ABSTRACT

Expression and purification of JAZ3 protein and a transcriptional factor MYC2 from *Arabidopsis thaliana*

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Jasmonate is a plant hormone that regulates development, growth and wound response. There are many jasmonate derivatives in nature. Particularly, jasmonyl isoleucine serves as a bioactive molecule in the jasmonate signaling pathway. In fact, the jasmonate signaling is regulated by interactions among four different proteins, including MYC2, JAZ, NINJA, and TOPLESS. Among them, MYC2 and JAZ have

critical role in the jasmonate signaling pathway. JAZ binds to MYC2 and represses the jasmonate signaling. Especially, intermolecular interaction of JAZ and MYC2 is attributable to JID motif of MYC2 and Jas motif of JAZ. Nonetheless these interactions play a crucial role in the jasmonate signaling pathway, molecular details of JAZ and MYC2 remain unknown, mainly due to the insoluble properties of each JAZ and MYC2 protein *in vitro*. In this thesis, I report various approaches that could yield a protein crystal of Jas and JID motif in JAZ and MYC2, respectively. Eight fusion proteins were produced. In those fused proteins, two different constructs for Jas motif and four for JID region are linked using Tobacco Etch Virus (TEV) protease cleavage sequence. Various chromatographic analysis indicated that JAZ forms a complex with MYC2 by cleaving the TEV protease cleavage site. Although crystallization of the complex has not been achieved at this moment, this study provides valuable information for producing the JAZ–MYC2 complex *in vitro*.

Keywords: jasmonate, jasmonyl isoleucine, JA signaling, MYC2, Jasmonate–ZIM domain3

Studunt number: 2008–21341

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GFC	gel filtration chromatography
IMAC	immobilized metal affinity chromatography
JA	jasmonate
JA-Ile	jasmonyl isoleucine
JAZ	Jasmonate-ZIM domain
JID	JAZ-interacting domain
NLS	nuclear localization sequence
SDS PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SER	surface entropy reduction

INTRODUCTION

Jasmonate (JA), a kind of oxylipin, is a phytohormone derived from linolenic acid. Chloroplast and peroxisome are known to sites of JA synthesis (1). Concretely, JA synthesis is mediated by lipoxygenase isozyme(2), allene oxide synthase(3), and allene oxide cyclase(1) in the chloroplast and 12-oxo-phytodienoic acid reductase(4), acyl-CoA oxidase, multifunctional protein MFP and L-2-ketoacyl-CoA thiolase(5) in the peroxisome.

Using reverse genetics, it was found that JA regulates the defense system and wound responses as well as male sterile in plants(6). When biosynthesis of linolenic acid, a JA precursor, was blocked by mutation in *Arabidopsis thaliana*, the mutant plant shows a low JA level and high mortality against insects' attack (7). In addition, JA is involved in many aspects of plant development, including root growth, seed germination, tuber formation, tendril coiling, nyctinasty, trichome formation, senescence, flower development, anther development and dehiscence, female organ development and filament elongation(8). Recent studies indicate that derivatives of JA are indeed bioactive molecules: methyl jasmonate (MeJA) and jasmonyl isoleucine (JA-Ile). MeJA is a volatile compound that plays a role in interplant communication (9), inhibits the germination of non-dormant seeds and stimulates the germination

of dormant seeds(10). Another type of JA derivative, JA-Ile is synthesized by ATP-dependent JA-amido synthetase jasmonate resistant 1(JAR1)(11, 12). This adduct between JA and Ile is involved in programmed cell death and leaf senescence for defence(13). When *A. thaliana* has wound, the JA-Ile concentration is increased in wild type plant whereas relatively small increased in *jar1-1* mutant (14).

There are two key proteins that control JA signaling: MYC2 and jasmonate-ZIM domain (JAZ). In normal condition, JAZ binds to MYC2 incorporated with specific DNA sequence. Wounded or invaded by insects or microorganisms, *A. thaliana* promotes synthesis of JA-Ile. When JA-Ile level reaches to a high level, JAZ protein is released from MYC2 leading to expression of JA-dependent gene (Figure 1a). *A. thaliana* MYC2 (accession number: NP_174541; 623 amino acids) is a transcriptional factor found in the nucleus using GFP-fused MYC2(15) and regulates JA signaling. MYC2 positively regulates *Ethylene Response Factor1* and *Octadecanoid-Responsive Arabidopsis AP2/ERF59* related to JA-mediated resistance to insects and tolerance to oxidative stress. On the other hand, MYC2 negatively regulates *PDF1.2*, *CHIB* and *PR4* related to the JA-responsive pathogen defense genes (16, 17). Recent biochemical study revealed that MYC2 has two structural domains, JAZ-interacting domain (JID; 93 - 158 amino acid) and basic helix-loop-helix domain (bHLH; 448

– 528 amino acid) (Figure 1b) (18). In particular, the JID interacts with Jas motif in JAZ protein (see below).

A. thaliana JAZ3 (accession number: NM_112667; 352 amino acids) belonging to the JAZ protein family serves as a repressor of JA signaling by binding to MYC2. JAZ3 has two domains, ZIM domain (172 – 207 amino acid) and Jas motif (302 – 327 amino acid) (Figure 1b) (19). The ZIM domain containing a TIFY motif is responsible for mediating homo– or heteromeric interactions between JAZ proteins(20),(21). Unlike ZIM domain, Jas motif was initially identified to interact with JID domain in MYC2. Later, this Jas motif was further characterized to interact with JA–Ile and Coronatine Insensitive1 (COI1). Therefore deletion of Jas motif results in resistance of degradation of JAZ protein and JA–insensitivity (22).

JAZ and MYC2 interact in a dose–dependent manner of JA (Figure 1a). When JA–Ile concentration is below a critical level, JAZ binds to MYC2, resulting in transcriptional repression of early JA–response genes. Later it was found that general corepressor, TOPLESS, also binds to JAZ intermediated by Noble Interactor of JAZ (23). When concentration of JA–Ile is increased enough, JAZ is separated from MYC2 and makes complex with COI1 and JA–Ile. Then COI1 makes Skp1/Cullin/F–box type E3 ubiquitin ligase complex, and triggers degradation of JAZ3 by 26S proteasome to proceed expression of early–JA responsive gene.

