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총 설

Symbionin Produced by Intracellular Symbionts, which has Molecular Chaperone Activity and Novel Histidine Protein Kinase

O-Yu Kwon† and Won-Sik Kim

Department of Anatomy, College of Medicine, Chungnam National University, Taejon 301-130, Korea

Abstract

Symbionin, a homologue of *E. coli* GroEL, produced by an intracellular symbiont of the pea aphid, has molecular chaperone activity both *in vitro* and *in vivo,* and it is able to tarnsfer its high-energy phospholy group to other compounds through its autophosphorylation and phosphotransferase activity. The symbionin is a novel histidine protein kinase and a sensor molecular of the two-component pathway.

Key words : *Symbionin, chaperone activity, histidine profein kinase, phosphorylation*

Introduction

Pea aphid, *Acyrthosiphon pisum,* harbors prokaryotic intracellular symbionts in the huge bacteriocytes in which of each cytoplasm is filled with thousands of symbionts, which are differentiated specifically to accommodate the symbionts[1]. The date of estabilishment of the symbiotic association is estimated to be 160-280 million years ago based on 16S rDNA molecular clock calibrated by aphid fossile[2]. The aphid and its intracellular symbionts are in a closely mutualistic relationship, and neither of them is able to propagate without the other[3]. Aposymbiotic aphids are markedly under sized body and sterile, and then symbionts are essential for the normal growth and reproduction of the aphid. They are maternally inherited generation to generation, and have not been successfully cultured outside the host bacteriocytes[4]. In view of the location and nutritional role of the symbionts in host insects, it is generally believed that they originated from gut bacteria taken in which diet by the host insect in the evolutionary past. According to the 16S rDNA phylogeny of bacteria, symbionts belong to the r-subdivision of the class Proteobacteria, and they are a class relative of *E. coli* in taxonomy[5,6].

In contast to the selective synthesis of symbionin by the symbionts in the bacteriocytes, the symbiont when outside of the host cells synthesizes several hundred proteins during about 2-3 hour. Symbionin is substantially the only protein produced by the symbionts *in vivo,* which is supposed to play a pivotal role in this symbiotic system[7]. DNA analysis of the symbionin showed that symbionin shares about 85% identical, at the amino acid sequence level, to the GroEL which is a major *E. coli* stress protein against heat shock[8]. Like GroEL, symbionin is a tetradecmeric(14-mer) homo-oligomer of 63kDa subunit[9]. Since the GroEL protein is a member of the "chaperonin" class of molecular chaperones, the close identity of symbionin to this *E. coli* pro-

† Corresponding author

tein raised the possibility that symbionin is also able to a stress protein which functions as a molecular chaperone[10, 11]. In fact, it has been shown that symbionin is able to molecular chaperone actions both *in vitro* and in vivo in mutant cells of *E. coli*[8].

When the isolated symbionts are exposed to heat shock, they synthesize several kind of stress proteins, one of which is phosphorylated form of symbionin[12]. It has been guessed that symbionin has the ability to phosporylate itself autocatalytically. Autophosphorylated symbionin is an energy-coupling protein able to transfer the high energy phosphate bond from ATP to other compounds through its autophosphorylation[13]. It has been also demonstrated that symbionin increases its chaperonin activity upon autophosphorylation. The 14-mer symbionin is disassembled more readily than the 14-mer GroEL, and in the presence of Mg-ATP the correct 14-mer structure is restored autogeously from monomeric (1-mer) symbionin molecules[14]. The reassembled symbionin not only had the same molecular mass as native symbionin but also exhibited the same ATPase and phosphotarnsferase activity. Phosphorylation site of symbionin has been determined at which is histidine-133. The results above make suggestion that symbionin functions as a histidine protein kinase, or a sensor molecule, of the two-component pathway known in other organisms. However, symbionin is not similar in amino acid sequence to any so far known histidine protein kinase[15, 16], and then symbionin is supposed as a novel histidine protein kinase.

