion of *Dictyostelium discoideum*

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Synopsis

We studied the effects of heavy metal ions on cell contact of *Dictyostelium discoideum*, EDTA-resistant cell contact, which appears in an aggregation-competent stage after starvation begins. Heavy metal ions were added after starvation began, and their effects on EDTA-resistant cell contact were investigated for the cells at the aggregation-competent stage. As a result, EDTA-resistant cell contact was significantly affected in cells treated with heavy metal ions: Hg^{2+}, Cd^{2+}, Pb^{2+} and Cu^{2+} of more than 10^{-16} M.

Introduction

Dictyostelium discoideum has been used as a suitable organism to clarify the molecular mechanism in cell-to-cell adhesion in multicellular formation, differentiation and signal transduction pathways. It is known that *D. discoideum* cells retain two types of cell adhesion: EDTA-sensitive and EDTA-resistant cell contacts. However, a little information is available on the roles of EDTA in the assay system of cell adhesion[1),2),3)]. Beug *et al.*[1)] have reported that EDTA was replaced by EGTA in an assay system of cell contact. According to their results, Ca^{2+} ions seem to be functional in EDTA-sensitive cell contact but not in EDTA-resistant cell contact. It remains to be studied whether the other ions are replaced by Ca^{2+} ions in EDTA-sensitive cell contact and whether no other ions besides Ca^{2+} ions are functional in EDTA-resistant cell contact. Recently, some researchers have shown some interesting effects of heavy metal ions on biological activities[4),5),6)], although little is known about how heavy metal ions cause such effects on biological activities. This study aims at elucidating the effect of heavy metal ions on EDTA-resistant cell contact.

Material and Methods

Cell culture and development. Cells of *D. discoideum* strain AX2-214 were grown axenically while shaking at 150 rpm at 22℃, harvested at a density of 5 x 10^6 cells/ml, washed free of nutrient medium and adjusted to 1 x 10^7 cells/ml in 17 mM Soerensen phosphate buffer pH 6.1 (standard buffer). Heavy metal ions at an appropriate concentration were added to cell suspension immediately after starvation began. Cells were agitated on a rotatory shaker at 150 rpm for 8 h in the presence of heavy metal ions.

Gel electrophoresis and immunoblotting. Sodium dodecyl sulfate (SDS)-polyacrylamide electrophoresis in 10% gel was carried out using the standard method of Laemmli[7)]. To obtain particulate fractions, cells were frozen, thawed and centrifuged at 15,000 rpm for 20 min. The particulate fractions were dissolved in sample buffer for SDS-polyacrylamide gel electrophoresis. The

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method of Towbin *et al.*[8] was used for transfer to nitrocellulose (BA85, Schleicher & Schuell). The nitrocellulose sheets were incubated with polyclonal antibodies against csA with a molecular weight of 80 kDa (80-kDa csA), which appears at the aggregation-competent stage and is involved in EDTA-resistant cell contact, as described in a previous paper[9]. Staining was performed with goat anti-rabbit IgG conjugated with horseradish peroxidase (E.Y Labs). Reactivity with concanavalin A was determined by incubating the nitrocellulose filters with horseradish-peroxidase conjugated concanavalin A (E.Y Labs).

Pulse experiments and particulate fractions. Cells of 1 x 10^7/ml were incubated for 8 h with 0.5 mCi/ml of [^{35}S]-methionine (ARS-110, American Radiolabeled Chemicals, Inc., USA) in one milliliter of standard buffer, and the labeled cells were washed in standard buffer. The 100 μl of suspended cells was centrifuged at 1,000 x g for 5 min and the cell pellets were dissolved in Clear-sol I (Nacalai tesque) for determination of radioactivity in a liquid scintillation counter. To obtain particulate fractions, cells were frozen, thawed, and centrifuged in a microcentrifuge. The supernatant was supplemented with 9 volumes of acetone, and precipitates and particulate fractions were dissolved in sample buffer for SDS-polyacrylamide electrophoresis.

Assay of cell agglutination. Cell agglutination was measured by the method of Beug *et al.* [1]. Cells were washed with standard buffer, adjusted to 1 x 10^6/ml and rotated at 40 rpm for 20 min at 22 ℃. Cells were counted in a hemacytometer under a light microscope. Doublets were scored as unaggregated cells. The percent of cell adhesion inhibition (%) was calculated as follows: $E-E_0/ 100-E_0$ X 100 (%); E, single cells (%) in the presence of heavy metal ions; E_0, single cells (%) in the absence of heavy metal ions.

