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Abstract

It has been shown that the single-domain intrabody 2H9-L against the hepatitis C virus (HCV) capsid (core) protein inhibits the viral propagation and NF-κB promoter activity induced by the HCV core. In this study, 2H9-L fused with the FLAG tag sequence was expressed in both *Escherichia coli* and silkworm pupae and then purified. In addition, the full-length and its C terminal deletions of the HCV core protein, i.e., 1–123 amino acid residues (C123), 1–152 amino acid residues (C152), 1–177 amino acid residues (C177) and 1–191 amino acid residues (C191), were expressed as fusion proteins with a 6 × His tag at their N-terminus in *E. coli* and then purified. Approximately 175 and 132 μg of the intrabody were purified from 100 ml of *E. coli* culture and 10 silkworm pupae, respectively, by affinity chromatography. The C123, C152, C177 and C191 HCV core protein variants were purified to approximately 152, 127, 103 and 155 μg, respectively, from 100 ml of *E. coli* culture. An ELISA in which the intrabodies were immobilized revealed that the intrabodies purified from both hosts were bound to all HCV core protein variants. However, their binding to the C191 appeared to be weak compared to their bindings to the other HCV core protein variants. When C152 was immobilized in the ELISA, the binding of each intrabody to the core protein was also observed. These purified intrabodies can be used in biochemical analyses of the inhibitory mechanism of HCV propagation and as protein interference reagents, thus providing a potential pathway to developing a new type of antiviral drug.

Keywords: Intrabody, Hepatitis C virus, Core protein, Silkworm pupae, *Escherichia coli*

1. Introduction

Antibodies have been widely used in various fields, basic research, diagnostics and disease therapy because they have stringent specificities to antigens. In nature, antibodies are composed of two heavy chains and two light chains, thus forming heterotetramers. Each chain is linked by disulfide bonds, and its heavy chains are *N*-glycosylated [1]. These protein modifications are required for the expression of functional antibodies, which often prevent recombinant production [2].

As recombinant DNA technologies have advanced, antibodies can now be engineered and redesigned to create antibodies with smaller sizes, higher affinity and higher stability [3]. Recombinant antibody fragments, including antigen-binding fragments (Fabs), single-chain fragment variables (scFvs) and minibodies, lack the fragment crystallizable (Fc) region of the native antibodies, but they still have the binding capacity to antigens. These antibodies have been used for immunotherapy and in vivo imaging [4, 5].

One of these engineered antibodies, single-domain antibodies, has been developed based on the heavy or light chains of the native antibodies. In addition, camelid and shark high-affinity single V-like domains (V_HH and V_{NAR}) have been used to create soluble single-domain antibodies [6]. These single-domain antibodies are used as predators for affinity chromatography, chaperones for the cocrystallization of recombinant proteins, regulators of protein aggregation and modulators of enzyme function [7]. The molecular weight of these single-domain antibodies is approximately 15 kDa, which is less than that of native antibodies, Fabs and scFvs. Their smaller molecular weight allows single-domain antibodies to be expressed as intracellularly soluble proteins instead of native antibodies, which are normally secreted into the extracellular fraction. Those antibodies that are expressed intracellularly are called intracellular antibodies or “intrabodies”.

These intrabodies, which are expressed intracellularly, can block the function of a protein in cells by directly binding to intracellular antigens [8]. Therefore, the in vivo knockdown of intracellular proteins can be conducted using intrabodies as well as siRNA and miRNA [9]. These intrabodies can also target specific proteins in the endoplasmic reticulum (ER) and nucleus with the addition of ER- and nucleus-targeting signals to the intrabodies. In addition, intrabodies can be injected directly into human cells for analytical and therapeutic purposes [10].

In a previous study, several single-domain intrabodies against the HCV core protein were generated from the anti-core monoclonal antibodies and tested for their binding to the core and their inhibition of core-mediated biological activities. Among the tested intrabodies, 2H9-L, which is composed of the light chain of mouse immunoglobulin G (IgG), was found to function as an inhibitor of both HCV production and the host cell signaling pathway activated by HCV [11]. In this study, the 2H9-L intrabody was expressed in *Escherichia coli* and silkworm and was purified. Several forms of the viral core protein were also bacterially expressed and purified, and their binding ability to 2H9-L was then evaluated.

2. Materials and methods

2.1. Materials

The gene of the intrabody against the HCV core protein (2H9-L) that was isolated previously was used in this study [11]. Silkworm pupae were purchased from Ehime Sansyu (Ehime, Japan).