Molecular structure and function of symbionin

Symbionin, a homologue of *E. coli* GroEL, which exists as a double-doughnut structure under E. M., consisting of two rings of seven 63kDa subunits demonstrated on SDS-, and native-gel, sharing similiar molecular structure properties with GroEL. According to molecular analysis of symbionin, it is more than 85% identical on the amino acid level and more than 75% identical on the DNA level to GroEL. In addition the antibody against symbionin has same ability for recognizing GroEL as a antigen. In the presence of Mg-ATP, denatured symbionin was reassembled without any kind of enzymes. And in the presence of GroES, denatured rubisco(ribulose biphosphate carboxylase/oxygenase) was also reassembled by symbionin, the resulting rubisco which has complete Co_2 fixation ability. In *in vivo* experiment GroE mutant tansformated with a expression vector containing isolated symbionin-gene showed complete properties like wild type of *E. coli*. The results suggested that symbionin has molecular chaperone activity like GroEL. Despite its structural and functional simility to GroEL, it, however, has a specific other function for maintaining intracellular system which is phosphorylated autocatalytically, and that it is able to tansfer the phosphate group to ADT and GDT, and hence has phosphotransferase activity. This functional difference between symbionin and GroEL raises the question of what structural difference causes it. The answer to this question will provide a clue to a further understanding of the structure and function of chaperonin in general.

Autophosphorylation and phosphotansferase activity of symbionin

The isolated symbionts were labeled with [^{32}P]orthophosphate at 39℃ for 30 min., and the symbionts were collected by centrifugation and homogenized. After discard cell debris, the resulting labeled proteins were resolved by SDS-PAGE and two-dimentional PAGE. Autophosphorylated symbionin(hyper-phosphorylationed form) was detected at 63kDa on SDS-PAGE and was more acidic than that of authentic symbionin in 2-D gel, and the phosphorylating activity was also observed with the immunocomplex of symbionin with anti-symbionin antibody. Since the phosphorylating activity survived heat treatment, it is noteworthy that symbionin could be autophosphorylated without any kind fo protein kinases.

In fact, an autophosphorylated symbionin, which is suggested that is an energy-coupling protein able to tansfer the high energy phosphate from ATP to other compounds through itself autophosphorylation. It has been also demonstrated that symbionin increases its molecular chaperone activity upon autophosphorylation. [^{32}P] labeled autophosphorylated symbionin was incubated with a molecular excess of ADP in the phosphorylation buffer at 37℃ for 60 min. The reacting proteins were passed by ultrafiltration to remove free [^{32}P], the resulting mixture was subjected to HPLC, and the eluate was counted for radioactivity. As a result, a significant amount of radioactivity was found in ATP.

To confirm whether symbionin has phosphotransferase activity, symbionin was incubated with [r-^{32}P]ATP and unlabeled GDP, a large amount of radioactivity was found in GTP, this result suggests that phosphate was transfered from ATP to GDP mediated symbionin as a phosphotransferase through autophosphorylation. However, GroEL used instead of symbionin catalyzed of ATP, but not phosphate tarnsfer to GDP under the same conditions. In addition when symbionin was treated with anti-symbionin antiserum, its phosphotransferase activity was entirely abolished, and then suggesting that the autophosphorylated symbionin is an energy-carrier mediated nothing of any kind of kinases. Symbionin probably has a dual function, serving as a phosphotansferase on the one hand, and on the other, as an intermediary substrate that mediates the phosphate transfer.

