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A highly efficient cell penetrating peptide pVEC-mediated protein delivery system into microalgae

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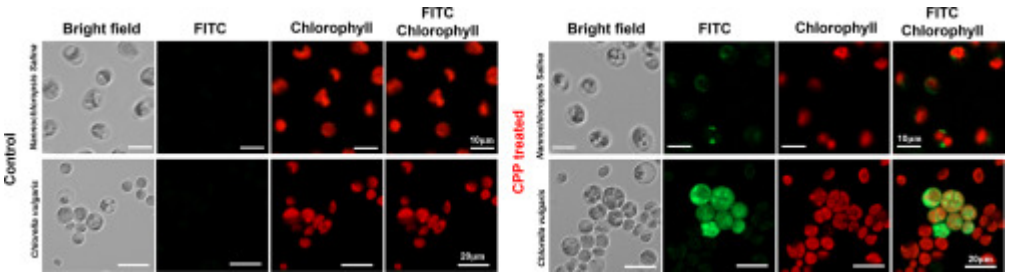
Abstract

Interest in microalgae has significantly increased due to its potential as a promising bioresource in various fields. However, a technique for intracellular delivery of proteins into microalgae has not established yet, although it is potentially valuable for advanced algal research. Here, we propose a cell penetrating peptide pVEC-mediated protein delivery tool for microalgae. We discovered that the peptide pVEC from vascular endothelial cadherin was more effective in transporting exogenous proteins into algal cells than other peptides including R9, Transportan, TAT and Penetratin. pVEC-mediated tool was shown to deliver proteins of various sizes from 6 kDa to 150 kDa into wild-type Chlamydomonas reinhardtii and to also work in other algal species such as Nannochloropsis *salina* and Chlorella vulgaris when delivering a 66 kDa protein. In addition, we show that our proposed pVEC-mediated protein delivery system is based on both the endocytic and non-endocytic pathways simultaneously through a mechanism study using inhibitors such as low temperature, *N*-ethylmaleimide, 5-(*N*-ethyl-*N*-isopropyl) amiloride, chlorpromazine, sodium azide, and methyl-beta-cyclodextrin. The results show that the proposed simple and efficient protein

delivery tool could contribute to advanced algal research for a wide array of applications for microalgae in various industries.

Graphical abstract



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Introduction

In the last few decades, microalgae, eukaryotic photosynthetic microorganisms that can grow at a rapid rate and live in harsh conditions [1], have been considered as a promising bioresource. Microalgae accumulate diverse nutraceuticals and pharmaceutical compounds in their cell bodies that can be used in the food and pharmaceutical industry [1], [2], [3]. Some research has shown that microalgae not only can generate valuable products but also can effectively fix carbon dioxide which could be part of the solution for the carbon dioxide gas emission issue [4], [5], [6]. Recently, microalgae have received significant attention as a bioresource to produce biodiesel because they accumulate lipids in their cell bodies ranging between 20% to 50% of their dry cell weight under certain conditions [1], [7], [8]. Many studies have shown that the conventional biofuel production system based on the agricultural biomass, which has severe limitations, could be replaced with an advanced biodiesel production system that uses specific algal species [8], [9]. However, for the practical use of microalgae in biodiesel production, improving their biological characteristics has been considered essential, especially the photosynthetic efficiency, growth rate, biomass production rate and oil contents in the cell body. For that reason, it has been a major focus of many basic biological science or genetic engineering studies [10], [11].

Intracellular delivery of proteins is an important technique in biological science. Delivery of proteins into cells enables the investigation of protein-protein interactions and their functions in a cell system [12], [13], [14], [15], [16]. Considering that most biological reactions are protein based reactions, the intracellular delivery of proteins is an excellent

approach for fundamental biological research which is the groundwork for future applications. Recently, the delivery of proteins has become a hot issue after the development of the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system which enables genome editing with high target specificity and simplicity [17], [18]. While numerous studies have covered the delivery of molecules such as proteins into mammalian cells, only a few studies have investigated the intracellular delivery of molecules into microalgae due to the difficulties of passing through the algal cell wall and the membrane barrier and the relatively limited amount of research compared to mammalian cell lines [19], [20].

Here, we investigated the highly efficient delivery of proteins into microalgae using the cell penetrating peptide pVEC, an amphipathic 18-amino acid-long peptide derived from vascular endothelial cadherin, in its non-covalent form. In our previous study investigating the penetration of cell penetrating peptides conjugated with fluorescein isothiocyanate (FITC), we reported that pVEC is the most effective cell penetrating peptide among the various cell penetrating peptides for *Chlamydomonas reinhardtii* [21]. Without any effort to covalently link target proteins to the cell penetrating peptide by chemical method, proteins of various sizes from 6 kDa to 150 kDa were effectively delivered into wild-type *Chlamydomonas reinhardtii* (*CC-124*) with pVEC in this study. In addition to *Chlamydomonas reinhardtii*, pVEC effectively delivered proteins into the *Chlorella vulgaris* and *Nannochloropsis salina* which are regarded as promising species for biofuel production. Furthermore, we investigated whether the endocytic pathway or non-endocytic pathway (direct pathway) are involved in the delivery of the proteins in a mechanism study using several endocytosis inhibitors. This study will greatly contribute to the basic biology of algae as a new molecular manipulation tool. Additionally, our system will be used in genetic engineering associated with the CRISPR/Cas9 system as an efficient cas9 delivery tool in the future.

Section snippets

Microalgae growth condition and preparation

Microalgae *Chlamydomonas reinhardtii* (CC-124) and *Chlorella vulgaris* were cultivated in tris-acetate phosphate (TAP) medium under continuous illumination, constant agitation (about 140 rpm), and constant temperature (25 °C). *Nannochloropsis salina* was cultivated in a modified F2N media consisting of 15 g/L sea salt (Sigma-Aldrich, USA), 10 mM Tris–HCl

(pH7.6), 427.5mg/L $NaNO_3$, 5mL/L trace metal mixture (4.36g/L Na_2 EDTA·$2H_2O$, 3.15g/L $FeCl_3$·$6H_2O$, 10mg/L $CoCl_2$·$6H_2O$, 22mg/L ZnSO4·$7H_2O$, 180mg/L $MnCl_2$·4H

Screening of cell penetrating peptides for the delivery of proteins

Previously, there have been only a few studies regarding the application of CPPs in microalgae such as the translocation of cell penetrating peptides (CPPs) labeled with FITC [19], [21], [25], delivery of small dsRNA fragments by a CPP [25] and delivery of proteins with a covalently linked form [19]. Most of these studies used the arginine (R)-rich cell penetrating peptide (R7~R9) for the delivery of molecules which is one of the most well-known cell penetrating peptides. In a previous report,

Conclusions

Although microalgae have been seen as a promising bioresource, a lack of knowledge on molecular manipulation of algal cells compared with that for mammalian cell lines remains a barrier to understanding advanced algal science. This study presents a pVEC-mediated protein delivery system for microalgae for the first time as a new intracellular molecular delivery method which requires no effort to conjugate the target protein to the cell penetrating peptide. The pVEC-mediated protein delivery

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> *Citation Excerpt :*
>
> …Among various non-viral vectors, cell-penetrating peptides (CPPs) have recently received considerable attention as delivery systems because they are composed of natural amino acids. CPP sequences consist of positive lysine and arginine residues that impart cationic properties allowing their interaction with lipid plasma membranes [17,18]. However, exposed cationic peptides without any shielding effects are very vulnerable to serum and salt conditions.…

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