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学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目：
Title of Dissertation

Application of precursor proteins with steric hindrance for blocking the process of protein import into chloroplasts *in vitro*
（立体障害を有する前駆体蛋白質が葉緑体への蛋白質輸送に及ぼす影響）

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Most of the nuclear encoded chloroplastic precursor proteins are imported into chloroplasts through protein translocation machinery (translocon) embedded in the outer and inner envelope membranes, after they are synthesized as precursors in the cytosol with their N-terminal transit peptide. Biochemical analyses of protein translocation intermediates (PTIs) formed during early stages of protein import into chloroplasts under restricted energy conditions identified many components involved in protein translocation. Whereas limited information is available about the latter stages of import, as it is difficult to monitor the precursor behaviour once it was released from the early PTIs. In order to address this problem, we have attempted to obtain post-early intermediates to plug translocation channel by precursor proteins carrying a tightly-folded structure at their C-termini. To this end, two strategies were employed to develop such kind of precursors: a precursor protein carrying a tightly folded domain; and a precursor protein post-translationaly attached with the folded structure. In case of the first strategy, a large quantity of precursor in a soluble form is required, if one wants to isolate PTIs enough for biochemical analyses. To this end, we prepared the

recombinant precursor protein which is overexpressed in *Escherichia coli* in soluble form and whose chloroplastic targeting signal was fused to dihydrofolate reductase from *E. coli*, known to fold tightly in the presence of its substrate analogue methotrexate. If the precursor was treated with methotrexate prior to the import reaction, the amount of processed precursor was reduced. However, the processed precursor was recovered in the soluble fraction after fractionation, indicating that methotrexate was released from the precursor, which suggested the presence of strong unfolding activity within chloroplasts. For the second strategy, biotinylated precursor proteins bound post-translationaly to monovalent streptavidin or its stable variant was applied for an *in vitro* chloroplastic protein import assay. Despite the strong non-covalent interaction between biotin and streptavidin, streptavidin or its stable variant seemed to be dissociated from the biotinylated precursors, thus translocation of this precursor was not prevented. These results also suggested the presence of strong unfolding activity within chloroplasts, regardless of whether this activity is exhibited by an unknown unfoldase present at the surface of chloroplasts or by a mechanical disruption of the biotin-streptavidin interaction due to the strong pulling force into chloroplasts.