Determinatation of phosphrylation site in symbionin

Despite of GroEL and symbionin are more than 85% identical to each other at the amino acid level, sharing the ATPase activity and chaperonin activity in common, in contrast to symbionin, GroEL, which does not have phosphotarnsferase activity. It is possible that symbionin is also an energy-coupling protein and a kind of histidine protein kinase which autophosphorylates its histidinyl residues. In most cases, energy-coupling proteins are histidine protein kinases. Symbionin, which contains only 3 histidinyl residues, two of which have been substituted for alaninyl residues comparing GroEL. These two histidine codons, in common, have been created as a result of three consecutive base substitution at the symbionin gene, a very rare phenomenon. To determine the phosphorylated residue of symbionin, the autocatalytically phosphorylated ^{32}P-labeled symbionin purified through HPLC was digested with TPCK-treated trypsin, followed by isolation of ^{32}P-labeled peptide by reverse-phase HPLC using an ODS-H column. The ^{32}P-labeled symbionin fragment was sequenced on a gas-phase peptide sequencer, which is -Glu -Glu -Leu -Lys -**His** -Leu -Ser -Val -Pro -Cys- (-GAA. GAA. TTA. AAA. **CAT**. TTA. TCT. GTA. CCA. TGT-), in contrast to its bacterial sequence of -Glu -Glu -Leu -Lys -**Ala** -Leu -Ser -Val -Pro -Cys- (-GAA. GAA. CTG. AAA. **GCG**. CTG. TCC. GTA. CCA. TGC-). The substituted codons are indicated by broad and underline, respectively. Phosphorylated residue of symbionin was directly demonstrated as a following experiment, the radioactive fraction from the HPLC was hydrolyzed with 5.7N HCl or 3N KOH and the resulting hyrdolysate was analyzed on TLC using a silica gel plate. The silica gel plate showed the radioactive spot, indicating that the site of autophosphorylation in symbionin is histidine-133 from the 1st-Met. The amino acid sequence of symbionin around the autophosphorylation site is conserved well, however, only autophosphorylation site is changed by resulting of 3 nucleotides substitution, suggesting that symbionin was evolutionally accumulated for intracellular endosymbionts in the symbiotic system.

Phosphoryl-group transfer from symbionin to other endosymbiotic protein

As described above, symbionin is autocatalytically phosphorylated to produce the phosphorylated symbionin containing a high-energy phosphate bond, and a po-

rtion of that is transferred to ADP and GDP, suggesting that symbionin itself behaves just as a histidine protein kinase, or a sensor molecule, in the two-component pathway. In the known two-component pathways, the sensor molecules transfer their phosphoryl group to "response regulators". As an initial step to identify the partner protein corresponding to a response regulate that accepts the phosphoryl gruop from the autophosphorylated symbionin, the following experiment was carried out. The symbionts were isolated from aphid and lysed for SDS-PAGE. After electrophoresis, proteins were transferred on a PVDF membrane, and the phosphoryl group transfer was determnined by incubating the PVDF blot with ^{32}P-labeled symbionin. The resulting radioactive signals were detected by a major band at 42kDa and three minor species migrating at 20, 25, and 31 kDa. Four additional poor bands 55, 60, 63, and 66 kDa were also detected. The radioactive bands survived successive treatments with detergent, 1M NaCl, 1N KOH, and 10% acetic, suggesting that the phosphoryl gruop is covalently linked to these polypeptides. It is unlikely that the radioactive signal was due to the bound ^{32}P-labeled symbionin itself, because when the radiolabeled blot was incubated with anti-symbionin antiserum, only one among 8 species of radiolabeled polypeptides cross-reacted, namely that of 63kDa, corresponding to symbionin or phosphorylated symbionin. Especially, a main protein of 42kDa shares high homology at amino acid level with OmpF which is one of the important *E. coli* membrane protein has an ability for singal transduction. However both proteins, 42kDa and OmpF, have not same immune response activity against anti-symbioin antiserum, and then it is supposed that 42kDa protein is an unique acceptor of phosphoryl group from ^{32}P labeled symbionin. It is possible that at least some of these polypeptides are response regulators and/ or those chaperoned by symbionin in the symbionts.

An additional interesting is that monomeric symbionin has potent autophosphorylating and phosphoryl group transferring activity. The initial rate of autophosphorylation of monomeric symbionin was almost twofold higher than that of native symbionin. This may be simply due to increased accessibility of the phosphate moiety to His-133 because of the change of the higher-order structure of monomer symbionin. However, symbionin is not similar in amino acid sequence to any histidine protein kinase so far known, and then symbionin, symbiont chaperonin, is a novel type of histidine protein kinase for maintaining symbiotic system specifically differentiated through long-time of evolution.