As described above, the role of JAZ and MYC2 in a JA signaling has well been studied. Unlike the vast amount of genetic and biochemical information, little has been disclosed for a protein structure in the JA signaling pathway. The only example is the COI1–ASK1 complex with JA–Ile and JAZ1 degron peptide (24). Here, I report various approaches to produce a soluble protein for the JAZ–MYC2 complex, in particular for Jas and JID motif in JAZ and MYC2, respectively. This study provides valuable information for producing the JAZ–MYC2 complex *in vitro*.

MATERIALS AND METHODS

Cloning of JAZ3-TEV-MYC2 fusion proteins

A. thaliana JAZ3 (GeneBank accession number NM_112667) and *A. thaliana* MYC2 (GeneBank accession number NM_102998) were obtained by polymerase chain reaction (PCR). Nucleotide sequence correspond to 295-330 and 295-352 of JAZ3 protein and 1-166, 1-307, 1-324, and 1-453 of MYC2 protein were amplified respectively by PCR. Then, each of the JAZ3 fragments and MYC2 fragments were linked with nucleotide sequence corresponding to Tobacco Etch Virus (TEV) protease cleavage site, 5'-GAAAACCTGTATTTTCAGGGC-3', by PCR. A total of eight different constructs were produced, including J330-TEV-M166, J330-TEV-M307, J330-TEV-M324, J330-TEV-M452, J352-TEV-M166, J352-TEV-M307, J352-TEV-M324, and J352-TEV-M452 (Table 1). Each PCR product was inserted into the pET-28a vector (Merck) designed for expression of N-terminal His_6-tagged protein.

Using N-terminal His-tagged J352-TEV-M324 as a template, four mutants were prepared by PCR with mutagenic primers (Table 2). Specifically, nucleus localization sequence (16) deletion mutant (J352-TEV-M324_dNLS) and surface entropy reduction mutants (J352-TEV-M324_SERs)(25) were made.

Evaluation of expression level and solubility of JAZ3-TEV-MYC2 fusion proteins

The SDS PAGE analysis was carried out to identify the solubility and expression level of eight of the JAZ3-TEV-MYC2 fusion proteins. Each of the J330-TEV-M166, J330-TEV-M307, J330-TEV-M324, J330-TEV-M452, J352-TEV-M166, J352-TEV-M307, J352-TEV-M324, and J352-TEV-M452 was expressed in *Escherichia coli* BL21-CodonPlus (DE3) ® (Agilent Technologies). The cells were grown at 37℃ in 5 mL of LB broth containing 50 μg/ml kanamycin and 50 μg/ml chloramphenicol. When the optical density of the culture medium reached to 0.65 at 600 nm, 0.5 mM isopropyl β-D-thiogalactopyranoside was added for expressing recombinant proteins. Then the cells were further induced at 20 ℃ for 16 h.

The cells were harvested using 1 min of centrifugation at 12,300 g and resuspended in 1 ml of ice-cold buffer A (50 mM Tris-HCl, 100 mM NaCl, 5 % glycerol, pH 8.0). The resuspended cells were ruptured by sonication for 1 min with 25 % amplitude at 4 ℃. The disrupted cells were centrifugated for 10 min at 12,300 g to collect supernatant and pellet fraction. The levels of expression and solubility were evaluated by 15 % sodium dodecyl sulfate

polyacrylamide gel electrophoresis (SDS PAGE) under denaturing condition.

Expression and purification of JAZ3-TEV-MYC2 fusion proteins and mutants of J352-TEV-M324

N-terminal His-tagged J352-TEV-M307, J352-TEV-M324, J352-TEV-M453, J352-TEV-M324_dNLS, J352-TEV-M324_SER1, J352-TEV-M324_SER2 and J352-TEV-M324_SER3 were expressed in *E. coli* BL21-CodonPlus (DE3)® (Agilent Technologies). The cells were incubated and collected as same way written above, except LB broth volume, 1.6L. The cells were resuspended in 60 ml of ice-cold buffer A (50 mM Tris-HCl, 100 mM NaCl, 5 % glycerol, pH 8.0) and ruptured by sonication for 10 min with 34 % amplitude. The disrupted cells were centrifugated for 1 h at 30,000 g to obtain supernatant at 4 ℃. The supernatants were purified by immobilized metal affinity chromatography (IMAC) (His Trap HP column 1ml, GE Healthcare) with elution buffer B (buffer A containing 500mM imidazole) (Table 3). The eluted protein after IMAC was purified by gel filtration chromatography (GFC) (HiLoad 16/60 Superdex 200 prep grade, GE Healthcare) with buffer A. The purified protein was concentrated.

Methylation of J352-TEV-M324

Overexpressed N-terminal His-tagged J352-TEV-M324 was purified using IMAC with buffer A (50 mM HEPES, 100 mM NaCl, pH 7.5) and elution buffer B (buffer A containing 500 mM imidazole). Methylation protocol was identical with that of previous study (26). 700 μL of 1 M dimethylamine borane complex solution was added and immediately 1400 μL of 1 M formaldehyde solution was added to the 35 ml of purified protein (about 2.0 mg/ml) and gently mixed at 4 ℃ for 2 h. Above reaction was repeated 1 more time. 350 μL of 1 M DMAB solution was added and gently mixed at 4 ℃ for 16 h. 4375 μL of 1M Tris (pH 7.5) was added to stop the reaction. The reaction mixture was centrifugated to remove aggregates at 30,000 g for 1 h and collected the supernatants. The supernatants were dialyzed against 2 L of 25 mM Tris, 100 mM NaCl, 5% glycerol, pH 8.0 for 2 h for 3 times and purified using GFC and concentrated.

Proteolysis of J352-TEV-M324 and methylated J352-TEV-M324 with TEV

After each of the J352-TEV-M324 and methylated J352-TEV-M324 was purified using IMAC, the imidazole in the purified protein solution was removed using desalting column (HiPrep 26/10

Desalting, GE Healthcare) with 50 mM Tris, 100 mM NaCl, 5% glycerol, pH 8.0. Then each of the eluted protein solutions was subjected to TEV protease (molar ratio of protein : TEV protease = 50 : 1) and incubated at 22 ℃ for 16 h. The proteins were purified using IMAC and GFC and concentrated.

