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Exploration of novel transporter proteins for oligosaccharides using ATPase (MsiK)

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Research aims

Streptomycetes are saprophytic soil bacteria which decompose biopolymers (protein, starch, cellulose, hemicelluloses, and chitin). To deepen our knowledge of recognition and decomposition systems for biopolymers in streptomycetes, we have investigated a degradation system for chitin, which is an *N*-acetylglucosamine polymer, in *Streptomyces coelicolor* A3(2). The bacterium possesses at least 13 (putative) chitinase genes (*chi*) in its genome, and the transcription of 8 among the 13 *chi* genes is induced in the presence of chitin and its degradation product *N, N′*-diacetylchitobiose $[(GlcNAc)_2]$[1–3]. $(GlcNAc)_2$ uptake is supposed to be required for transcriptional induction of the *chi* genes, as the *msiK* gene, encoding an ATP-hydrolyzing component of $(GlcNAc)_2$ transporters, is essential for induction of chitinase production[4]. Hurtubise *et al*.[5] have already demonstrated that uptake of cellobiose and xhlobiose is essential for induction of cellulase and xylanase production, respectively, in *Streptomyces lividans*. Uptake of disaccharides is thus assumed to be a prerequisite for induction of corresponding polysaccharide-hydrolases.

In streptomycetes, uptake systems for maltose, cellobiose, xylobiose, and $(GlcNAc)_2$ have been identified as disaccharide transporters[6–10]. All of these uptake systems are, interestingly, ABC (ATP-binding cassette) transporters and supposed to act with the above-mentioned MsiK protein[4,11]. *msiK* seems to be transcribed constitutively, whereas *malE*, *cebE*, *bxlE*, and *dasA*, encoding sugar-binding proteins of ABC transporters, are induced by corresponding sugars; i.e. maltose, cellobiose, xylobiose, and $(GlcNAc)_2$, respectively[4].

There are 37 sets of genes for (putative) ABC transporters for OSPs (oligosaccharides and polyols) on the *S. coelicolor* A3(2) genome (ABCISSE database). It is noteworthy that in streptomycetes, in contrast to other bacteria, a gene for the ATP-hydrolyzing component is not located among or with each of the 37 gene clusters, which encode the other components (a solute-binding protein and two membrane proteins). MsiK is, thus, supposed to act with those components for OSP transporters as a common component. However, transport substrates for 28 of the 37 transporters remain, up to now, unclear. This study aims to investigate novel ABC transporter components for OSP, whose uptake systems are unidentified, by obtaining proteins that interact with MsiK in the presence of corresponding transport substrate. We report here the development of a system to obtain proteins interacting with MsiK.

Methods

msiK and its flanking region (including the putative promoter sequence) were amplified by using primers to produce C-terminally His-tagged MsiK protein under the control of the putative native promoter. The amplified DNA fragment (1295 bp) was cloned and its sequence was confirmed to be identical to that registered in the genome database (http://www.sanger.ac.uk/Projects/S_coelicolor/). The cloned fragment was integrated into a multi-copy plasmid vector pWHM3 to obtain the resulting plasmid pCMK02. The *msiK*-null mutant ASC3[4] was transformed with pWHM3 or pCMK02. To investigate the localization of MsiK, *S. coelicolor* A3(2) transformants were cultivated according to a method described previously[3], with some modifications. Cytoplasmic and envelope fractions were prepared from the harvested mycelia by sonication, centrifugation, and ultracentrifugation. Nickel affinity column chromatography was done according to a described method [12].

Results

In *S. coelicolor* A3(2) ASC3 (pMCK02), a protein band corresponding to 45 kDa was detected by immunoblot analyses using *anti*-MsiK antiserum or *anti*-His-tag antibodies, while no band was detected with the antiserum or the antibodies in its vector control strain ASC3 (pWHM3). In contrast, using the *anti*-MsiK antiserum, a protein band (40 kDa) was observed in the wild type strain carrying the plasmid vector pWHM3. The data demonstrated that His-tagged MsiK protein ($MsiK^{His}$) was successfully produced in the strain ASC3 (pCMK02). Irrespective of the kind of sugar in the culture media, MsiK protein was found in the cell envelope fraction of *S. coelicolor* A3(2) wild type strain M145. $MsiK^{His}$ was also detected in the envelope fraction of the strain ASC3 (pCMK02). The data implied that MsiK protein, regardless of the C-terminal His-tag, interacted with membrane proteins, which may act as transporters. $MsiK^{His}$ was obtained by nickel affinity column chromatography from the cell lysate of ASC3 (pCMK02), although other proteins were co-eluted. To investigate proteins interacting with $MsiK^{His}$, the composition of eluted

proteins was compared by SDS-PAGE among M145 (pWHM3), ASC3 (pWHM3), and ASC3 (pCMK02). Some protein bands were specifically detected in ASC3 (pCMK02). It was thus concluded that proteins interacting with $MsiK^{His}$ were gained by the chromatography.

Conclusion

We successfully produced C-terminally His-tagged MsiK protein ($MsiK^{His}$) in the *S. coelicolor* A3(2) *msiK*-null mutant carrying pCMK02. $MsiK^{His}$ was detected in cell-envelope fraction of the transformant, as native MsiK protein in the wild type strain M145. The data suggested that $MsiK^{His}$ interacted with membrane proteins as does native MsiK. By obtaining $MsiK^{His}$ from the *S. coelicolor* A3(2) transformant using nickel affinity column chromatography, some proteins, which would interact with $MsiK^{His}$ *in vivo*, were obtained. We shall explore novel sugar transporter proteins acting with MsiK, by identifying the probable MsiK-interacting proteins.

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