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論文内容の要旨

Introduction

A number of organisms accumulate compatible solutes in their cells in response to environmental stresses such as high salinity, dehydration, and low temperature. Among such compatible solutes, glycinebetaine is widely found from bacteria to higher plants and animals. Glycinebetaine is a quaternary ammonium compound that contains positive and negative charges within a molecule. The physiological functions of glycinebetaine have long been argued. It has been suggested that glycinebetaine protects the cells from salt stress by keeping osmotic balance with the environment, and by stabilizing the quaternary structure of complex proteins. Regardless of numerous studies, there has been no direct evidence on the role of glycinebetaine in protecting the cells from those stresses. It cannot be ruled out that some others biochemicals, which are also accumulated in response to the stresses, play a significant role in the protections.

In the present study, the protective role of glycinebetaine was studied in vivo by establishing the biosynthesis of glycinebetaine in the cyanobacterium, Synechococcus sp. PCC 7942 that does not accumulate glycinebetaine. Choline oxidase of a soil bacterium, Arthrobacter globiformis converts choline to glycinebetaine without a requirement of any cofactors. The codA encoding this enzyme was cloned from Arthrobacter globiformis, and was introduced into Synechococcus sp. PCC 7942. The resultant transformed cells accumulated glycinebetaine, and consequently acquired the ability to tolerate multiple stresses.

Cloning of the codA gene

The codA gene was isolated from Arthrobacter globiformis as follows: First, the amino-terminal sequence of 21 amino acid residues of the choline oxidase of Arthrobacter globiformis was determined. A DNA fragment corresponding to this amino acid sequence was amplified by PCR. The obtained DNA fragment was used as a probe for screening a genomic DNA library of Arthrobacter globiformis to clone the codA gene. The gene contained an open reading frame of 1,641 bp which encoded a polypeptide of 547 amino acid residues. Based on the sequence analysis and comparison with known flavoproteins, the amino-terminal region of the deduced amino acid sequence of choline oxidase was identified as a putative FAD-binding site of the protein.

Transformation of *Synechococcus* sp. PCC 7942 with the codA gene

The codA gene was inserted into the plasmid pAM1044 that contained the conII promoter, a spectinomycin-resistance cartridge, and intergenic regions of the chromosomal DNA of Synechococcus sp. PCC 7942 which allowed the integration

of the insert into the chromosome by homologous recombination. The plasmid pAM1044/codA gene was introduced into the cyanobacterium Synechococcus sp. PCC 7942, and the resultant transformed strain was designated PAMCOD. The control strain designated PAM was also produced by transforming Synechococcus sp. PCC 7942 with the plasmid pAM1044. Analysis by PCR indicated that the inserts were integrated into the chromosomes and all the copies of native chromosomes had been replaced by the recombinant one. Western blot analysis showed that the codA gene was expressed under the control of the conII promoter in the cells of strain PAMCOD. The accumulation of glycinebetaine in the cells of strains PAM and PAMCOD was determined by ^{1}H NMR spectroscopy. No traces of glycinebetaine were detected in the cells of strain PAM. By contrast, the cells of strain PAMCOD accumulated glycinebetaine at intracellular levels of 60-80 mM.

Tolerance to salt stress

The effect of accumulation of glycinebetaine in protecting the cells from salt stress was evaluated in terms of growth, accumulation of chlorophyll, and photosynthetic activity. In the presence of 0.4 M NaCl, the growth of the PAM cells was completely inhibited, whereas the PAMCOD cells were able to grow after a lag period. The PAM cells did not accumulate chlorophyll under these conditions, while the PAMCOD cells synthesized chlorophyll. The photosynthetic activities of both strains decreased during the initial period of incubation in the presence of 0.4 M NaCl. However, the PACOD cells could subsequently recover their photosynthetic activity, and then the cells started to proliferate again. These results suggest that the accumulation of glycinebetaine in the cytoplasm enhances the tolerance to salt stress in the PAMCOD cells. However, some lag time is necessary before the effectiveness of glycinebetaine in protecting the cells against salt stress becomes apparent.

Tolerance to low- and high-temperature stresses

The PAMCOD cells were able to grow at 20℃ and 42℃ at which the growth of the PAM cells was remarkably retarded. The effect of accumulated glycinebetaine on tolerance of photosynthesis to temperature stress was also examined. The results are described as follows:

(1) The photosynthesis of the PAMCOD cells was more resistant to low temperatures ranging from 0℃ to 10℃ in darkness than that of the PAM cells.