Crystallization

The purified protein solutions were screened for crystallization at 22 ℃ using the sitting-drop method with 576 conditions. 96-well plates were used in sitting drop method using screening kits from Hampton Research, Emerald BioStructures and QIAGEN: WIZARD 3·4, Protecomplex, pH Clear, PEG/Ion Screen, Index, and PACT. The drops consists of 1.0 μl and 2.0 μl of protein solution mixed with the same volume of reservoir solution. Each sitting drop was positioned with 60 μl of reservoir solution. The wells were sealed with HD CLEAR tape (Shur Tech Brands, LLC).

RESULTS

Expression and purification of JAZ3-TEV-MYC2 fusion proteins and their mutants

SDS PAGE analysis shows that proteins are appeared with the expected molecular weight (Figure 2a-e). Six fusion proteins, including J330-TEV-M307, J330-TEV-M324, J330-TEV-M453, J352-TEV-M307, J352-TEV-M324 and J352-TEV-M453, were soluble, but J330-TEV-M166 and J352-TEV-M166 were not. Given that J352-TEV-M307, J352-TEV-M324, and J352-TEV-M452 had been expressed as a soluble form in a higher level than the others, subsequence experiments were carried out using these three different fusion proteins.

The recombinant proteins, J352-TEV-M307, J352-TEV-M324, and J352-TEV-M453 were expressed and purified using IMAC and GFC (Figures 3 - 5). The purified protein was concentrated.

J352-M324 Complex

When J352-TEV-M324 is reacted with TEV protease, the fusion protein is divided into JAZ3: 290-352 and MYC2:1-324. The mixture of JAZ3: 290-352 and MYC2:1-324 form a complex (J352-

M324 complex). The resulting J352-M324 complex is then purified with IMAC and GFC (Figure 6), and was concentrated to 7.5 mg/ml and 13.7 mg/ml, respectively, for crystallization screening under 576 conditions.

Methylated J352-TEV-M324

J352-TEV-M324 has 13 lysine residues. According to MALDI-TOF analysis, there would be about 12~13 methylated residues (data not shown). The methylated J352-TEV-M324 was purified using GFC (Figure 7). The methylated J352-TEV-M324 solution was concentrated and crystallization screened with 6.5 mg/ml and 17.8 mg/ml of protein solution.

Methylated J352-M324 Complex

After J352-TEV-M324 had been reacted with TEV protease, the J352-M324 complex was methylated and purified with GFC (Figure 8). The methylated J352-M324 was eluted earlier with each GFC cycle. It seems that the methylated J352-M324 complex makes multimer gradually.

Mutants of J352-TEV-M324

As the J352-TEV-M324 was more soluble and stable than J352-TEV-M307 and J352-TEV-M453, nucleus localization sequence

deletion mutant and surface entropy reduction mutants were made with J352-TEV-M324.

dNLS mutant was deleted from 119 to 122 of MYC2. SERs were substituted some aspartate, glutamate or lysine to alanine (Table 4). These mutants were purified using IMAC and GFC (Figure 9 - 12). The purified protein was concentrated.

Discussion

Protein structural information is essential for describing details of molecular interactions. Therefore, structure determination of JAZ3 and MYC2 would be a clue to identifying interaction between these two proteins and furthermore for better understanding the JA signaling. In this study, I have produced eight different types of the JAZ3–MYC2 complex and screened for a crystal of the complex.

Previous approach to produce the JAZ3 and MYC2 had been failed, in which two proteins were tried to be expressed in a co-expression system (data not shown). In this study, JAZ3 and MYC2 were fused with TEV proteolysis sequence, ENLYFQ, as a linker. In particular, MYC2–TEV–JAZ3 fusion proteins were insoluble (data not shown). Therefore, JAZ3 was fused in the N-terminal region and MYC2 was in the C-terminal region in the construct for the fusion proteins. Among those constructs, the J352–TEV–M324 fusion protein was most soluble according to GFC elution profile (Figure 3b, 4b and 5b).

Our attempts to crystallize the fused proteins were not successful. Subsequently, the fused proteins were subjected to TEV proteolysis, releasing the complex between two fragments, J352 and M324. The J352–M324 complex has similar GFC elution profile and solubility with J352–TEV–M324 fusion protein (Figure 4b and 6b).

As reported in previous study (26), methylated J352-TEV-M324 fusion protein and methylated J352-M324 complex show lower solubility than the unmethylated.

Unfortunately, NLS deletion mutant and the three SER mutants show similar or worse behavior as well as do not have improved solubility compared to the unmodified construct (Figure 4b, 9b, 10b, 11b, 12b).

Currently, I constructed a total of 24 different fusion proteins and they have been subjected to crystallization screen. Extensive searches including different buffer compositions, pHs, and protein concentrations were explored, but crystal for the complex has not been produced. Nevertheless, this study provides valuable information for producing the JAZ-MYC2 complex *in vitro* and paves a way for crystallizing the complex.

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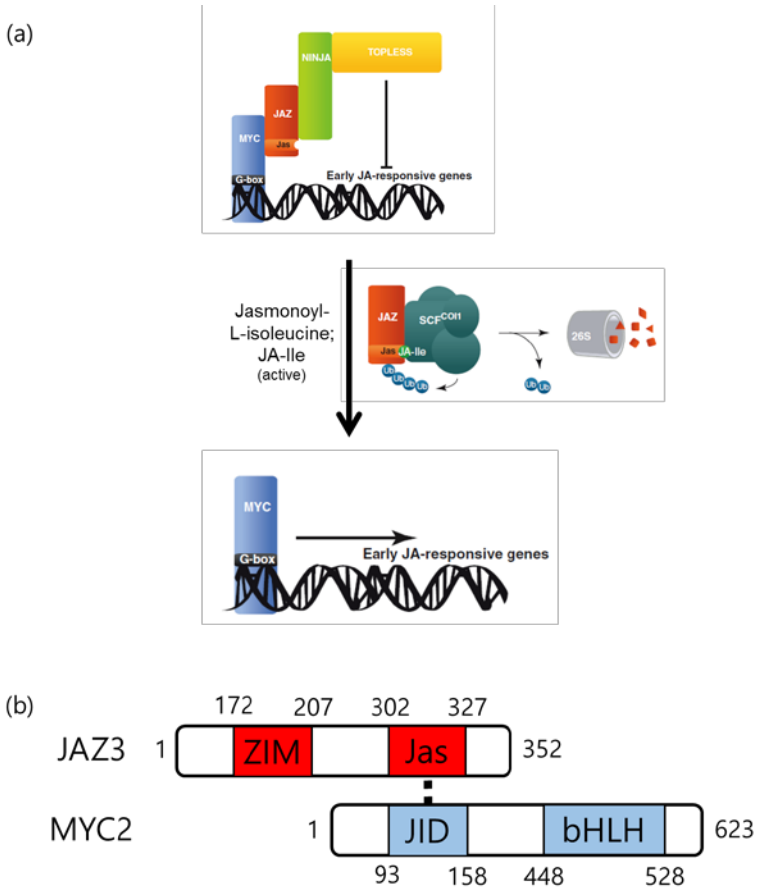