Results and Discussion

Effects of heavy metal ions on EDTA-resistant cell contact The influence of heavy metal ions on EDTA-resistant cell contact was investigated for the cells at the aggregation-competent stage as described in Materials and Methods. Heavy metal ions of Cu^{2+}, Hg^{2+}, Cd^{2+} and Pb^{2+} of more than 10^{-16} M showed inhibitory effects on EDTA-resistant cell contact (Figure 1). However, no inhibitory effect of heavy metal ions was observed in the cell contact in the assay without EDTA. It is believed that a cell adhesion molecule, csA, is directly involved in EDTA-resistant cell contact[9,10,11]. Inhibition of EDTA-resistant cell contact by heavy metal ions was thought to be due to the reduced amount of csA glycoprotein. However, the results of western blots with polyclonal antibodies against csA showed that there were no differences in the amounts of csA glycoprotein among cells treated with heavy metal ions of various concentrations, except 10^{-4} to 10^{-5} M of Cd^{2+} ions and 10^{-4} M of Hg^{2+} ions (Figure 2). These results suggest that substances other than csA are involved in EDTA-resistant cell contact.

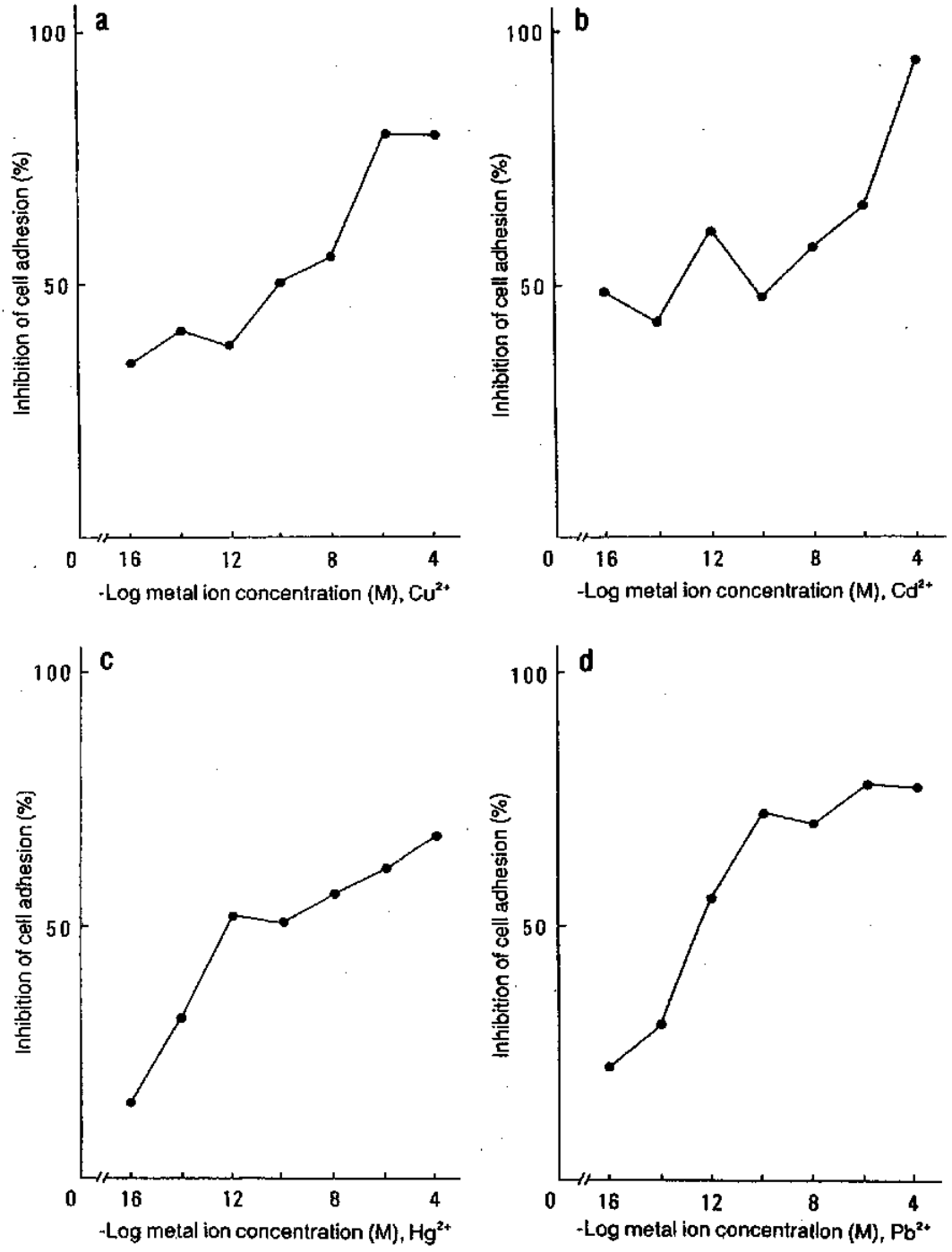

