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Variation of Surface Properties of *Streptomyces griseus* Cells after Heat Treatment with Liposome

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Surface properties of *Streptomyces griseus* cells, such as surface net charge and surface net hydrophobicity, were characterized by using the aqueous two-phase system (ATPS) method. It was found that the surface net charge of *S. griseus* cells was reduced after the POPC liposome-treatment of the cells at 41^{o}C. The surface net hydrophobicities of *S. griseus* cells and liposome-treated cells cultivated at 41^{o}C were about -122 and -510 kJ/mol, respectively, evaluated by the ATPS method. The above results show that the variation of the cell surface properties could be obtained by the cell-liposome interaction under heat stress conditions.

1. Introduction

Streptomyces griseus is a Gram positive bacterium, which is known to produce both chitinase and chitosanase enzymes by fermentation [1]. In our previous study, the utilization of liposomes and heat stress could enhance the production and release of chitosanase from *S. griseus* cells [2], implying that the interaction between chitosanase from *S. griseus* cells and liposomes under heat stress conditions could play an important role [2].

Liposome, a closed bilayer phospholipids membrane, has been used as a model of a cell membrane and has been used in various research activities. For example, it has been reported that the cationic liposome-DNA complex could be utilized to enhance gene delivery [3]. The utilization of liposome in drug delivery systems has also received a lot of interest [4]. Even though there is much interest in the interaction between liposomes and cell membranes such as binding, fusion, and endocytosis of liposomes with the cells mainly focusing on their electrostatic interaction [5, 6, 7, 8], the ability to control the interaction between the liposomes and biological cells is still limited owing to the lack of a quantitative approach.

. In the previous study, it has been reported that heat stress and liposomes enhanced the production and release of chitosanase from *S. griseus* cells [2]. It is thus important to understand the variation of the cell surface properties of these cells after liposome treatment under heat stress conditions. The surface properties of *E. coli* and *S. cerevisiae* have been successfully evaluated by using the ATPS method [9]. The purpose of this study is to characterize the variation of the surface net charge and the surface net hydrophobicity of *S. griseus* cells and liposome-treated cells under heat stress conditions.

2. Experimental

Poly (ethylene glycol) (PEG 1540, 4000, 6000; Mw 1.5 kD, 3kD and 7 kD, respectively) and dextran (Dex) (60~90k, 100~200k; Mw 60-90 kD and 100-200 kD, respectively) were purchased from Wako Pure Chemicals Ltd. (Osaka, Japan). The basic composition of the systems (the total weight is 5 g) for the partitioning of the cells was 7~13 wt% PEG 1540, 4000, 6000 and 7~13 wt% Dex 60-90k, 100-200k. The ATPSs were prepared by mixing the stock solutions of 30 wt% PEG and 30 wt% Dex with the cell suspension as described below. The pH values of these systems were adjusted by the addition of high concentration HCl and NaOH solutions.

Streptomyces griseus cells were obtained by growing in seed culture media as previously reported [2]. After centrifugation, the cells were washed several times with distilled water. The final concentration of these cells was adjusted to about $1x10^6$ CFU prior to use. This cell suspension was treated by heat stress at 41^oC in the absence and presence of 100 nm POPC liposome (0.1 mM as final concentration) for 1 h.

The surface net hydrophobicity (*HFS*) of the *S. griseus* cells and the liposome-treated cells was analyzed by using the aqueous two-phase partitioning method [10], in a similar manner to that for amino acids, protein and bacterial cells [11]. The surface net charge (*Z*) of the *S. griseus* cells was determined on the basis of the partition coefficient of *S. griseus* ($K_{cell} = C_{top} / C_{bottom}$) in the top and bottom phases of the ATPS containing different types of salts, which give positive and negative electrostatic potentials between the two phases, with the same anion ionic strength under various pH conditions. The cell concentrations in the top and bottom phases were determined by UV absorption at 600 nm. The calculation of the surface net charge (*Z*) of the *S. griseus* cells was performed as follows [12]:

$$\ln K_1 = \ln K_{cell,NaCl} = \ln K_0 + \gamma_1 Z \quad (1)$$

$$\ln K_2 = \ln K_{cell,Na_2SO_4} = \ln K_0 + \gamma_2 Z \quad (2)$$

$$\Delta \ln K = \ln K_1 - \ln K_2 = (\gamma_1 - \gamma_2) Z = \Delta\gamma Z \quad (3)$$

where γ_1, γ_1 are the electrostatic potentials between the two phases with NaCl and Na_2SO_4, respectively, and $\Delta\gamma$, an increment of the electrostatic potential, was determined based on the partitioning of amino acids ($\Delta\gamma = 0.056$).

3. Results and Discussion

The surface net charge of the *S. griseus* cells and liposome-treated cells was evaluated at various pH values by using ATPS method. **Figure 1 (a)** shows a typical example of the pH dependence of cell partitioning in the PEG 4000 (9%)/Dex 100-200k (9%) system with NaCl or Na_2SO_4 under heat stress conditions. The partition coefficient of the *S. griseus* cells was dependent on the pH in the different ATPS.

