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学 位 論 文 名	Study on Membrane-Membrane Interactions Induced by Heat Stress and Applications to Produce Chitosanase and Its LIPOzyme from *Streptomyces griseus* （熱ストレス誘導型膜－膜間相互作用および *Streptomyces griseus* からの Chitosanase 酵素 /LIPOzyme 生産への応用に関する研究）
論 文 審 査 委 員	（主査） 教　授　久保井亮一 （副査） 教　授　實川浩一郎　　教　授　田谷　正仁　　准教授　馬越　　大

論 文 内 容 の 要 旨

The production of biofunctional oligochitosan has widely attracted many researchers because of its various applications in food, pharmaceutical, cosmetics and so on. Chitosanase is known as a good enzyme that effectively hydrolyzes chitosan to small functional oligochitosans. A lot of studies have focused on the enhanced production of chitosanase from microbial cells and/or the effective ways to use chitosanase for such purposes. Recently, however, the importance of membrane-membrane interaction has been shown to play the crucial roles for the enhanced production and secretion of intracellular enzymes. In this study, the membrane-membrane interaction induced by the heat stress is studied to prepare new-type catalyst (chitosanase-LIPOzyme) that shows significantly higher in catalytic efficiency and stability than those of conventional chitosanase. The chitosanase-LIPOzyme prepared in this study represents a novel example of hydrolysis LIPOzyme that can be prepared from the enzyme harboring signal peptide associated-liposomes interacting cell membrane under stress conditions.

In chapter I, the effective production and secretion of chitosanase from *S. griseus* cells were studied by the treatment of these cells with the neutral 1-palmitoyl- 2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC) liposomes together with heat and/or oxidative stress. The effects of the liposomes and heat treatment on the cell growth and chitosanase production were first analyzed. The possible effects of the heat stress on the conformation and activity of chitosanase, as well as on the membrane fluidity of liposomes, were systematically investigated to elucidate the driving force of the heat induced liposome-chitosanase interaction. The interaction of liposomes with target chitosanase and cell lipid membrane play important roles for the enhanced production and secretion of chitosanase from these cells under heat stress condition. The fundamental investigations of the oxidative stress (hydrogen peroxide) affecting on the lipid and protein peroxidation of cell membrane of *S. griseus* pretreated with and without liposomes were further carried out to optimize the conditions for enhanced production and secretion of chitosanase. The interaction of liposomes with cell membrane could significantly prevent the oxidative damage on cell membrane; as a consequence, the growth and production of chitosanase from these cells pretreated with liposomes and heat stress at 41 °C were enhanced even under oxidative stress condition.

In chapter II, the surface properties of *S. griseus* cell treated with the heat and/or oxidative stress in the presence of liposomes were quantitatively evaluated in order to optimize conditions for the interaction of liposomes with cell membrane and secreted chitosanase under such stress conditions. The characterization of

surface net charge and hydrophobicity (*HFS*) of *S. griseus* cells was carried out by using aqueous two-phase partition system (ATPS). The surface hydration of cell membrane and those of various kinds of liposomes together with their hydrogen bond stability under different stress conditions were systematically evaluated by using Fourier transform infrared spectrometer (FTIR). The results have shown that the heat treatment enhanced *HFS* value relating to the increasing membrane fluidity, and reducing surface hydration (water loss) of cell membrane under heat stress condition. Based on these fundamental investigations, the interactions of liposomes with the cell membrane such as the adsorption, fusion, and internalization could be effectively induced by the hydrophobic, electrostatic, and hydrogen bond interactions through the stresses mediated variation of surface properties of cell membrane and liposomes.

In chapter III, on the basis of fundamental results obtained in the chapter I and II, the preparation method, the heat stress induced chitosanase secretion which resulted in the chitosanase-LIPOzyme (chitosanase bound-liposome membrane), was further investigated as a case study. The secreted chitosanase containing signal peptide (SP) associated with the lipid membrane of liposomes interacting cell membrane of *S. griseus* cell pretreated with inhibitor of signal peptidase under heat stress condition. Characterization was made based on the protein molecular weight, the catalytic efficiency and stability of these LIPOzymes under stress conditions. The stress resistant chitosanase-LIPOzyme prepared showed higher catalytic efficiency and stability as compared to those of conventional chitosanase under various stress conditions. Finally, the effective production of biofunctional oligochitosans by using chitosanase-LIPOzymes was also shown.

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論文審査の結果の要旨

細胞は生体膜ならびに細胞内部の膜群(細胞内小器官)から構成される．従来から，生体膜ならびに細胞内膜の特性に着目して，遺伝子導入，DDS，タンパク質生産の改善など，各種の応用が試みられている．しかし，従来のアプローチでは，ゲノム(Genome)およびプロテオーム(Proteome)というアプローチが一般的であり，細胞内に特定機能を有するタンパク質・酵素を発現させる手法に限定して研究が進められている．生体膜の主要構成成分であるリン脂質二重膜であるものの，そのものの機能については着目されていなかったのが現状である．

本学位論文では，生体膜のストレス応答機能を有効利用した物質生産/材料調製プロセスを開発する事を目的として、ストレス条件下で誘導される生体膜(細胞質膜)とリポソーム膜(モデル生体膜)の相互作用を体系的に検討した．有用生体高分子として知られるOligochitosanあるいはglucosamineを生産する酵素であるChitosanaseを*Streptomyces griseus*菌から生産分離するプロセス，または，Chitosanaseをリポソーム表層に提示したChitosanase-LIPOzyme (Liposome + Enzyme)という材料開発について応用されている．第１章では，リポソーム共存下における*S.griseus*菌からのChitosanase生産・放出に及ぼす熱ストレス負荷効果について検討されている．熱ストレスを最適に負荷する事により，通常の培養条件と比較して，約2倍程度のChitosanaseが得られる事を示した．同様に他のストレス(酸化ストレス)についても検討しており，リポソーム共存下で熱/酸化ストレスを適切に負荷する事により，Chitosanase生産・放出特性を改善できる事を示した．第２章では，熱ストレス条件下におけるリポソームと生体膜との相互作用について体系的に検討されている．生体膜ならびにリポソームの表面特性を，水性二相分配法・FTIR法を用いて解析し，膜膜間相互作用における物理化学的特性の役割について検討し，静電的相互作用に始まり，膜の疎水環境中における水素結合安定性，ならびに，膜表層の水和状態が重要である事を示した．第３章では,熱ストレスにより膜間相互作用を制御する手法を確立した．そのケーススタディとして，*S.griseus*菌からのChitosanase生産・放出を改善する手法を述べると共に，Signal Peptideを保持したChitosanaseをリポソーム表層に提示したChitosanase LIPOzymeの調製について検討されている．最終的に調製されたChitosanase-LIPOzymeは，(i)従来のChitosanaseと比較して活性が５倍程度であり，(ii)酸/アルカリ/熱などの各種ストレスに対する耐性が高く，(iii)膜モジュールとの併用が可能である事から，工業的な応用の可能性も指摘されている．

以上の様に，本学位論文では，ストレス条件下における生体膜-リポソーム膜間相互作用を体系的に検討を進めると同時に，ストレス制御型物質生産/材料創成の手法についても示されている．よって，博士（工学）の学位論文として価値のあるものと認める．