Fig. 1 Effects of heavy metal ions on EDTA-resistant cell contact. Cells were incubated in the presence of indicated concentrations of heavy metal ions. Abscissa: final concentration of (M);Cu^{2+} (a); Cd^{2+} (b); Hg^{2+} (c); Pb^{2+} (d) in standard buffer. Ordinate: percent of cell adhesion inhibition (%), calculated as described in Materials and Methods.

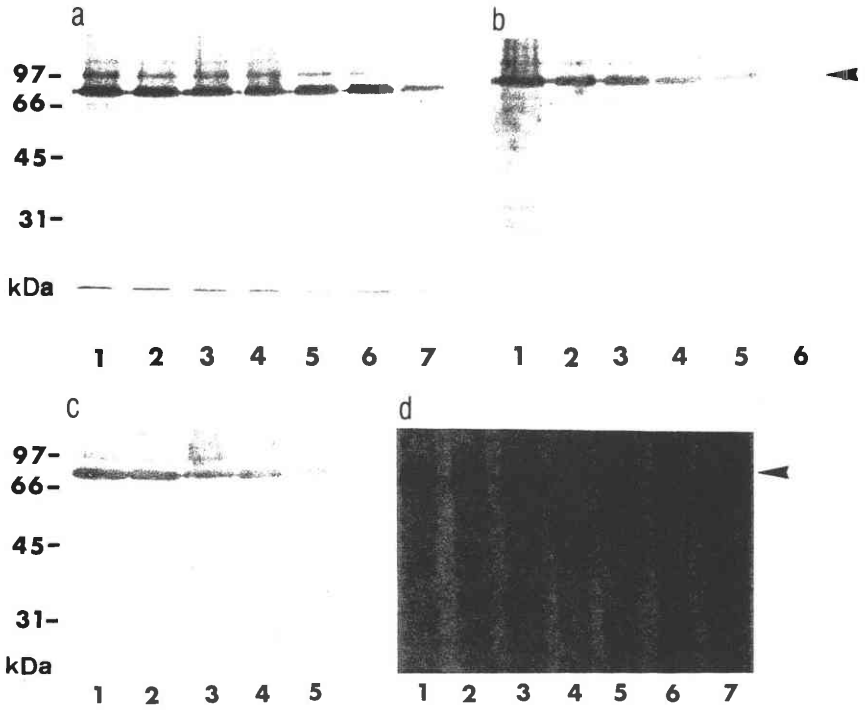

Fig. 2 Detection of csA glycoprotein from cells treated with heavy metal ions. Particulate fractions equivalent to 1×10^6 cells from cells treated with heavy metal ions were separated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose filters. The filters were incubated with polyclonal antibody against purified csA. Staining was done as described in Materials and Methods. Heavy metal ions used in final concentration (M) were the following: in

(a): without Cu^{2+} (lane 1), with Cu^{2+}(lanes 2, 10^{-12}; 3, 10^{-10}; 4, 10^{-8}; 5, 10^{-6}; 6, 10^{-5}; 7; 10^{-4});

(b): without Cd^{2+} (lane 1), with Cd^{2+} (lanes 2, 10^{-12}; 3, 10^{-10}; 4, 10^{-8}; 5, 10^{-6}; 6, 10^{-5});

(c): without Hg^{2+}(lane 1), with Hg^{2+}(lanes 2, 10^{-10}; 3, 10^{-8}; 4, 10^{-6}; 5, 10^{-5});

(d): without Pb^{2+}(lane 1), with Pb^{2+} (lanes 2, 10^{-12}; 3, 10^{-10}; 4, 10^{-8}; 5, 10^{-6}; 6, 10^{-5}; 7, 10^{-4}). Arrowheads indicate the position of csA.