2.2. Construction of recombinant vectors and BmNPV bacmids

To express the intrabody in *E. coli*, pCold III vector (Takara Bio, Shiga, Japan) was used. The gene of the intrabody was amplified by a polymerase chain reaction (PCR) using the IB-E-F and IB-E-R primer set (Table 1) and was inserted into the *Nde* I-*Eco*RI site in the pCold III vector. The constructed vector was transformed into *E. coli* SHuffle (New England Biolabs Japan, Tokyo, Japan) to express the intrabody.

To express the intrabody in silkworm pupae, the *Bombyx mori* nucleopolyhedrovirus (BmNPV) bacmid system was used. The gene of the intrabody was amplified by PCR using the IB-Bm-F and IB-Bm-R primer set (Table 1) and was inserted into the pENTR D-TOPO vector (Thermo Fisher Scientific K.K., Yokohama, Japan) by topo-cloning. Using the constructed vector, the intrabody gene was inserted into the pDEST8 vector (Thermo Fisher Scientific K.K.) by LR cloning. Finally, the intrabody gene was inserted into the BmNPV bacmid using the recombinant pDEST8 vector by the BmNPV Bac-to-Bac system [12]. Recombinant BmNPV bacmid was extracted from a white colony after transforming the recombinant pDEST8 vector into *E. coli* BmDH10bac, and it was used to express the recombinant intrabody in the silkworm pupae.

To express the HCV core protein in *E. coli*, pCold II vector (Takara Bio) was used. Four types of HCV core protein variants (Fig. 1), whose gene codings of 1–123, 1–152, 1–177, and 1–191 amino acids were amplified by PCR using HCV-core-F and each reverse primer set (Table 1). The gene of the HCV JFH-1 core protein was used as a template [13]. Each amplified gene was inserted into the *Eco*RI-*Not* I site in the pCold II vector. Each recombinant vector was transformed into *E. coli* BL21 (DE3) to express each

HCV core protein.

2.3. Expression of the intrabody and each HCV core protein in E. coli and its purification

An *E. coli* strain that harbored each expression vector was cultivated in Luria-Bertani (LB) medium at 37°C until the optical density reached 0.5, which was followed by cooling on ice. After adding 1 mM isopropyl-β-D-thiogalactopyranoside to the culture medium, the cells were cultivated again at 15°C for 24 h. The cells were collected by centrifugation at 10000 × g. Cells that expressed the intrabody were suspended with 50 mM Tris-HCl (pH 7.5) that contained 200 mM NaCl. Cells that expressed the HCV core protein variants were suspended with 50 mM Tris-HCl (pH 7.5) that contained 200 mM NaCl and 1% *n*-dodecyl-β-D-maltopyranoside (DDM). Cells were disrupted by sonication, and the homogenate was centrifuged at 20000 × g. The supernatant was collected and used for the purification of the intrabody and each core protein.

To purify the intrabody, DDDDK-tagged Protein PURIFICATION GEL (Medical & Biological Laboratories, Nagoya, Japan) was used. The supernatant was applied to a DDDDK-tagged Protein PURIFICATION GEL column, and the column was washed by 50 mM Tris-HCl (pH 7.5) that contained 600 mM NaCl and 0.5% Triton X-100. The intrabody was eluted with 100 mM glycine-HCl (pH 3.5) that contained 500 mM NaCl and was immediately neutralized with 1 M Tris-HCl (pH 8.0) that contained 500 mM NaCl. The purified intrabody was dialyzed with phosphate-buffered saline (PBS, pH 7.4) and used for further analyses.

To purify each core protein, TALON Metal Affinity Resin (Clontech Laboratories, Mountain View, CA, USA) was used. The supernatant was applied to the TALON Metal

Affinity Resin column, and the column was washed with 20 mM Tris-HCl (pH 7.6) that contained 300 mM NaCl, 10 mM imidazole and 1% DDM. Each core protein was eluted with 20 mM Tris-HCl (pH 7.6) that contained 300 mM NaCl, 200 mM imidazole and 1% DDM. The purified core proteins were dialyzed with PBS and used for further analyses.

2.4. Expression of the intrabody in silkworm pupae and its purification.

Ten micrograms (45 µl) of recombinant BmNPV bacmid that contained the gene of the intrabody was mixed with 5 µl of DMRIE-C reagent (Thermo Fisher Scientific K.K.) and incubated for 30 min at room temperature. The mixture was injected into a silkworm pupa, and the pupa was incubated for 6 d at 25°C. The pupa was suspended with 1.5 ml of Tris-buffered saline (TBS) that contained 0.1% Triton X-100 (TBST) and 10 µl of 200 mM 1-phenyl-2-thiourea. The pupa was disrupted by sonication, and the suspension was centrifuged at 12000 × g. The supernatant was filtered using a 0.45-µm syringe filter, and the filtrate was used to purify the intrabody.