The inactivation of photosynthesis in darkness at low temperature has been suggested to be caused by the phase transition of lipids of plasma membrane from the liquid-crystalline state to the phase-separated state. The author found that the temperature of phase transition of the PAMCOD cells was shifted to lower temperature than that of the PAM cells. Whereas, the chemical analyses indicated that the transformation with the codA gene did not alter the fatty-acid composition of the plasma membrane. Thus, these results suggest that the

presence of glycinebetaine in the cytoplasm enhances the tolerance of photosynthesis to low temperature in darkness by decreasing the temperature for phase transition.

(2) The photosynthesis of the PAMCOD cells was more resistant to low-temperature stress in light (photoinhibition) at the temperature range about 20 ℃ than that of the PAM cells. The extent of photoinhibition in vivo results from the balance between two processes, the initial damage to D1 protein that is followed by the repairment of photosystem Ⅱ activity by a newly synthesized D1 protein. The author found that photosynthetic activity of the PAMCOD cells could recover from photoinhibition with faster rates than of the PAM cells, and that the presence of lincomycin, the inhibitor of protein synthesis, subtracted the tolerance of photosynthetic activity of the PAMCOD cells. These findings suggest that the recovery process of photosynthesis from photoinhibition is accelerated in the PAMCOD cells. Therefore, it can be suggested that the inhibition of growth of the PAM cells at 20℃ was initially caused by photoinhibition.

(3) The tolerance of photosynthesis to high-temperature stress in darkness and light was not changed in the PAMCOD cells as compared with that of the PAM cells. It is likely that the concentration of accumulated glycinebetaine of about 80 mM was not high enough to be effective in the stabilization, since 1 M glycinebetaine is required for stabilizing the photosystem Ⅱ complexs. These results may suggest that some mechanisms other than the stabilization of photosynthesis are involved in the acquisition of tolerance to high temperature in the PAMCOD cells.

審査結果の要旨

申請者Patcharaporn Deshniumの申請論文「Genetically engineered alteration of stress tolerance in *Synechococcus*：protective roles of glycinebetaine」は、植物細胞のモデルであるラン藻*Synechococcus*にグリシンベタイン合成酵素の遺伝子を導入し、得られた形質転換ラン藻の高塩ストレスおよび低温、高温ストレスに対する耐性を評価することにより、グリシンベタインの保護効果をin vivoの系で解析したものである。

この論文は四つの章に分かれている。第一章は生物の環境ストレス応答についての概論であり、第二章は土壌細菌*Arthrobacter globiformis*からグリシンベタイン合成酵素コリンオキシダーゼの遺伝子（*codA*）の単離、第三章は*codA*遺伝子のラン藻*Synechococcus*への導入、第四章は形質転換ラン藻の環境ストレス耐性の解析を記述している。

グリシンベタインは、耐塩性植物に多く蓄積されることが従来から知られており、また光合成活性を高塩や高温による傷害から保護することがin vitroの実験から判っている。申請者は、グリシンベタインを蓄積しないラン藻*Synechococcus*にグリシンベタインの生合成経路を確立させることにより、生体内におけるグリシンベタインの保護効果を明らかにするを目的とした。この際、土壌細菌*Arthrobacter globiformis*が有するコリンオキシダーゼが、コリンから一段階でグリシンベタインを合成することに注目し、この細菌からコリンオキシダーゼの遺伝子*codA*を単離してラン藻の形質転換に用いた。得られた形質転換ラン藻は細胞内に60から80mMのグリシンベタインを蓄積し、野性株が生育できない高塩濃度でも生育できるようになった。その原因の一つとして、形質転換体の光合成活性が高塩ストレスに対してより耐性になっていることを示した。さらに、形質転換体は低温や高温のストレスに対しても野性株より耐性となった。形質転換体の低温耐性の機構に関しては、光化学系II活性が低温下で起こる光傷害から回復する過程がグリシンベタインの蓄積により促進されることを示した。

以上のように、申請者Patcharaporn Deshniumの研究は生物学の研究として高い水準を持っており、博士論文としてふさわしいものであると判断した。なお、これらの成果の一部はPlant Mol. Biol. 誌に発表されている。

また、審査委員全員の立ち会いの下で、申請者Patcharaporn Deshniumに口答諮問を行った。申請論文の内容、背景、基礎的な知識について質問し、回答について検討した結果、十分な学力を有すると判断した。英語の語学力については、英語による口答諮問、英文で書かれた申請論文、および筆頭著者として国際誌で発表した論文を検討した結果、研究者として十分な語学力を持っていると判断した。