In the case of the ATPS containing 400 mM NaCl, the partition coefficient of the cells decreased with increasing pH. On the contrary, the partition coefficient of these cells increased with increasing pH in the ATPS containing 200 mM Na_2SO_4. It has been reported that the surface net charge of the biomolecules can be calculated from the partitioning behavior in ATPS having positive and negative electrostatic potentials between the two phases. The electrostatic potential of the above two types of ATPS was first evaluated on the basis of the partition behavior of charged amino acids (arginine and glutamine) where their surface net charge could be calculated. On the basis of the electrostatic potential of the ATPS, the surface net charge of the *S. griseus* cells was calculated and plotted in **Fig. 1 (b)**. From similar calculations, the surface net charge of liposome-treated *S. griseus* cells under heat stress is also shown in **Fig. 1 (b)**. The values of the isoelectric point (pI) of the *S. griseus* cells and the liposome-treated cells, determined as the crossing-point [12], are shown as 3.2 and 2.3, respectively.

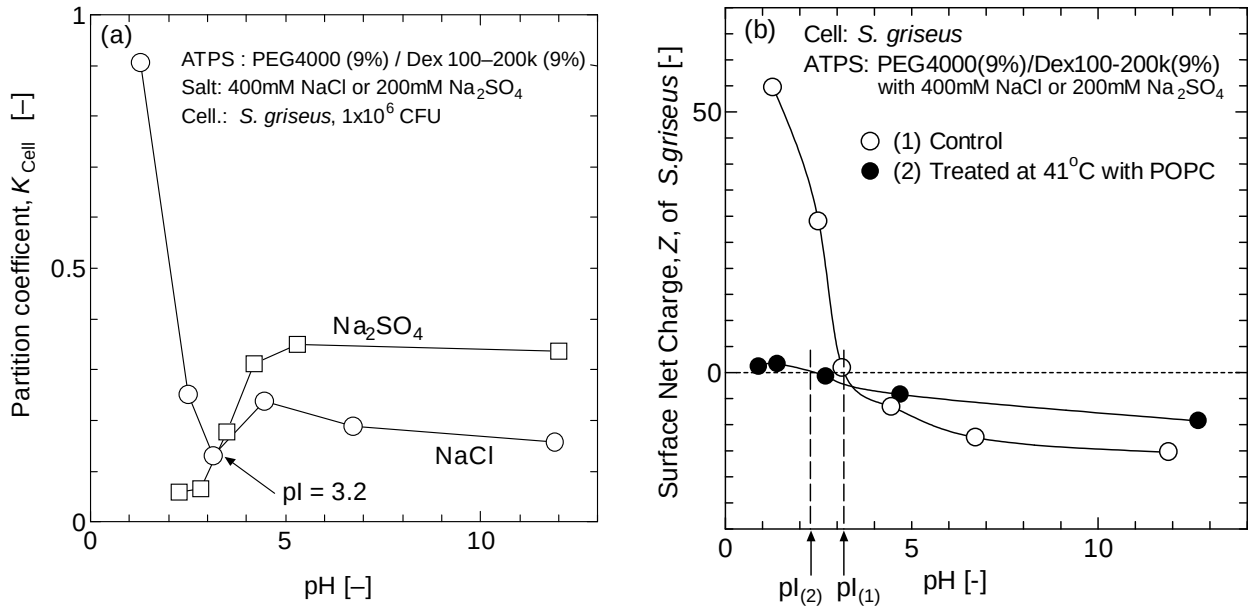

Fig. 1 Dependence of the partition coefficient (a) and surface net charge (b) of *S. griseus* cells and liposome-treated cells evaluated by the ATPS method on the pH.

Figure 2 (a) shows the summary of the electrostatic properties of the two types of cells. The surface net charge (*Z*) and the isoelectric point (pI) values of the *S. griseus* cells were similar to that of *E. coli* W3110 strains in a stationary phase cultivated at 37°C. However, the values were varied after heating at 41°C with POPC liposomes, resulting in a significant reduction of the surface net charge of the *S. griseus* cells (*Z* reduced from -13.1 to -5.8 at pH 7.5). This observation implies that the zwitterionic POPC liposomes might aggregate on the surface of the *S. griseus* cell to reduce its surface net charge and pI value.

