A Study on the Uptake of Cadmium (Cd^{2+}) in Cd-resistant Chinese Hamster V79 Cells

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Introduction

We previously isolated cadmium-resistant cells (Cd^r) from Chinese hamster V79 cells in culture.¹⁾ The inducibility of metallothionein and the rate of synthesis of glutathione in Cd^r cells were not different from those in Cd-sensitive cells (Cd^s). Only the rate of accumulation of Cd^{2+} was markedly decreased as compared to the rate in Cd^s cells. In the present study, we investigated the mechanism for the decreased accumulation of Cd in Cd^r cells, together with the biochemical features of plasma membrane purified from the Cd^r and the Cd^s cells.

Methods

Cells, plated in 12-well plates at 1.2×10^5 cells/ml/well and incubated for 20 h, were incubated in the medium that contained $1 \mu\text{Ci/ml}$ ^{109}Cd ($0.1\text{--}10 \mu\text{M}$ as CdCl_2) for specified times. The cells were then lysed and the radioactivity was counted. The plasma membrane fraction was purified by a method of Percoll gradient centrifugation. The membrane proteins were separated by SDS-PAGE and were transferred to a PVDF membrane. The membrane was probed in 10 mM Tris-HCl, pH 7.4, with 0.1 M KCl, 0.1 mM Zn- $(\text{CH}_3\text{COO})_2$, and $1 \mu\text{Ci/ml}$ ^{109}Cd for 10 min at room temperature and Cd-binding proteins were detected by autoradiography.

Results and Discussion

Cd^r and Cd^s cells took up Cd^{2+} in a time-dependent manner but the rate of the uptake by

Cd^r cells was about 15% of that by Cd^s cells. No difference in the rate of efflux of Cd^{2+} was observed between Cd^r and Cd^s cells. Kinetic studies of the uptake of Cd^{2+} showed that the K_m values for Cd^r and Cd^s cells were $31.95 \mu\text{M}$ and $3.15 \mu\text{M}$, respectively. The $V_{\max}$ values were $0.31 \text{ pmol/h/mg protein}$ for Cd^r cells and $0.46 \text{ pmol/h/mg protein}$ for Cd^s cells. The rate of uptake of Cd^{2+} by Cd^s cells was very high at pH 7.4 and pH 8.0 relative to the rates at pH 6.0 and pH 6.8. In contrast, the uptake of Cd^{2+} by Cd^r cells was lower at all pH values than that by Cd^s cells. These results suggest that the affinity of Cd^{2+} for the molecules that are involved in the transport of Cd^{2+} differs markedly between these two cell types. Mersalyl acid, an impermeable SH blocker, inhibited the uptake of Cd^{2+} by Cd^s cells while Cd^r cells were insensitive to the inhibition, suggesting that SH group in the plasma membrane plays a role in the uptake of Cd^{2+} .

The Cd-blotting of the plasma membrane proteins revealed two major differences between Cd^r and Cd^s cells. The level of a 110-KDa Cd-binding protein (Cd-BP) which was detected in Cd^s cells was specifically reduced in Cd^r cells. In contrast, an 82-KDa Cd-BP, which was not observed in Cd^s cells, was detected in Cd^r cells. These results suggest that the observed changes in Cd-BP represented qualitative changes due to differences in the affinity for Cd^{2+} .

Reference

- 1) T. Ochi, *Chem.-Biol. Interact.*, 78, 207 (1991).