Figure 1. Control of the JA signaling. (a)Model of JA signaling control (27)

In a low JA-Ile level, JAZ which makes complex with NINJA and TOPLESS binds to MYC to block the JA-responsive gene expression. When JA-Ile level is high enough, SCF^{COI1} complex promotes JAZ for ubiquitination and degradation. As a result, released MYC modulates early JA-responsive genes expression. (b) JAZ3 and MYC2

intermolecular interaction. The residue numbers are indicated at and the dashed line shows intermolecular interaction between Jas and JID.

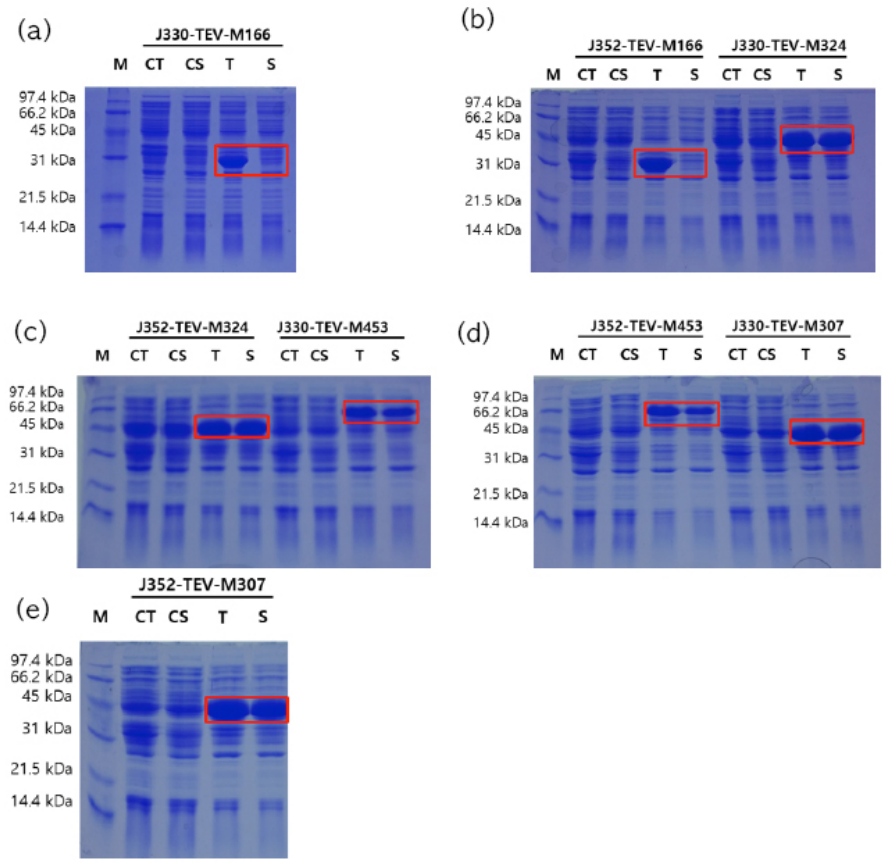

Figure 2. SDS-PAGE analysis of the JAZ3-TEV-MYC2 fusion proteins. (a) J330-TEV-M166, (b) J352-TEV-M166 and J330-TEV-M324, (c) J352-TEV-M324 and J330-TEV-M453, (d) J352-TEV-M453 and J330-TEV-M307, (e) J352-TEV-M307.

The expressed fusion proteins are indicated with red box. M: Marker; CT: Control Total fraction; CS: Control Soluble fraction; T: Total fraction; S: Soluble fraction of fusion proteins. In each panel, two independent expression was carried out with and without isopropyl β-

D-thiogalactopyranoside, respectively. In control experiment IPTG did not added.

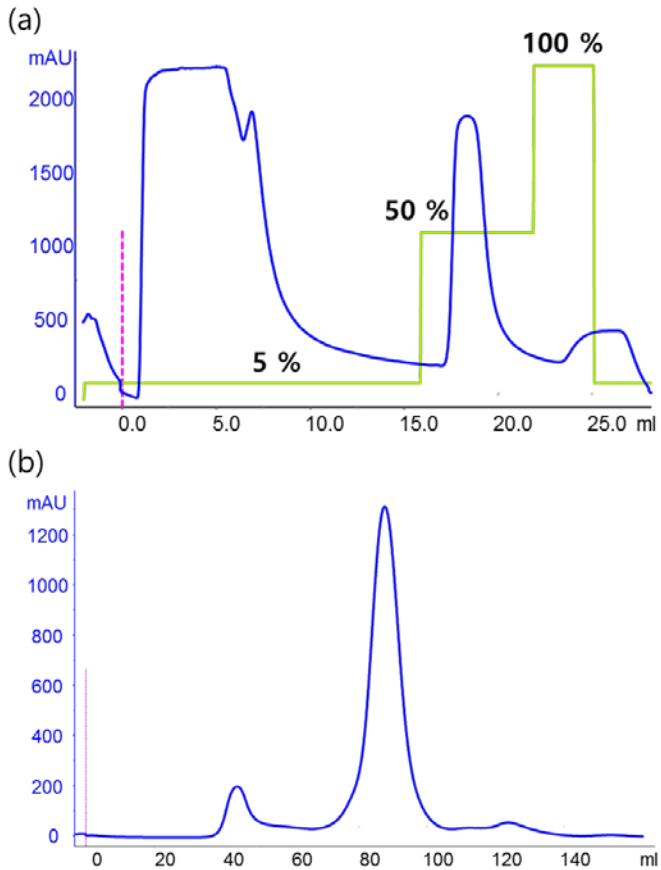

Figure 3. The purification profile of the J352-TEV-M307.