Molecules affected by treatment of heavy metal ions To study the influence of heavy metal ions on protein synthesis, the incorporation of [^{35}S]-methionine into proteins was determined in the presence of Cu^{2+} ions. [^{35}S]-methionine incorporation was reduced to about 50% of untreated control in the presence of 10^{-4} M Cu^{2+} ions (Table 1). As described before, no differences were found in the synthesized amount of csA glycoprotein among cells treated with 10^{-4} to 10^{-12} M Cu^{2+} ions (Figure 2).

Table 1 Incorporation of [^{35}S] -methionine into cells in the presence of Cu^{2+} ions.

Cu^{2+} concentrations (M)	0	10^{-8}	10^{-4}
incorporation rate (cpm/10^6 cells)	26,029	24,277	13,242

[^{35}S]-methionine was added to one millilter of cell suspensions (1×10^7/ml) with or without Cu^{2+} ions immediately after starvation began. After starvation for 8 h, 100 μl of cell suspentions was washed in standard buffer and the incorporation of [^{35}S]-methionine was measured as described in Materials and Methods.

Western blots with concanavalin A indicated the changes in both 97-kDa and 56-kDa bands in particulate fractions from aggregation-competent cells treated with 10^{-4}, 10^{-5} Cu^{2+}, 10^{-5} M Cd^{2+} and 10^{-5} M Hg^{2+} ions (Figure 3). On the other hand, the cells treated with 10^{-4} to 10^{-6} M Cu^{2+} ions showed a remarkable loss of EDTA-resistant cell contact ability (Figure 1). Taken together, these results suggest that molecules other than csA are directly or indirectly involved in EDTA-resistant cell contact and that the function of the molecules are drastically inhibited by heavy metal ions.

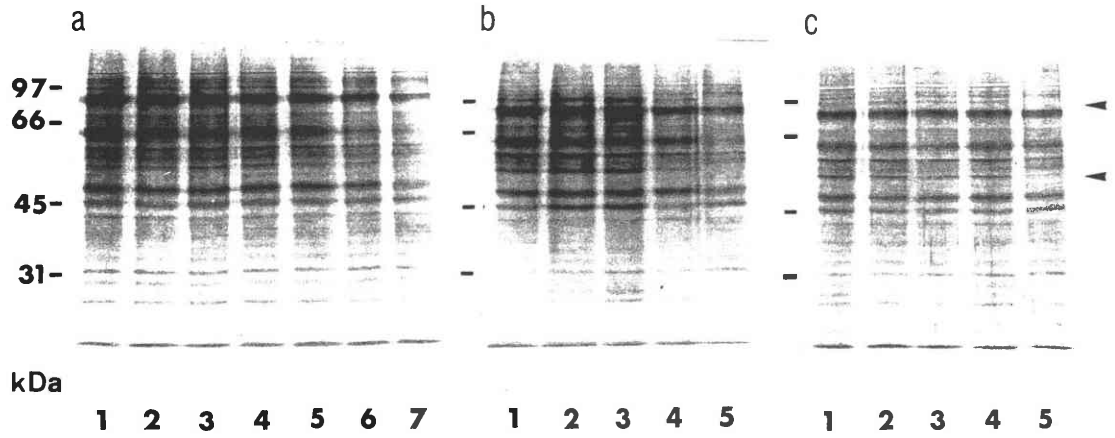

Fig. 3 Differences of protein profiles among aggregation-competent cells treated with each heavy metalion. Particulate fractions from aggregation-competent cells starved for 8h in the presence of each heavy metal ion were separated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose filters. The filters were incubated with concanavalin A as described in Materials and Methods.

(a), cells were developed without Cu^{2+} (lane 1), with Cu^{2+} (final concentration, M) (lanes 2, 10^{-10}; 3, 10^{-8}; 4, 10^{-7}; 5, 10^{-6}; 6, 10^{-5}; 7, 10^{-4}).

(b), without Cd^{2+} (lane 1), with Cd^{2+} (final concentration, M) (lanes 2, 10^{-10}; 3, 10^{-8}; 4, 10^{-6}; 5, 10^{-5}).

(c), without Hg^{2+} (lane 1), with Hg^{2+} (final concentration, M) (lanes 2, 10^{-10}; 3, 10^{-8}; 4, 10^{-6}; 5, 10^{-5}). Arrowheads indicate positions of 97-kDa and 56-kDa bands.