Discussion

Symbionts housed by the aphid bacteriocytes selectively synthesize a chaperonin called symbionin, which is more than 85% identical with GroEL at the amino acid sequence level, has an oligomeric structure similar to that of GroEL. When isolated symbionts exposed to heat shock, a kind of enviromental stress, synthesize a phosphorylated form of symbionin. The mechanisim of phosphorylation of symbionin by heat shock demonstrated that symbionin is autocatalytically phosphorylated to produce a protein with a high-energy phosphate bond, which is transferred to ADP, GDP and another proteins located in the symbionts, suggesting that symbionin has dual functions as an enzyme and as an inermediary substrate for signal transduction at intracelluar symbiotic system. These characteristic properties of symbionin resemble those of the energy-coupling protien with phosphotarnsferase activity that regurates various types of bacterial adaptive responses. The peptide sequnce analysis and TLC analysis showed that symbionin is phosphorylated at a histidine residue at position 133 from 1st-Met. Camparing the bacterial histidine protein kinase domain with amino acid sequence of symbionin autophosphorylated site, it has no similarity despite of showing same phosphotransferase activity. It suggesting that symbionin is a novel type of histidine protein kinase that functions specifically in the signal transduction

in the symbionts. In this regard, it should be noted that His-133, the phosphorylation site of symbionin, has been created as a result of three consecutive base substitutions between the symbionin gene and GroEL. It is possible that in the symbiont, symbionin chaperones the substrate polypeptides on the hand, and on the other hand, through its autophosphorylation. The exact nature of the symbionin will hopefully lead to the unveiling of a still unknown property of eukaryotic symbiosis system and chaperonin activity is coupled with its ATPase activity through the cycle of phosphorylation/ dephosphorylation of this protein. Thus, when we understand fully the mechanism underlying the accumulation of the phosphorylated form of symbionin under stress, we will be very close to a full understanding of the energetic aspect of the chaperonin activity in general. Finally here we present the hypothetic schema of the two-component pathway in intracellular symbiotic system, which is showed in Fig. 1.

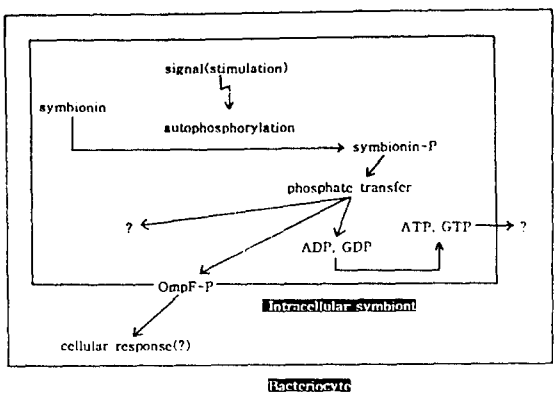

Fig. 1. Presumed signal transfer mechanism associated intracellular symbiont in the symbiotic system.

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초록 : Symbionin은 세포내 공생미생물이 생산하는 molecular chaperone 활성을 가진 색다른 histamine protein kinase이다

권오유 · 김원식†(충남대학교 의과대학 해부학교실)

대장균의 GroEL과 상동성을 가지는 symbionin이 진딧물의 세포내 공생미생물에서 유일하게 생산된다. 이것은 *in vitro*와 *in vivo*에서 molecular chaperone 활성을 가지는 것과 함께 자가인산화(autophosphorylation)와 인산기전이효소(phosphotransferase)의 활성에 의해서 고에너지 인산기를 다른 곳에 줄 수 있다. Symbionin은 two component pathway의 센서분자의 역할을 하며, 지금까지 알려진 것과는 다른 성질을 가진 protein kinase이다.