Elution of proteins was indicated in blue line recording an absorbance at 280 nm. (a) IMAC. The green line represents the relative amount of buffer B. (b) GFC. The second peak corresponds the fusion protein.

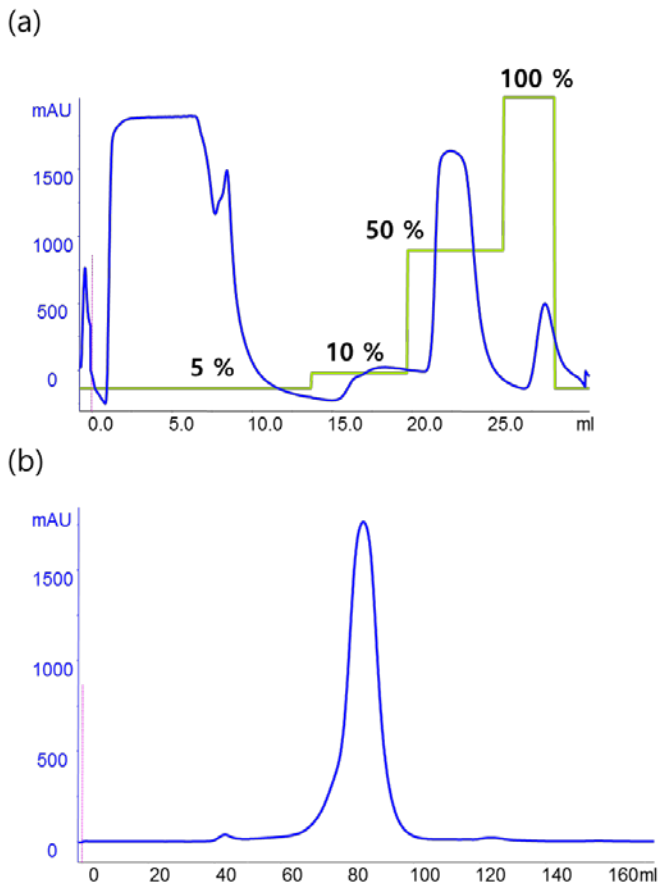

Figure 4. The purification profile of the J352-TEV-M324.

Elution of proteins was indicated in blue line recording an absorbance at 280 nm. (a) IMAC. The green line represents the relative amount of buffer B. (b) GFC. The second peak corresponds the fusion protein.

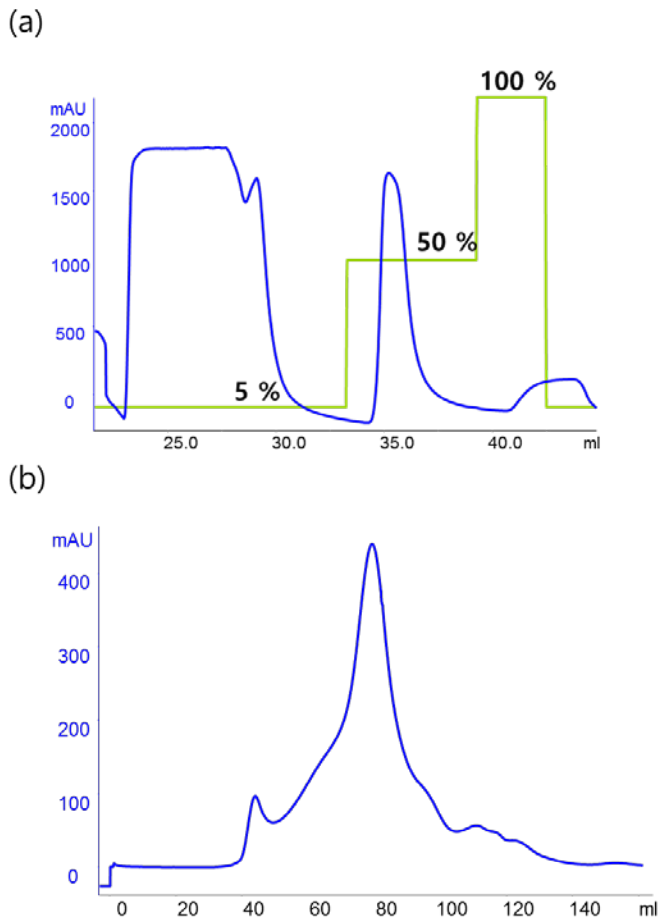

Figure 5. The purification profile of the J352-TEV-M453.

Elution of proteins was indicated in blue line recording an absorbance at 280 nm. (a) IMAC. The green line represents the relative amount of buffer B. (b) GFC. The second peak corresponds the fusion protein.

