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Effects of Heavy Metal Ions on EDTA-sensitive Cell Contacts of *Dictyostelium discoideum*

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Synopsis

The effects of heavy metal ions on the EDTA-sensitive cell contacts, which exist from growth-phase stage of *Dictyostelium discoideum*, was investigated. EDTA-sensitive cell contacts of cells at the growth-phase stage were analyzed in the presence of heavy metal ions. Heavy metal ions Hg^{2+}, Cd^{2+} and Cu^{2+} inhibited EDTA-sensitive cell contacts at concentrations higher than 10^{-5} M, whereas Pb^{2+} did not show any recognizable effects at the same concentration range. The possible mechanisms of action of these metal ions are discussed.

Introduction

Dictyostelium discoideum has been used as a suitable organism for elucidation of molecular mechanisms of cell-to-cell adhesion during multicellular formation, differentiation and signal transduction pathways. *D. discoideum* cells display two types of cell adhesion: EDTA-sensitive and EDTA-resistant cell contacts. However, little is known about the potential targets of EDTA in the assay system of cell adhesion[1),2),3)]. Beug *et al.* have reported that EDTA could be replaced by EGTA in the assay system of cell adhesion. Their results suggest that Ca^{2+} ions play an important function in the EDTA-sensitive cell contacts, although it remains to be studied whether other ions can replace Ca^{2+} ions in EDTA-sensitive cell contacts. Some researchers have shown interesting effects of heavy metal ions on biological activities, such as chemotaxis, enzyme activity, and gene expression[4),5),6)]. This study aims at elucidating the effects of heavy metal ions on the actual EDTA-sensitive cell contacts.

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 by centrifugation and adjusted to 1 x 10^7 cells/ml in 17 mM Soerensen phosphate buffer pH 6.1 (standard buffer).

Preparation of particulate fractions and gel electrophoresis. Sodium dodecyl sulfate (SDS)-10% polyacrylamide gel electrophoresis was carried out using the standard method of Laemmli[7)] and staining with silver[8)]. 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 supernatant was removed as samples for cytosol fractions,

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supplemented with 9 volumes of acetone and centrifuged at 15,000 rpm for 20 min. The precipitates were dried under reduced pressure and dissolved in sample buffer for SDS-polyacrylamide gel electrophoresis.

Assay of cell agglutination. Cell agglutination was measured by a modification of 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°C. Cells were counted in a hemacytometer under light microscope. Single cells and doublets were scored as unaggregated cells. The percent of cell adhesion inhibition (%) was calculated as follows: E - E_0/ 100-E_0 (%); where E stands for single cells (%) in the presence of heavy metal ions; E_0, single cells (%) in the absence of heavy metal ions. Heavy metal ions at an appropriate concentration were mixed in test tubes with 1 ml of cell suspension at growth-phase rotated as described above.

Results and Discussion

Effects of heavy metal ions on EDTA-sensitive cell contacts The influence of heavy metal ions on EDTA-sensitive cell contacts was investigated for cells at the growth-phase stage as described in Materials and Methods. The EDTA-sensitive cell contacts was inhibited by Cu^{2+}, Hg^{2+} and Cd^{2+} ions, whereas Pb^{2+} showed no effects (Figure 1). It is conceivable that these inhibitory heavy metal ions displace Ca^{2+} ions at the functional sites involved in the formation of EDTA-sensitive cell contacts.

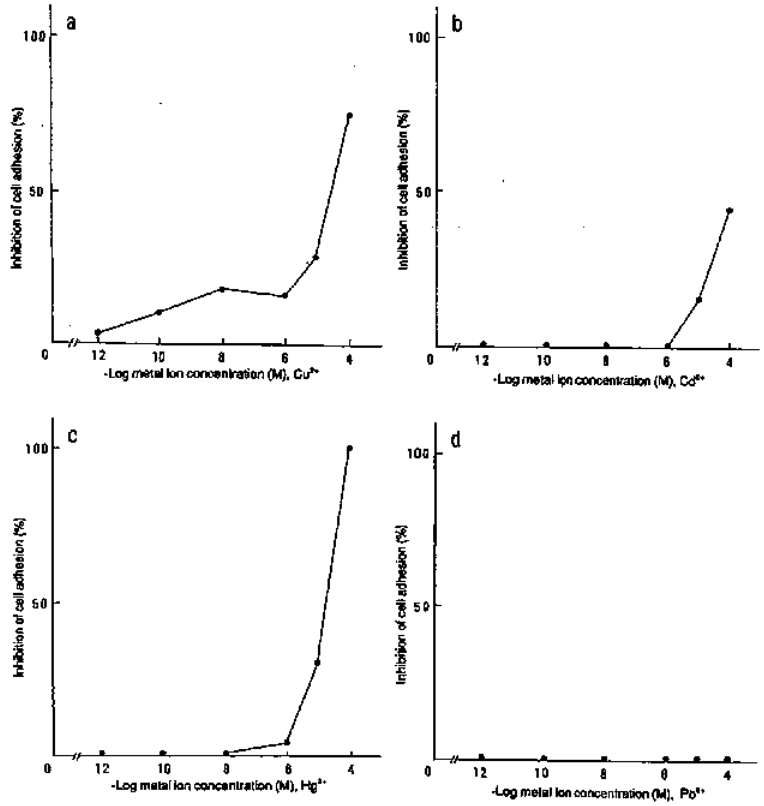

Figure 1. Effects of heavy metal ions on EDTA-sensitive cell contacts. Cells at growth-phase stage were incubated in the presence of heavy metal ions at indicated concentrations. Abscissa: final concentration of heavy metal ions (M); Cu^{2+} in (a), Cd^{2+} in (b), Hg^{2+} in (c), Pb^{2+} in (d). Ordinate: percent inhibition of cell adhesion (%). Percent inhibition was calculated as described in Materials and Methods.

