氏名	坂　本　敏　夫
学位（専攻分野）	博士（理学）
学位記番号	総研大甲第９２号
学位授与の日付	平成　６　年　３　月２４日
学位授与の要件	生命科学研究科　分子生物機構論専攻 学位規則第４条第１項該当
学位論文題目	Fatty acid desaturases of cyanobacteria and the modification of membrane lipid unsaturation.
論文審査委員	主　査　教　授　村　田　紀　夫 教　授　藤　田　善　彦 教　授　西　村　幹　夫 助教授　岡　田　清　孝

論文内容の要旨

The physical properties of biological membranes depend on the fatty acid unsaturation of membrane lipids. Cyanobacteria respond to a decrease in ambient temperature by desaturating fatty acids of membrane lipids to compensate the decrease in membrane fluidity at low temperature. Fatty acid desaturases are enzymes that introduce double bonds into hydrocarbon chain of fatty acids. The enzymes play an important role to control the fluidity of membrane lipids in the acclimation of cyanobacterial cells to low temperature.

In the cyanobacterium, *Synechocystis* PCC6803, polyunsaturated fatty acids are synthesized by sequential introduction of double bonds into C_{18} fatty acids esterified to the *sn*-1 position of glycerolipids by four distinct desaturases, and each of the desaturases catalyzes the desaturation of fatty acids at a specific position: the Δ6, Δ9, Δ12 or ω3 position. At the beginning of the present studies, the *desA* gene that encodes the Δ12 desaturase had been isolated from *Synechocystis* PCC6803 [Wada, H., Gombos, Z. and Murata, N. (1990) *Nature* 347: 200-203], but no other desaturase genes of cyanobacteria, or of higher plants had been reported.

In order to resolve the role of unsaturation of membrane lipids, it is necessary to control the degree of membrane lipid unsaturation by genetic manipulation of the fatty acid desaturases. In addition, the structural information of desaturases is requisite to understand the reaction mechanism of the fatty acid desaturation.

The author isolated cyanobacterial desaturase genes: the Δ12 desaturase genes (*desA*) from three strains of cyanobacteria, i.e.,

Synechocystis PCC6714, *Synechococcus* PCC7002 and *Anabaena variabilis*; the ω3 desaturase gene (*desB*) from the cyanobacterium, *Synechocystis* PCC6803; and the Δ9 desaturase genes (*desC*) from *A. variabilis* and *Synechocystis* PCC6803. The membrane-lipid unsaturation of cyanobacteria was altered by manipulating the isolated desaturase genes.

Cyanobacterial *desA* genes were isolated from *Synechocystis* PCC6714, *Synechococcus* PCC7002 and *A. variabilis* by cross-hybridization with DNA probes derived from the *desA* gene of *Synechocystis* PCC6803. It appeared that the *desA* genes of *Synechocystis* PCC6714, *Synechococcus* PCC7002 and *A. variabilis* encode proteins of 349, 347 and 350 amino acid residues, respectively. The amino acid sequences of the products of the *desA* genes revealed the presence of four conserved domains. The transformation of *Synechococcus* PCC7942 with the *desA* genes from the three strains was associated with acquisition of the ability to introduce the second double bond at the Δ12 position of fatty acids.

Cyanobacteria respond to a decease in temperature by desaturating fatty acids of membrane lipids to compensate the decrease in membrane fluidity. Among various desaturation reactions in cyanobacteria, the desaturation at the ω3 position of fatty acids is the most sensitive to the change in ambient temperature. The author isolated a gene for the ω3 desaturase, *desB*, from the cyanobacterium, *Synechocystis* PCC6803, using a heterologous hybridization probe derived from the *desA* gene of the same cyanobacterium. The *desB* gene encodes a protein of 359 amino-acid residues and of molecular mass of 41.9 kDa. In order to manipulate the fatty acid unsaturation of membrane lipids, the *desB* gene in *Synechocystis* PCC6803 was mutated by insertion of the

kanamycin-resistance gene cartridge. The resultant mutant was unable to desaturate fatty acids at the ω3 position. On the other hand, the *desA* and *desB* genes were introduced into cells of another cyanobacterium, *Synechococcus* PCC7942, which can desaturate fatty acids of membrane lipids only at the Δ9 position. The resultant transformant was capable of desaturating fatty acids at the Δ9, Δ12 and ω3 positions. These results demonstrate that the disruption and the introduction of the *desB* gene enable us to genetically manipulate the fatty acid unsaturation of membrane lipids in cyanobacteria.