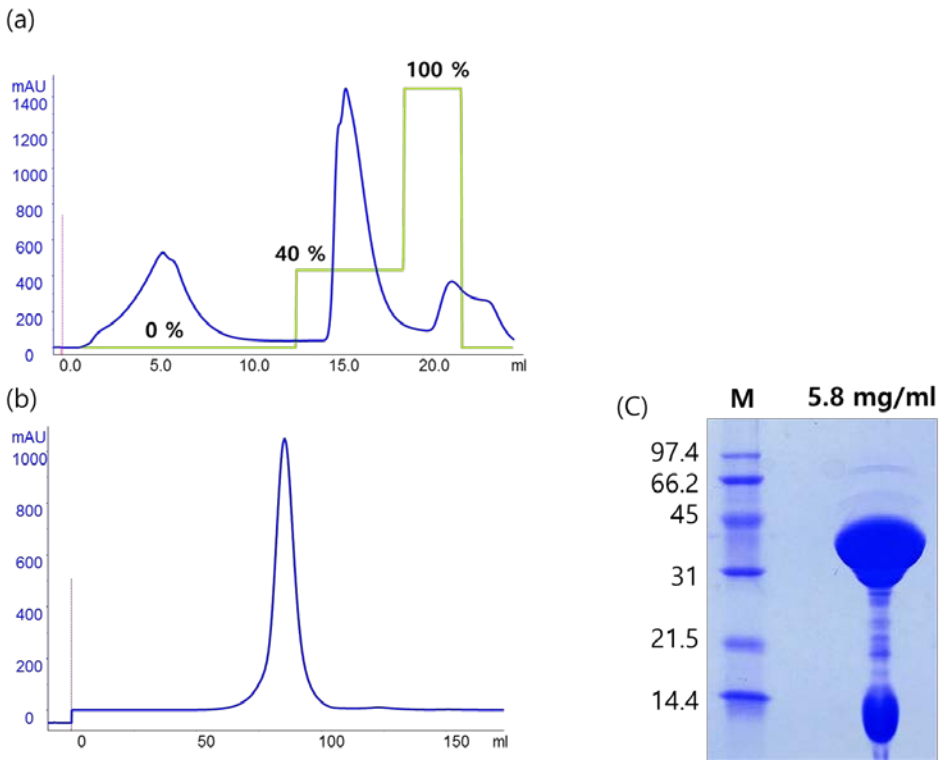

Figure 6. The purification profile of the J352–M324 complex.

Elution of proteins was indicated in blue line recording an absorbance at 280 nm. (a) IMAC. The green line represents the relative amount of buffer B. (b) GFC. (c) SDS PAGE analysis. J352–M324 complex after GFC (5.80 mg/ml) shows two bands correspond to M324 at an upper band and J352 at a lower band.

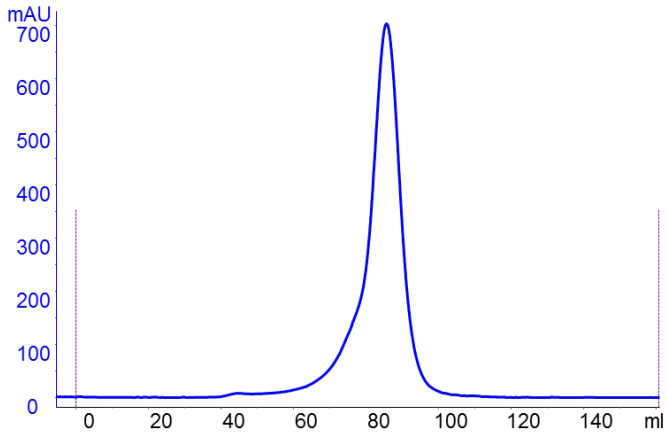

Figure 7. The GFC purification profile of the methylataed J352-TEV-M324 complex.

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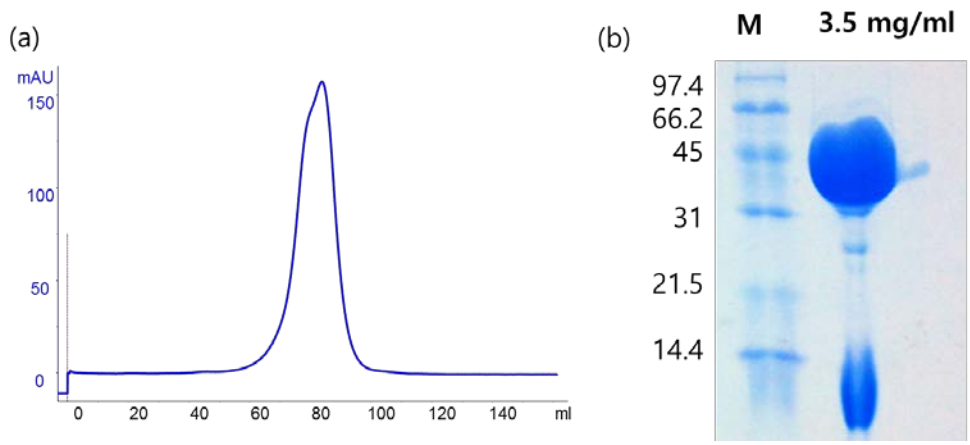


Figure 8. The purification profile of the methylated J352–M324 complex.

(a) GFC. Elution of proteins was indicated in blue line recording an absorbance at 280 nm. (b) SDS PAGE analysis. Methylated J352–M324 complex after GFC (3.50 mg/ml) shows two bands correspond to M324 at upper band and J352 at lower band.

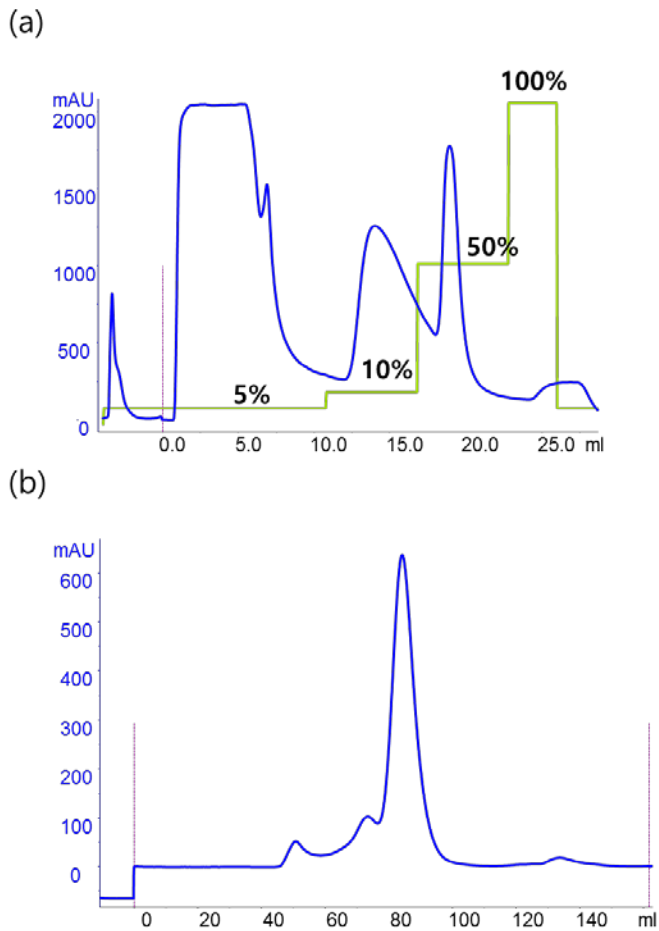


Figure 9. The purification profile of the J352-TEV-M324_dNLS.