Fluorography with [^{35}S]-methionine incorporation in particulate fractions and cytosol fractions of aggregation-competent cells treated with 10^{-4} M Cu^{2+} ions was carried out to detect the molecules affected by the treatment of Cu^{2+} ions. A few different bands were detected between treated and untreated cells by fluorography (Figure 4). They were identified as a 42-kDa band in cytosol fractions

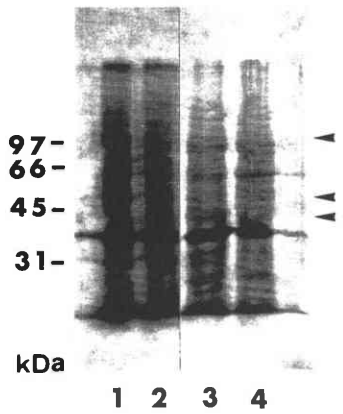

Fig. 4 Labeling of particulate and cytosol fractions equivalent to 1×10^{6} cells developed for 8 h with or without Cu^{2+} ions of 10^{-4} M (final concentration). [^{35}S]- methionine of 0.5 mCi was added to one milliliter of cell suspensions (1×10^{7}/ml) and cells were starved for 8 h without (lanes 1, 3) or with Cu^{2+} ions of 10^{-4}M (lanes 2, 4). Particulate (lanes 1, 2) and cytosol fractions (lanes 3, 4) were prepared as described in Materials and Methods. Arrowheads indicate positions of 97-kDa and 51-kDa bands (lanes 1, 2) and 42-kDa bands (lanes 3, 4).

and 97-kDa and 51-kDa bands in particulate fractions. Some of the molecules might be directly or indirectly involved in EDTA-resistant cell contact. It is possible that heavy metal ions interfere with the interactions among these substances or block their functions through the interactions with these substances in EDTA-resistant cell contact. There is indeed evidence to show that heavy metal ions interact with carbohydrates[12]. Moreover, the formation of complexes of Cu^{2+} ions with amino sugars or suitable polyols has been reported[13),14)]. As would be expected, some molecular shift bands were observed among substances treated with Cu^{2+} ion by silver staining, western blots or fluorography. However, if the interaction of heavy metal ions with carbohydrates was weak, heavy metal ions could be easily dessociated from carbohydrates and it might be difficult to detect different bands between treated and untreated cells. Using an instrument equipped with a biosensor might be useful for confirming the existence of a weak interaction between heavy metal ions and carbohydrates.

References

1) H. Beug and G. Gerisch: J. Cell Biol., **56**, 647-658 (1973)
2) M. Sussman and C. Boschwitz: Dev. Biol., **44**, 362-368 (1975)
3) D.R. Fontanna: Differentiation, **53**, 139-147 (1993)
4) L. Elling: Glycobiology, **5**, 201-206 (1995)
5) E.S. Hujanen, S.T. Seppä and K. Virtanen: Biochim. Biophys. Acta., **1245**, 145-152 (1995)
6) M. Wagner, C.L. Klein, H. Kleinert, C. Euchenhofer, U. Forstermann and C.J. Kirkpatrick: Pathobiology, **65**, 241-252 (1997)
7) U.K. Laemmli: Nature, **227**, 680-685 (1970)
8) H. Towbin, T.Staehelin and J.Gordon: Proc, Natl, Acad. Sci. USA.,**76**, 4350-4354 (1979)
9) M. Yoshida: J. Biochem, **101**, 1233-1245 (1987)
10) A. Noegel, C. Harloff, P. Hirth, R. Merkl, M. Modersitzki, J. Stadler, U. Weihart, M. Westphal and G. Gerisch: EMBO J, **4**, 3805-3810 (1985)
11) C.Harloff, G. Gerisch and A.A. Noegel: Genes & Development, **3**, 2011-2019 (1989)
12) M. Yoshida, S. Ishida and Y. Iizuka: Cell Struct. Funct, **16**, 383-390 (1991)
13) S.J. Angyal: Adv. Carbohydr. Chem., **47**, 1-43 (1989)
14) B. Radomska, H. Kozlowski, P. Decock, B. Dubois and G. Micera: J. Inorganic Biochem., **33**, 153- 159 (1988)
15) S.J. Angyal: Carbohydr. Res., **200**, 181-188 (1990)

接着活性に対する重金属の効果

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摘　要

粘菌細胞 *(Dictyostelium discoideum)* の接着様式のひとつであるEDTA-resistant cell contactにおける EDTAの役割を検討する目的で重金属を用いて、EDTA-resistant cell contact 活性を調べた。その結果、10^{-16}M以上のHg^{2+}、Cd^{2+}、Pb^{2+}、Cu^{2+}によりEDTA-resistant cell contact 活性は効果的に阻害された。