Alternatively heavy metal ions may interact directly with cell adhesion molecules involved in EDTA-sensitive cell contacts independently of Ca^{2+} ions. The Hg^{2+} ions of 10^{-4} M caused lysis of one-third of the treated cells, suggesting that Hg^{2+} ions at this concentration are toxic to the cells at the growth-phase stage.

Molecules affected by treatment of heavy metal ions Particulate fractions or cytosol fractions from the cells at the growth-phase stage, which were treated for 20 min at 22°C with Cd^{2+} or Cu^{2+} ions at various concentrations (10^{-4}~10^{-8} M), were subjected to SDS-polyacrylamide gel electrophoresis and stained with silver to detect proteins affected by treatment of heavy metal ions (Figure 2). In the samples derived from cells treated with Cd^{2+} or Cu^{2+} ions at 10^{-4} M, a molecular shift was observed in a few bands, while no differences were found among cells treated with the other concentrations. In particulate fractions from growth-phase cells treated with 10^{-4} M Cd^{2+} or Cu^{2+}, 97-kDa and 41-kDa bands were detected as molecular-shift bands, while 80 to 90-kDa bands were identified as molecular-shift bands in cytosol fractions. Gp 24 and gp 126 are found to be candidate molecules involved in EDTA-sensitive cell contacts[9),10)]. It is believed that gp 24 is a cell adhesion molecule involved in EDTA-sensitive cell contacts[11)]. In this study, the 97-kDa, 80~90-kDa and 41-kDa bands were identified as molecules affected by treatment with Cd^{2+} or Cu^{2+} in growth-phase cells. It is possible that the molecular shift occurred by the interactions of these substances with heavy metal ions. The 97-kDa and 41-kDa bands observed in treatment with Cu^{2+} or Cd^{2+} ions might be good candidates for functional molecules in EDTA-sensitive cell contacts through a Ca^{2+} bridge[12),13)], although it remains to be clarified whether these molecules could function as calcium-binding proteins.

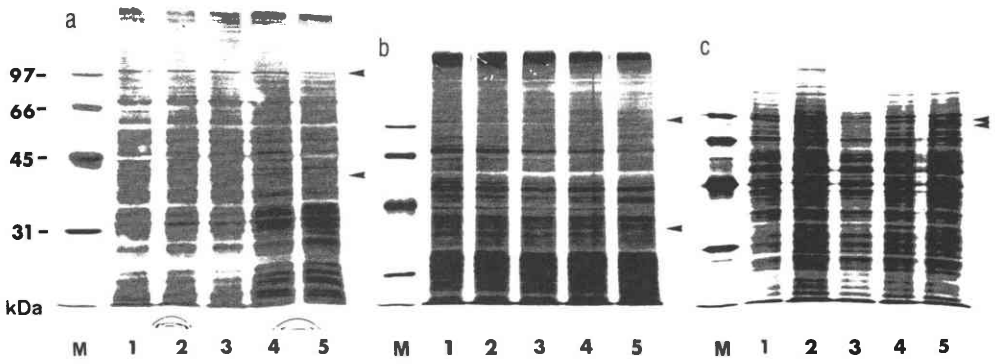

Figure 2. Electrophoregrams of particulate fractions or cytosol fractions from growth-phase cells treated with heavy metal ions. Particulate fractions equivalent to 1×10^6 cells and cytosol fractions equivalent to 2×10^6 cells were subjected to SDS-polyacrylamide gel electrophoresis and stained with silver. In (a), particulate fractions from cells treated with Cu^{2+} (final concentration, M) were applied; lane 1, without Cu^{2+}; 2, 10^{-8}; 3, 10^{-6}; 4, 10^{-5}; 5, 10^{-4}. In (b), particulate fractions from cells treated with Cd^{2+} (final concentration, M) were applied; lane 1, without Cd^{2+}; 2, 10^{-8}; 3, 10^{-6}; 4, 10^{-5}; 5, 10^{-4}. In (c), cytosol fractions from cells treated with Cu^{2+} (final concentration, M) were applied; lane 1, without Cu^{2+}; 2, 10^{-8}; 3, 10^{-6}; 4, 10^{-5}; 5, 10^{-4}. Arrowheads indicate positions of 97-kDa and 41-kDa bands (a, b), and 80~90-kDa bands (c). Lane M is molecular mass markers.

We have previously reported that EDTA-sensitive cell contacts might be mediated by the interaction of carbohydrates on each cell surafce at the growth-phase stage[14)]. Heavy metal ions could interfere with the interactions among the substances involved in EDTA-sensitive cell contacts or block their functions through the interactions with these substances. There is indeed evidence to show that heavy metal ions interact with carbohydrates[15)]. The formation of complexes of Cu^{2+} ions with amino

sugars or suitable polyols has also been reported[16),17)]. However, if the interaction of heavy metal ions with carbohydrates were weak, heavy metal ions might easily be detached from carbohydrates and it might be difficult to detect different bands between treated and untreated cells.

Identification of the complexes of heavy metal ions and carbohydrates is in progress.

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Ca^{2+}依存性細胞接着に対する重金属の効果

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摘　要

粘菌細胞 *(Dictyostelium discoideum)*の増殖期から存在しているCa^{2+}依存性細胞接着(EDTA-sensitive cell contacts)の機構解析を重金属を用いて行った。その結果、10^{-5} M 以上のHg^{2+}、Cd^{2+}、Cu^{2+}処理においてCa^{2+}依存性細胞接着が効果的に阻害された。一方、Pb^{2+}処理では阻害効果は認められなかった。