Elution of proteins was indicated in blue line recording an absorbance at 280 nm. (a) IMAC. The green line represents the relative amount of buffer B. (b) GFC. The third peak corresponds the fusion protein.

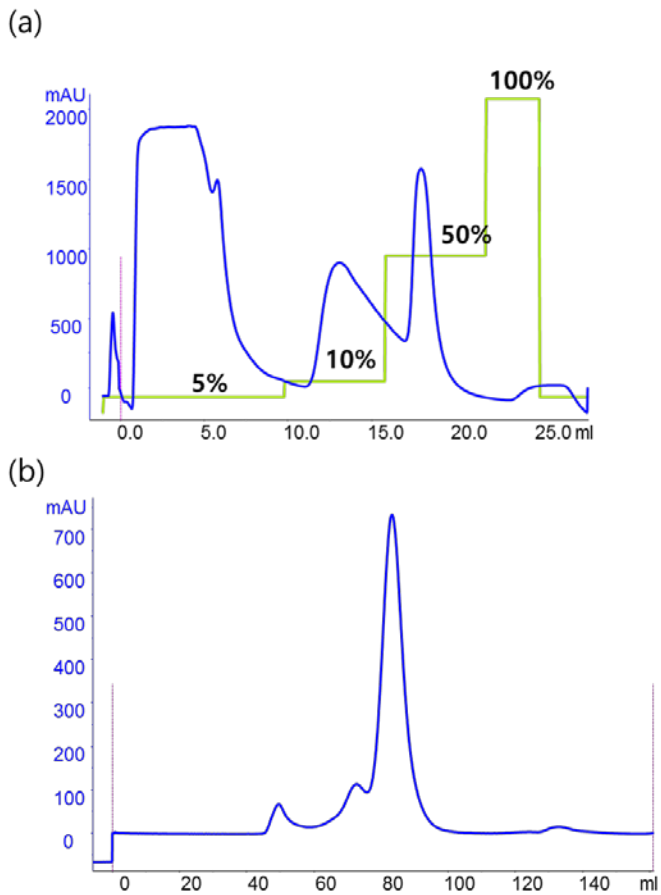


Figure 10. The purification profile of the J352-TEV-M324_SER1.

Elution of proteins was indicated in blue line recording an absorbance at 280 nm. (a) IMAC. The green line represents the relative amount of buffer B. (b) GFC. The third peak corresponds the fusion protein.

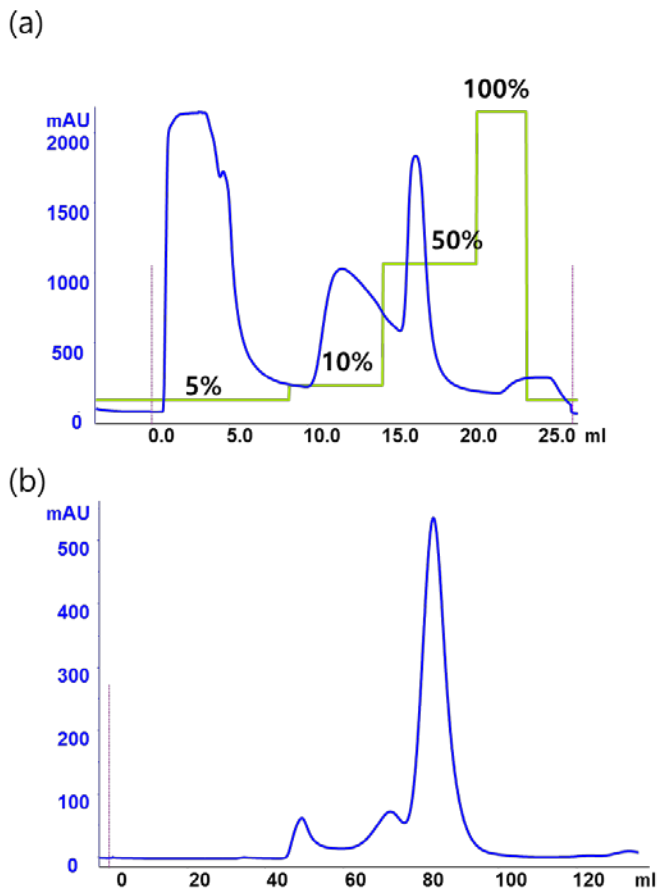


Figure 11. The purification profile of the J352-TEV-M324_SER2.

Elution of proteins was indicated in blue line recording an absorbance at 280 nm. (a) IMAC. The green line represents the relative amount of buffer B. (b) GFC. The third peak corresponds the fusion protein.

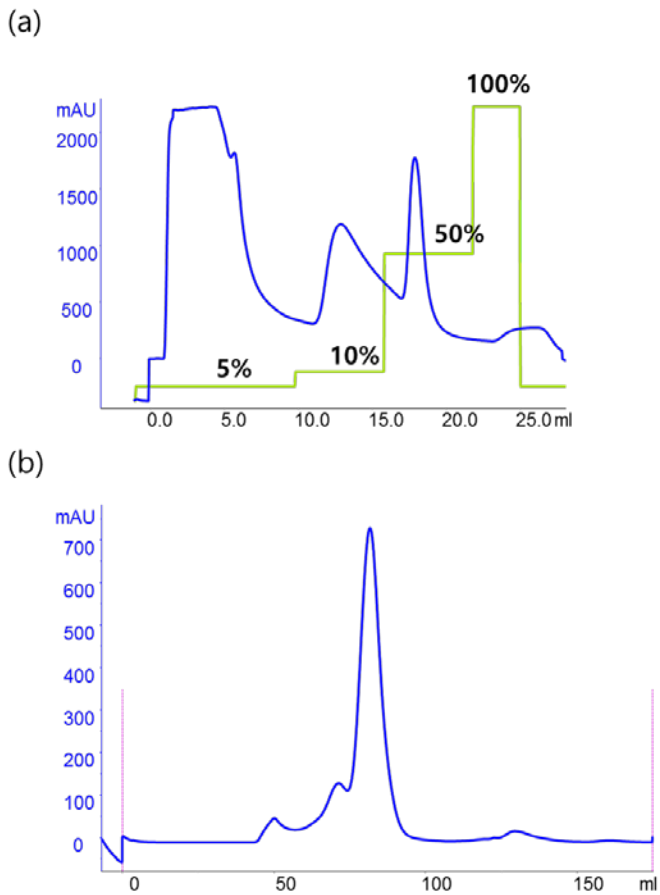


Figure 12. The purification profile of the J352-TEV-M324_SER3.