To purify the intrabody, Anti-FLAG M2 Affinity Gel (Sigma-Aldrich Japan, Tokyo, Japan) was used. The purification was conducted with the same method as the purification of the intrabody from *E. coli* and using a DDDDK-tagged Protein PURIFICATION GEL column. However, to purify the intrabody from silkworm pupae, this affinity chromatography was performed twice.

The amount of purified proteins was measured by Pierce BCA Protein Assay Kit (Thermo Fisher Scientific K. K.).

2.5. SDS-PAGE and western blotting

Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using 10% or 12% acrylamide and subsequently subjected to western blotting. After SDS-PAGE, the proteins were blotted onto a polyvinylidene fluoride membrane using the Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad, Hercules, CA, USA). After blocking in 5% skim milk in TBST (pH 7.6), the membrane was incubated in 10000-fold diluted monoclonal anti-polyhistidine-horseradish peroxidase (HRP) antibody produced in a mouse (Sigma Aldrich Japan, Tokyo, Japan) for the detection of each HCV core protein. Alternatively, 3000-fold diluted anti-HCV core monoclonal antibody (GenTex, Irvine, CA, USA) and 10000-fold diluted sheep anti-mouse IgG-HRP (GE Healthcare Japan, Tokyo, Japan) were used as primary and secondary antibodies for each HCV core protein detection, respectively. The intrabody was detected using 10000-fold diluted anti-DYKDDDDK tag monoclonal antibody (Wako Pure Chemical, Osaka, Japan) and 10000-fold diluted sheep anti-mouse IgG-HRP (GE Healthcare Japan) as primary and secondary antibodies. Detection was performed using Immobilon™ Western Chemiluminescent HRP Substrate (Merck Millipore, Billerica, MA, USA). Specific bands were detected on a Fluor-S MAX Multi-Imager (Bio-Rad).

2.6. Enzyme-linked immunosorbent assay (ELISA)

For ELISA, 10 μg of purified intrabody was immobilized onto a well of an ELISA plate, and the wells were blocked with PBS that contained 2% skim milk for 2 h at room temperature. The wells were washed by PBS that contained 0.1% Tween 20 (PBST) 3

times. After the PBST was removed in the wells, 10 μg of each purified core protein was added to each well, and the plate was incubated for 1 h at room temperature. The wells were washed by PBS that contained 0.1% Tween 20 (PBST), followed by adding 3000-fold diluted anti-polyhistidine monoclonal antibody-HRP conjugate (Sigma Aldrich Japan) and incubating for 1 h at room temperature. The wells were washed by PBST 3 times, and the HRP reaction was then conducted. One hundred microliters of substrate (0.1 mg/ml 3,3′,5,5′-tetramethylbenzidine in 100 mM sodium acetate (pH 6.0) with 0.2% (v/v) of 30% hydrogen peroxide was added to each well and incubated at room temperature until a blue color developed in each well. The HRP reaction was stopped by the addition of 50 μl of 1 N H_2SO_4 solution, and the absorbance of each well was measured at 450 nm.

Alternatively, 5 μg of purified core protein was immobilized on a well of a SuperEpoxy2 glass slide (Arrayit, Sunnyvale, CA, USA) in the presence of protein printing buffer (Arrayit). The glass slide was washed with Blockit blocking buffer (Arrayit) 3 times and blocked with the same buffer for 30 min at room temperate. The glass slide was washed with PBS 3 times, and each amount of purified intrabody was added to a well of a SuperEpoxy2 glass slide. The glass slide was incubated for 1 h and washed with PBS 3 times. Anti-DYKDDDDK tag monoclonal antibody (Wako Pure Chemical) diluted by 10000-fold with Blockit blocking buffer was added to the wells of the glass slide and incubated for 1 h at room temperature. After the glass slide was washed with PBS 4 times, Fluorescein (FITC) AffiniPure anti-Mouse IgG (H+L) (Jackson ImmunoResearch, West Grove, PA, USA) diluted by 500-fold with Blockit blocking buffer was added to the wells of the glass slide. The glass slide was incubated for 1 h at room temperature and washed with PBS 4 times. The fluorescence in each well of the

glass slide was detected using Molecular Imager FX (Bio-Rad).

3. Results

3.1. Expression of the intrabody in E. coli and silkworm pupae

In a previous report, the intrabody considered here (2H9-L) was isolated from mouse hybridomas derived from mice immunized with recombinant HCV core protein and was found to be composed of a single domain, only a light chain of IgG1 and its signal sequences at its N-terminal sequence [11]. This signal sequence enhances the intracellular expression level of this intrabody in 293T cells. The intrabody fused with the FLAG tag at its C terminus was expressed in *E. coli* and silkworm pupae. In both expression systems, the intrabody was expressed in a soluble fraction below 20 kDa (Fig. 2A). The estimated molecular weight of this intrabody is approximately 15 kDa, which indicates that the bands detected by western blotting are the recombinant intrabody being expressed.

