Interaction between a Type-II Dockerin Domain and a Type-II Cohesin Domain from *Clostridium thermocellum* Cellulosome

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Received September 24, 2003; Accepted November 21, 2003

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The interaction between the type-II dockerin domain of the scaffoldin protein CipA and the type-II cohesin domain of the outer layer protein SdbA is the fundamental mechanism for anchoring the cellulosome to the cell surface of *Clostridium thermocellum*. We constructed and purified a dockerin polypeptide and a cohesin polypeptide, and determined affinity constants of the interaction between them by the surface plasmon resonance method. The dissociation constant ($K_D$) value was $1.8 \times 10^{-9}$ M, which is a little larger than that for the combination of a type-I dockerin and a type-I cohesin.

**Key words:** *Clostridium thermocellum*; cellulosome; dockerin; cohesin; surface plasmon resonance

Cellulolytic clostridia such as *Clostridium cellulolyticum*,1,3 *Clostridium cellulovorans*,2 *Clostridium josui*,3 and *Clostridium thermocellum*4 are known to produce multi-enzyme complexes having high hydrolytic activity against crystalline cellulose and related plant cell-wall polysaccharides, termed cellulosome. A common feature of the clostridial cellulosomes is that they consist of a large number of catalytic components arranged around noncatalytic scaffolding proteins. The scaffolding proteins have been identified as CipA (or scaffoldin) in *C. thermocellum*,5 CipC in *C. cellulolyticum*,6 and CipA in *C. josui*.3 These proteins fundamentally consist of repetitive noncatalytic domains of about 140 residues, termed cohesin domains, and a carbohydrate-binding module (CBM), e.g., *C. thermocellum* CipA is composed of nine repeated cohesin domains, a CBM between the second and third of the cohesin domains, and a dockerin domain at its C-terminus. Each cohesin domain is a subunit-binding domain that interacts with a docking domain, termed dockerin, of each catalytic component, i.e., in *C. thermocellum*, a scaffolding protein CipA and nine catalytic components can be assembled into a cellulosome by the interaction between one of the cohesin domains of CipA and a dockerin domain of a catalytic component. Although a dockerin domain exists at the C-terminal region of CipA, it does not associate with cohesin domains of CipA itself but binds specifically to a set of polypeptides such as SdbA and OlpB located in the cell envelope7–9 which are thought to mediate attachment of the cellulosome to the surface of *C. thermocellum* cells. A combination of dockerin domains of catalytic components and cohesin domains of CipA was defined as type I and another combination of the dockerin domain of CipA and the cohesin domains of SdbA and OlpB as type II.

The interactions between dockerin domains and cohesin domains of type I have been quantitatively investigated, i.e., the dissociation constant for the interaction of the CelS dockerin polypeptide and the second cohesin domain polypeptide from CipA was determined to be smaller than $10^{-11}$ M by surface plasmon resonance (SPR) analysis10 and the binding constant of $(2.6 \pm 0.5) \times 10^9$ M$^{-1}$ was obtained for the interaction of the CelD dockerin polypeptide and the seventh cohesin domain polypeptide by isothermal titration calorimetry analysis.11 However, there is no report of quantitative analysis of the interaction between type-II dockerin and type-II cohesin. In this study, we constructed and purified the type-II dockerin polypeptide from CipA and the type-II cohesin polypeptide from SdbA and measured the dissociation constant for their interaction by the SPR method.

The plasmid used to produce the type-II dockerin polypeptide of *C. thermocellum* CipA was constructed as follows: The DNA region encoding the dockerin was amplified by PCR from pCip10212 with KOD dash DNA polymerase (Toyobo, Osaka, Japan) and a combination of PCR primers containing an artificial BamHI or HindIII site, 5'GGGGATCCGGAGACATGGTGGAGCAATTC-3' and 5'GGAAAGCTTTACTGTGGCGTGTAATCTTTGTGGTGG-3' (the BamHI and HindIII sites are...
underlined). The resulting PCR fragment was treated with BamHI and HindIII and cloned into pET-28a (+) (Novagen, Madison, Wisconsin), yielding pET-DocII. Plasmid pCT1830 is derivative of pQE-30 (Qiagen, Valencia, California) and contains a DNA fragment encoding the type-II cohesin of SdbA.7) Recombinant polypeptides encoded by pET-DocII and pCT1830 are referred to as rDocII and rCosII respectively in this study.