In cyanobacteria, oleic acid is synthesized by introducing a double bond into stearic acid bound to polar glycerolipids. This biosynthetic pathway in cyanobacteria is distinct from the other organisms. The author found an open reading frame in the nucleotide sequence of the 5' upstream region of the *desA* gene of *A. variabilis*, and its deduced amino-acid sequence showed similarity to stearoyl-CoA desaturases of rat, mouse and *Saccharomyces cerevisiae*. Thus, the author postulated that the open reading frame of *A. variabilis* encodes the cyanobacterial Δ9 desaturase. The gene was designated *desC*. Using a DNA fragment derived from the *desA* gene of *A. variabilis*, another *desC* gene was isolated from *Synechocystis* PCC6803. Upon expression of the *desC* gene of *Synechocystis* PCC6803 in *Escherichia coli*, the *E. coli* cells accumulated oleic acid, which is not synthesized in the wild-type cells of *E. coli*. The deduced amino-acid sequence of the *desC* gene of *Synechocystis* PCC6803 shows significant similarity to the stearoyl-CoA desaturases of rat (25%), mouse (24%) and yeast (25% in the internal region corresponding to the *desC*).

Recently, the Δ6 desaturase gene was isolated from *Synechocystis* PCC6803 [Reddy, A.S., Nuccio, M.N., Gross, L.M. and

Thomas, T.L. (1993) *Plant Mol. Biol.* 27: 283-300]. Thus, the author has obtained the structural information of all desaturases of *Synechocystis* PCC6803. Similarity in terms of the amino acid sequence between the Δ12 desaturase (*desA*) and the ω3 desaturase (*desB*) is 27%. A short region of consecutive amino-acid identity was found in the ω3 desaturase and the Δ12 desaturase. By contrast, the extents of similarity in terms of amino acid sequence between the Δ6 desaturase and the other desaturases, and those between the Δ9 desaturase (*desC*) and the other desaturase are very small, namely 11-16%. However, striking similarities were found among the hydropathic characteristics of these four desaturases of *Synechocystis* PCC6803. The predicted products of these genes contain two hydrophobic region, and, therefore, it is likely that they are membrane-bound proteins. The comparison of the local structures of the four desaturases of *Synechocystis* PCC6803 disclosed the conserved amino-acid residues among these desaturases. It is likely that the conserved amino-acid residues are responsible for the introduction of a double bond into fatty acids bound to glycerolipids.

論文の審査結果の要旨

生物は低温に曝されると膜脂質の脂肪酸の不飽和結合を増加させるが、これは温度低下に伴う膜の流動性の低下を補償して、低温下における生理活性を一定の水準に保つための適応現象である。脂肪酸不飽和化酵素は、膜脂質の脂肪酸に不飽和結合を導入する酵素であり、低温適応における流動性の調節において重要な役割を担っている。本研究では、ラン藻から脂肪酸不飽和化酵素の遺伝子を単離し、さらにその遺伝子操作によって膜脂質の不飽和度を改変した形質転換体を作成している。

先ず、ラン藻 Synechocystis PCC6803 の△12位の脂肪酸不飽和化酵素の遺伝子（desA）を用いて、他の３種のラン藻（Synechocystis PCC6714, Synechococcus PCC7002, Anabaena variabilis）より desA 遺伝子を単離した。この desA 遺伝子を、シャトルベクターpUC303 を用いて Synechococcus PCC7942 に導入し、得られた形質転換体において△12位での不飽和化が進行することにより、desA 遺伝子の機能を同定した。続いて、Synechocystis PCC6803 よりω3位の脂肪酸不飽和化酵素の遺伝子（desB）を単離しその構造を明かにした。desB の機能は、desB を破壊した Synechocystis PCC6803 の形質転換体において、ω3位での脂肪酸の不飽和化が進行しないことにより同定した。また、Synechococcus PCC7942 に desA と desB を同時に導入することにより、△12位およびω3位での脂肪酸の不飽和化を進行させることができた。さらに、Anabaena variabilis および Synechocystis PCC6803 より、△9位の脂肪酸不飽和化酵素の遺伝子（desC）を単離し、その構造を明かにした。desC の機能は、不飽和化酵素を持たない大腸菌内での発現により△9位での不飽和化がおこることにより同定した。

この研究の特徴は、従来単離されれていなかったラン藻のアシル脂質不飽和化酵素の２種類の遺伝子を単離できたことであり、その成果はラン藻および高等植物の低温適応における膜脂質の役割を解明する研究、および不飽和化反応のメカニズム（二重結合の導入部位を決定するメカニズム）を分子レベルで解明する研究に大いに貢献するものと思われる。なおこの成果の一部は既に Plant Molecular Biology 誌に採択されている。

以上の内容により、審査会は当博士論文に対して合格の判断を下した。