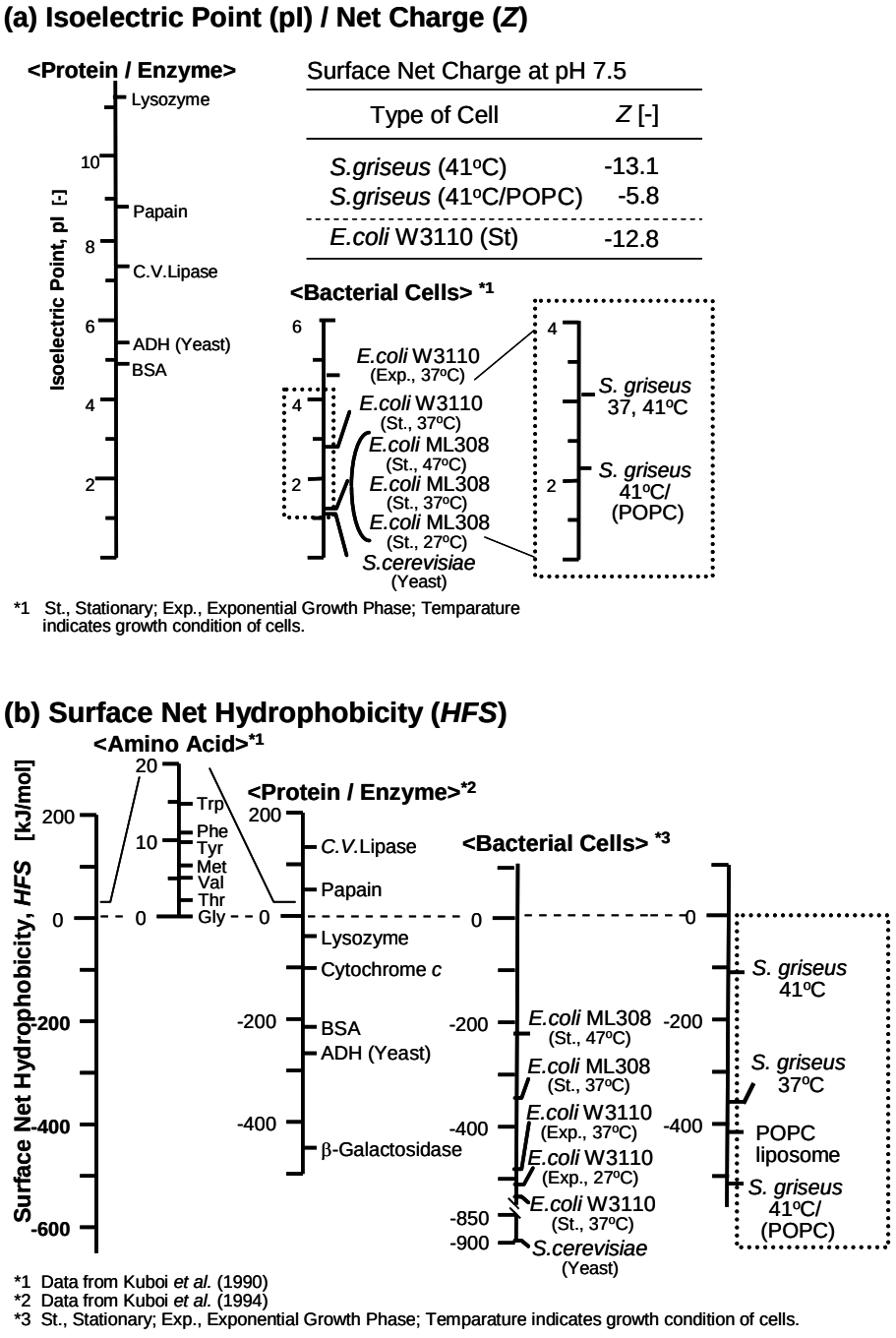

Fig. 2 Comparison of pI and *HFS* values of *S. griseus* cells and liposome-treated cells.

The surface net hydrophobicity (*HFS*) of the two types of cells was further evaluated by using the aqueous two-phase partitioning method at the determined isoelectric point (pI) value according to the previously reported method [9, 10, 11]. The obtained *HFS* values are shown in **Fig. 2 (b)**. The *HFS* values of *S. griseus* cells at 37 and 41°C were -358 and -122 kJ/mol, respectively. These were relatively high for

various types of bacterial cells shown here, showing that the surface of the *S. griseus* cells is basically hydrophobic. In the case of liposome-treated *S. griseus* cells at 41°C, the value was reduced to -510 kJ/mol, showing that the cell surface of the *S. griseus* cell became more hydrophilic through the interaction with POPC liposomes at 41°C. However, the *HFS* value of these cells cultivated at 37°C was not significantly changed in the presence and absence of liposome. These results also imply that the heat stress induced the direct interaction of hydrophilic POPC liposomes with the membrane of the *S. griseus* cells.

4. Conclusion

The surface properties of *S. griseus* cells such as surface net charge and surface net hydrophobicity are varied through its co-incubation with POPC liposomes at 41°C. This phenomenon is important to the understanding of the mechanism of the chitosanase release by *S. griseus* cells induced by heat stress at 41°C and POPC liposomes as reported in our previous paper [2]. The direct interaction of POPC liposomes with the membrane surface of these cells is considered to play an important role during the release of chitosanase under heat stress conditions. The values for *E. coli*, *Saccharomyces cerevisiae* and protein reported by Umakoshi et al. [9] and Kuboi et al. [11] were evaluated by the ATPS method.

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