Recombinant polypeptides, rDocII and rCosII, were purified by using Ni-NTA resin (Qiagen) from Escherichia coli BL21(DE3) harboring pET-DocII and E. coli M15 harboring pCT1830 respectively. The purified polypeptides gave a single band by SDS-polyacrylamide gel electrophoresis analysis (PAGE)13) or Tricine-SDS-PAGE14) (Fig. 1). Qualitative analysis using native PAGE confirmed the previous observations that the dockerin domain of CipA did not bind to the cohesin domains of CipA but bound to the cohesin domain of SdbA.15)

SPR with a BIAcore 2000 (BIAcore AB, Upplands, Sweden) was used to determine the kinetic and equilibrium constants of the cohesin–dockerin interaction. HBS buffer (10 mM HEPES, pH 7.4, 0.15 M NaCl) was used as an immobilization buffer, and 10 mM CaCl2, 0.005% surfactant P20 (BIAcore), and 50 mM Tris-maleate buffer, pH 6.5 was used as a running buffer, as described previously for SPR analysis of C. cellulolyticum proteins.16) The rCohII polypeptide was immobilized on a dextran matrix with free carboxylic groups (CM5 chip, BIAcore) employing conventional carbodiimide coupling chemistry and subsequent deactivation of excess active esters using ethanolamine (EDC/NHS coupling kit, BIAcore). The purified cohesin polypeptide was injected for 10 s, resulting in approximately 400–500 resonance units (RU) of immobilized protein. Two flow cells were prepared for the kinetic experiments: in one cell, the cohesin polypeptide was immobilized and an adjacent cell was used as a reference with no treatment. The flow cells were routinely equilibrated with running buffer. The ligand (rDocII) was diluted in running buffer and allowed to interact with the sensor surface by a 240-s injection. Three different concentrations of ligands were injected. Kinetic evaluation was performed with the BIAevaluation 3.1 software (BIAcore), fitting the three curves in each concentration series using the global fitting method. The sensorgrams obtained were evaluated with the biomolecular interaction analysis evaluation software (BIAcore) to calculate the kinetic constants. The data were interpreted on the basis of the simple model L+ A ⇌ LA, where L denotes the mobile ligand and A is the immobilized receptor. The sensor chip’s surface was efficiently regenerated by injection of 10 mM HCl between each protein injection. A typical sensorgram obtained is shown in Fig. 2. As a result of evaluation of sensorgrams, the dissociation and association rates, $k_{off}$ and $k_{on}$, for the interaction between rDocII and rCosII were estimated to be $2.2 \times 10^{-4}$ s$^{-1}$ and $1.3 \times 10^{9}$ s$^{-1}$m$^{-1}$, resulting in $1.8 \times 10^{-3}$ of $K_D$. Although a $k_{off}$ value less than $10^{-4}$ s$^{-1}$ cannot be correctly measured by BIAcore, the $k_{off}$ value obtained was within the measurable limit. Therefore, this $K_D$ value is reliable and is quite a bit larger than that for the interaction between the type I dockerin polypeptide from CelID and the seventh cohesin polypeptide from CipA ($3.8 \times 10^{-10}$ M), reported as binding constant of $2.6 \times 10^{8}$, determined by the ITC method,11) and that for the interaction between the type I dockerin polypeptide from CelS and the second cohesin polypeptide from CipA (smaller than $10^{-11}$ M), determined by the SPR method.10) The interaction between the first cohesin polypeptide from C. cellulolyticum CipC and the dockerin polypeptide from CelA was examined by SPR and its $K_D$ value was $2.5 \times 10^{-10}$ M.16) Species specificity of cohesin/dockerin interactions was reported between C. thermocellum and C. cellulolyticum, i.e., dockerin domains from these bacteria recognize cognate cohesin domains but not noncognate cohesin domains.17)

![Fig. 1. SDS-PAGE of the Purified Cohesin Polypeptide (A) and Tricine-SDS-PAGE of the Purified Dockerin Polypeptide (B).](image)

Gels were stained with Coomassie brilliant blue. (A) A 15% polyacrylamide gel was used. Lane 1, molecular mass standards (LMW; Pharmacia); lane 2, the purified rCosII. (B) A 16.5% Tricine polyacrylamide gel was used. Lane 1, molecular mass standards (polypeptide SDS-PAGE standards; Bio-Rad); lane 2, the purified rDocII.

![Fig. 2. SPR Sensorgrams Showing the Binding of rDocII onto the Immobilized rCosII.](image)

In the graph, curve 1 = injection of rDocII (7 nM); curve 2 = injection of rDocII (14 nM); curve 3 = injection of rDocII (28 nM).
Fierobe et al.18,19) constructed cellulosome chimeras using chimerical scaffolding proteins composed of a cohesin domain from Clostridium thermocellum and another cohesin domain from Clostridium cellulolyticum, and chimerical catalytic components containing one of the divergent dockerin domains. The combination of type-II cohesin and type-II dockerin is also useful for the construction of more complicated cellulosome chimeras.

In conclusion, this is the first report describing the quantitative analysis of the interaction between the type-II dockerin and the type-II cohesin domain from C. thermocellum. The combination of type-II cohesin and type-II dockerin appears to be useful for the construction of more complicated cellulosome chimera or novel artificial enzyme complexes.

Acknowledgments

This work was partly supported by a Grant-in-Aid for Scientific Research (A), no. 14206038, from the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

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