Elution of proteins was indicated in blue line recording an absorbance at 280 nm. (a) IMAC. The green line represents the relative amount of buffer B. (b) GFC. The third peak corresponds the fusion protein.

Table 1. Constructs of JAZ3−TEV−MYC2 fusion proteins.

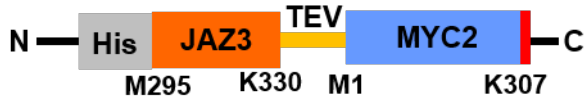
Name	Construction scheme (▮ − stop codon)
J330−T−M166	
J330−T−M307	
J330−T−M324	
J330−T−M453	
J352−T−M166	
J352−T−M307	
J352−T−M324	
J352−T−M453	

Table 2. The primer sequences used for mutation.

Name		Sequence	
dNLS	F 5' -	CAAAGGTGAAGAAGATAAAGCAAAC	
		TCGAGTTCGCCGCCGTTTTCTACTC	-3'
	R 5' -	GAGTAGAAAACGGCGGCGAACTCGAG	
		TTTGCTTTATCTTCTTCACCTTTG	-3'
SER1	F 5' -	GGTTATTACAAAGGTG**C**AG**C**AGAT**GC**	
		AGCAAACCCGAGACGG	-3'
	R 5' -	CCGTCTCGGGTTTGCT**GC**ATCT**G**CT**G**	
		CACCTTTGTAATAACC	-3'
SER2	F 5' -	CCATATTGCTTAGAC**GC**G**GC**GTCATC	
		GACAGATTGT	-3'
	R 5' -	ACAATCTGTCGATGAC**GC**C**GC**GTCTA	
		AGCAATATGG	-3'
SER3	F 5' -	GCTAGGTTTTTAGAG**GC**ACGC**GC**AG**C**	
		AAGGGTCACGAGCGTA	-3'
	R 5' -	TACGCTCGTGACCCTT**G**CT**GC**GCGT**G**	
		CCTCTAAAAACCTAGC	-3'

The bond-underlined letters indicate the mutated sequences.

Table 3. The concentration of buffer A and B in each steps of IMAC.

Construct	Buffer	Wash	Step 1	Step 2	Step 3
J352-T-M307	A	95 %	95 %	50 %	0 %
	B	5 %	5 %	50 %	100 %
J352-T-M324	A	95 %	90 %	50 %	0 %
	B	5 %	10 %	50 %	100 %
J352-T-M453	A	95 %	95 %	50 %	0 %
	B	5 %	5 %	50 %	100 %
J352-T-M324_dNLS	A	95 %	90 %	50 %	0 %
	B	5 %	10 %	50 %	100 %
J352-T-M324_SER1	A	95 %	90 %	50 %	0 %
	B	5 %	10 %	50 %	100 %
J352-T-M324_SER2	A	95 %	90 %	50 %	0 %
	B	5 %	10 %	50 %	100 %
J352-T-M324_SER3	A	95 %	90 %	50 %	0 %
	B	5 %	10 %	50 %	100 %

Table 4. The mutated amino acid sequences according to surface entropy reduction mutation.

	Template	Mutant
SER1	MYC2: 113EEDK116	MYC2: 113AADA116
SER2	JAZ3: 330KK331	JAZ3: 330AA331
SER3	JAZ3: 315KRKE318	JAZ3: 315ARAA318

ABSTRACT IN KOREAN

애기장대 유래 전사조절인자 JAZ3 와 전사인자 MYC2 단백질 복합체 발현 및 정제

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자스모네이트는 식물의 발달, 성장 및 상처 반응 등에 관여하는 식물 호르몬이다. 자연계에서는 자스모네이트에서 유래한 다양한 유도체가 존재하며, 특히 자스모닐 아이소루이신은 자스모네이트 신호 전달 과정에서 생체 활성을 가지는 물질이다. 자스모네이트 신호 전달은 MYC2, JAZ, NINJA 및 TOPLESS 로 구성된 네 가지 단백질의 상호작용에 의하여 조절된다. 그 중에서 특히 MYC2 및 JAZ 는 자스모네이트 신호 전달 과정에서 가장 중요한 역할을 한다. 구체적으로, JAZ 의 Jas 모티브가 MYC2 의 JID 도메인과 결합함으로써 자스모네이트 신호 전달을 억제한다. 이러한 상호 작용이 자스모네이트 신호 전달 과정에서 중요한

역할을 함에도 불구하고, *in vitro* 에서 JAZ 및 MYC2 가 불용성이기 때문에, 이들의 분자 구조를 밝혀는 데에는 어려움이 있었다. 이 논문에서는 JAZ 의 Jas 모티브와 MYC2 의 JID 도메인의 단백질 결정을 만들기 위한 많은 접근 방법에 관하여 기술한다. Jas 모티브를 포함하는 두 가지의 JAZ 단편 및 JID 도메인을 포함하는 네 가지의 MYC2 단편을 조합함으로써 8 가지의 융합 단백질을 발현하였으며, JAZ 단편과 MYC2 단편은 TEV 절단 서열로 연결하였다. 다양한 크로마토그래피 분석법 및 SDS-PAGE 분석법을 통해 확인한 결과, 융합 단백질이 TEV 단백질 분해 효소와 반응하여 MYC2 및 JAZ 가 복합체를 이루는 것을 확인하였다. 비록 복합체의 단백질 결정을 얻지는 못했지만, 본 연구는 *in vitro* 에서 JAZ-MYC2 복합체에 관한 중요한 정보를 제공한다.

주요어: 자스모네이트, 자스모닐 아이소루이신, 자스모네이트 신호 전달, MYC2, JAZ3

학번: 2008-21341