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Uptake of Ammine-Cobalt(III) Complexes into *Escherichia Coli* Cells

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Synopsis. The uptake of Co into *E. coli* cells was studied in various Co(III) ammine complexes. It was found to be independent of the incubating temperature and existence of Mg^{2+} ion, but dependent on the ligands, the uptake of Co being in the following order. $[Co(NH_3)_4(OH_2)_2]_2(SO_4)_3 > [Co(NH_3)_5(OH_2)]Cl_3 > [Co(NH_3)_6]Cl_3 \gg [Co(en)_3]Cl_3 > [Co(OH_2)_6]Cl_2$

Wide attention has been given to the activities or properties of various inorganic complexes of transition metals in biological fields. For example, it was found that *cis*-dichlorodiamineplatinum(II) suppresses the division of *E. coli* cells by inhibiting DNA synthesis.[1,2] Chromium, palladium, and rhodium complexes also have a similar function.[3,4] Several reports suggest that these complexes might become useful anti-cancer drugs by controlling the propagation of cancer cells in consequence of interaction between DNA molecule and the complexes.[5]

The effect of several cobalt(III) complexes which induce filamentous growth or inhibit the respiration of *E. coli* has also been reported.[6] On the assumption of quantitative estimation for the biological function of these complexes, the locomotion and distribution of the complexes in the bioorganism have not been fully elucidated. The uptake of several cobalt(III) complexes into the *E. coli* cells is given in this report.

Experimental

Escherichia coli B (ATCC 11246) was used as the test organism, which was grown in minimum nutrient media: K_2HPO_4(40 mM), KH_2PO_4(22 mM), $(NH_4)_2SO_4$ (7.6 mM), disodium citrate (2.1 mM), $MgSO_4$(0.8 mM), and glucose (27.8 mM) were dissolved in distilled water. Cobalt(III) complexes $[Co(NH_3)_6]Cl_3$, $[Co(en)_3]Cl_3$, $[Co(NH_3)_5(OH_2)]Cl_3$, and $[Co(NH_3)_4(OH_2)_2]_2(SO_4)_3$ were prepared by the usual methods.

E. coli was preincubated twice and incubated in 200 ml media at 30 °C with shaking. When turbidity of media become O.D. 0.7, the cobalt(III) complexes were added to make cobalt concentration 30 ppm. *E. coli* cells incubated with complexes for 1 h were harvested by centrifugation (5000×g 8 min), washed twice with 0.9% sodium chloride solution, dried at 105 °C until weights of harvest became constant within 0.3 mg, and ashed at 600 °C in an electric furnace. After the ashed cells had been dissolved in hydrochloric acid, the amount of cobalt was determined by atomic absorption analysis using a Hitachi 207 atomic absorption spectrophotometer.

Results and Discussion

Many mechanisms concerning the uptake of metal ions have been reported. For example, the uptake of the cobalt(II) into *E. coli* is an active transport linked with the energy metabolism,[7,8] and is suppressed in the presence of Mg^{2+} as shown in Table 1.

The uptake of cobalt(III) complexes is independent of the presence of Mg^{2+}. The amount of complexes taken up into the cell is found to be almost unchanged with incubation temperature. The results indicate that the uptake of these complexes depends on the physical adsorption on the cell surface, but not on the biological activity. This can be supported by the fact that it is difficult for hydrophilic ions to pass the biological membrane without active transport system.

Ease of uptake of the cobalt(III) complexes is in the following order: $[Co(NH_3)_4(OH_2)_2]_2(SO_4)_3 > [Co(NH_3)_5(OH_2)]Cl_3 > [Co(NH_3)_6]Cl_3 \gg [Co(en)_3]Cl_3 > [Co(OH_2)_6]Cl_2$. The amounts of taken-up complexes seem to be dominated by the number of aqua ligands. From the fact that $[Co(NH_3)_6]Cl_3$ is taken up about three times more than $[Co(en)_3]Cl_3$, the hydrophilicity of ligands seems also important in the uptake of complexes. The results indicate that the formation of hydrogen bond with cell surface *via* the hydrogen atom of ligands of the complex plays an important role in the uptake of the cobalt(III) complexes.

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TABLE 1. UPTAKE OF COBALT(III) COMPLEXES (μg Co/100 mg dried weight)

Temperature	3 °C	11 °C	20 °C	25 °C	30 °C	30 °C ($-Mg^{2+}$)
$[Co(NH_3)_6]Cl_3$	65.0	74.9	70.4	72.7	75.0	62.8
$[Co(NH_3)_5(OH_2)]Cl_3$	67.0	95.5	78.5	85.3	—	75.0
$[Co(NH_3)_4(OH_2)_2]_2(SO_4)_3$	111.0	154.6	121.0	77.5	—	109.4
$[Co(en)_3]Cl_3$	19.9	30.2	30.7	24.2	22.4	27.6
$[Co^{II}(OH_2)_6]Cl_2$	7.7	18.7	11.8	16.2	8.9	49.2

The content of cobalt in the whole cells of *E. coli* which were incubated with the ammine complexes at various temperatures between 3 and 30 °C.

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