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論文内容の要旨

Antioxidative stress-response functions of the biological cells can be induced and utilized for survival when the cells were exposed to oxidative stress condition. From the conventional viewpoint of molecular biology, the specific genes, such as *sod* or *cat*, are expressed under the oxidative stress condition to protect the cell from the reactive oxygen species (ROS) through the expression of antioxidative enzymes eliminating ROS, such as superoxide dismutase (SOD) and catalase (CAT). However, there are some biological cells, which defect the gene for the antioxidative enzymes, though they still survive under the oxidative stress condition. Such a contradictory mismatch between genotype and phenotype in the biological system could sometimes be caused by the potential functions of the biological membrane induced under the oxidative stress condition. In this study, the potential functions of phospholipid bilayer membrane (liposome) were systematically studied, focusing on the membrane recruited activity on its surface via the use of the minimal elements i.e. liposomes, metal ions, and the fragments of SOD.

In chapter I, the roles of membrane in reactivation of oxidatively-damaged enzyme were demonstrated to clarify the membrane function in biological systems. Under oxidative stress, SOD was observed to be fragmented, leading to enzymatic inactivation, and the loss of an α -helix neighboring its active center. The H_2O_2 -treated SOD, which lost their activity depending on stressed condition, was reactivated on the added POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) liposomes, resulting in the increase of enzymatic activity. When strong stress induced fragmentation of SOD, produced specific peptide fragments could interact with POPC liposomes. Liposome membrane was found to assist the conformational change of oxidized and fragmented superoxide dismutase (Fr-SOD) and accelerate the adsorption of metal ions on the fragment to give the original SOD-like activity. The mixture of all of these elements (fragmented SOD and POPC liposomes with Cu^{2+} and Zn^{2+}) gave not only the increase of the α -helix and β -sheet contents but also the induction of the high SOD-like activity. In this case, the POPC liposomes could act like molecular and metal chaperones for the stress-damaged peptides, resulting in the creation of a new SOD-like active center, i.e. the antioxidative LIP0zyme that continues to express the SOD-like enzymatic activity under the oxidative conditions.

In chapter II, characterization of the liposome recruited activity, LIP0zyme activity, was made by studying the effect of lipid composition of liposome and by analyzing the possible elemental steps of the formation of LIP0zyme. The specific peptide fragment, originated from SOD, was first analyzed, and its sequence was identified. The oxidized SOD fragment was found to be displayed on the liposome

surface through the following three steps: (1) binding (recognition), (2) refolding, and (3) reactivation on the membrane. Such interaction was considered to be related with the characteristics of the both fragments and liposomes caused by the combination of electrostatic, hydrophobic interactions, and hydrogen bonding between the peptide and liposome membrane. A strategy to elucidate the liposome-induced antioxidative activity by recruiting the minimal elements has finally been established, focusing on the “state of stressed membrane” (the membrane activity and toxicity).

In chapter III, conversion and regulation of the antioxidative function of the liposome were attempted as a case study of the strategy described in chapter II. Surprisingly, the CAT-like activity, which decomposes H_2O_2 , could be induced on the liposome surface which displays the SOD-fragments-metal ions (Cu/Zn) complex. Actually, after the fragment-metal complex was recovered by liposome membrane, under continuous oxidative stress of hydrogen peroxide, membrane could regulate both enzyme-like activities. The LIPOzyme or the membrane-recruited SOD fragment possessed not only SOD-like enzymatic activity but also CAT-like activity, resulting in the decomposition of all the ROS in the biological system. The SOD-like activity and CAT-like activity were found to be modulated depending on the state of the membrane. Unexpected response of living cell (algal cell) such as survival under the strong oxidative stress could be related to such membrane-recruited antioxidative activities of the LIPOzyme.

The obtained results demonstrated the potential roles of membrane in recognition, recruitment, modification, and regulation of active sites of the LIPOzyme. The present results are applicable in modulation of artificial enzyme to control the oxidative agents and in the deeper understanding of the cellular response against oxidative stress.

論文審査の結果の要旨

酸化ストレス(Reactive Oxygen Species; ROS)は、地球上で生きる限り不可避なストレスである。近年、アルツハイマー症や癌などの各種疾病と酸化ストレスとの関連性が指摘されている。生体細胞には、ROSを効率的に利用する分子機構が存在する。特に、ROSを触媒反応により消去する抗酸化酵素(Superoxide Dismutase (SOD)ならびにCatalase (CAT))が注目されているが、触媒反応により生成するROSが蓄積した場合、容易に失活するという問題点があった。

本学位論文では、ROSにより失活したSOD/CAT抗酸化酵素システムが、ストレス負荷(モデル)生体膜の潜在機能(分子認識、構造形成、活性中心構築ほか)により、再度、本来の機能を獲得するという現象について体系的に検討した。さらには、上記の基礎原理に基づいて抗酸化LIPOzyme(SOD/CAT LIPOzyme)を調製した。1章では、SOD LIPOzymeの調製方法について検討した。SOD生成物である過酸化水素が高濃度で共存する条件でSODの失活・フラグメント化現象を解析した。SODがフラグメント化されるにも関わらず、リボソーム(リン脂質二重膜から成る閉鎖小胞)を添加するのみで、本来のSOD活性を誘導できる事を示した(SOD LIPOzyme機能)。2章では、SOD LIPOzyme機能の誘導機構を評価した。各種表面特性の異なるリボソーム共存下におけるペプチド認識特性ならびにSOD活性を詳細に解析した結果、上記のSOD LIPOzyme活性獲得は、(i) フラグメント化したペプチド群からの特定ペプチドの認識、(ii) 構造形成、(iii) 金属吸着、(iv)活性中心構築により達成されている事を示した。上記の知見ならびにペプチドの解析結果に基づき、活性誘導の第1条件となる特定ペプチドの認識に際しては、リボソームとペプチド間の静電的相互作用、疎水性相互作用、ならびに水素結合が複合的に関与している事が示された。以上の解析結果は、LIPOzyme調製のためのスキームとして示されている。3章では、そのケーススタディとして、ストレス負荷修飾法によるSOD/CAT抗酸化LIPOzymeの調製について検討した。SOD LIPOzymeへの酸化ストレス負荷により、リボソーム膜上でのペプチドの構造状態ならびに活性中心を制御し、SODとCAT活性を同時に発現するSOD/CAT LIPOzymeを調製できる事を初めて示した。

以上の様に、本学位論文では、抗酸化LIPOzymeの調製と特性評価について体系的に検討を進めると同時に、リボソームをコア材料とした人工酵素/触媒の全く新しい調製方法も示されている。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。