The intrabody expressed from the extract of *E. coli* and silkworm pupae was purified by FLAG tag-based affinity chromatography. A single band of the intrabody was purified from *E. coli*, as shown in an SDS-PAGE gel stained with CBB (Fig. 2B). However, the intrabody from silkworm was not completely purified by single affinity chromatography. After second affinity chromatography, a single band of the intrabody was observed in an SDS-PAGE gel stained with CBB (Fig. 2B). The yield of the purified intrabody was 171 μg from 100 ml of *E. coli* culture and 132 μg from 10 pupae. We also attempted to express an intrabody that lacked its signal sequence in *E. coli*, and this intrabody was purified (data not shown). The yield of the purified intrabody that lacked its signal sequence was

38 μg from 100 ml of *E. coli* culture. This yield is lower than that of the intrabody that contained its signal sequence, which indicates that the signal sequence contributes to an increase in the intrabody's expression level, as previously reported [11].

3.2. Expression of the HCV core protein variants in E. coli

The HCV core protein can be divided into 3 domains, domain 1 (D1, 1–117 aa), domain 2 (D2, 118–177 aa) and a signal sequence of E1 protein (178–191 aa), as shown in Fig. 1 [13]. D1 is rich in basic amino acid residues and is hydrophilic. The functions of D1 include RNA binding, RNA chaperoning, and oligomerization. D2 contains two amphipathic α-helices and has roles in ER and lipid droplet membrane association. In this study, 4 types of HCV core protein variants (C123: 1–123 aa, C152: 1–152, C177: 1–177, C191: 1–191) were expressed in the soluble fraction of *E. coli* and were detected by both the anti-His antibody and anti-HCV core antibody (Fig. 3A). The expression pattern of C123 was almost the same as that of C152, while the expression pattern of C177 was almost the same as that of C191. In all variants, a main band was detected above 15 kDa. In C177 and C191, an additional band was also detected over the main band. These findings suggest that the main band could be a C123 variant and that the additional band could be C177. The estimated molecular weight of C123, C152, C177, and C191 is approximately 16, 18, 21, and 23 kDa, respectively. The molecular weight of both the main band and the additional band in Fig. 3A is 16–18 kDa. In all variants, some bands were also detected below 15 kDa.

Each HCV core protein variant was purified using affinity chromatography. Each HCV core variant was purified, but several bands were detected between 10 and 20 kDa

on an SDS-PAGE gel stained with CBB (Fig. 3B). The band patterns of these purified samples were almost the same as those in Fig. 3A. This finding indicates it is likely that the bands that were detected between 10 and 20 kDa came from expressed HCV core protein variants. The yield of each purified HCV core protein variant was 152 μg for C123, 127 μg for C152, 103 μg for C177 and 155 μg for C191, each from 100 ml of *E. coli* culture. When using Triton X-100 instead of DDM during these purifications, it was difficult to purify each HCV core protein variant from the *E. coli* extract.

3.3. Analyses of the binding of the intrabody to HCV core protein variants

ELISA was also performed to confirm the binding of both intrabodies to all of the HCV core protein variants. Bovine serum albumin was used as a negative control. Higher absorbance was observed when using each HCV core protein variant than when using the negative control, which indicates that both intrabodies have an ability to bind HCV core protein variants (Fig. 4). However, the binding of both intrabodies to C191 appeared to be weaker than their binding to other variants. To analyze the binding capacity of the intrabody to the HCV core proteins, immunoprecipitation was performed using purified intrabodies and each HCV core protein variant (Supplementary Fig. 1). The intrabodies that were purified from both *E. coli* and silkworm pupae bound to all of the HCV core protein variants. Similar to the ELISA, the binding of both intrabodies to C191 was weaker than their binding to the other HCV core protein variants.

The binding capacity of both intrabodies to C152 was confirmed using a glass plate. A SuperEpoxy2 glass slide was used with epoxy groups on its surface, so the proteins could be easily immobilized on the glass slide through amine groups, hydroxyl groups

and thiol groups. As the amount of each intrabody increased, the intensity of the fluorescence also increased (Fig. 5). Fluorescence was not observed when bovine serum albumin was used as a negative control instead of purified C152. The intrabody from silkworm pupae had a dissociation constant (K_d) of approximately 53 μM based on an equilibrium analysis when a surface plasmon resonance (SPR) experiment was performed by Biacore using C152 immobilized on the sensor chip (Supplementary Fig. 2).

4. Discussion

Intrabodies have a prominent role in biological research, including in functional genomics and proteomics, because the ectopic expression of the molecules inside living cells enables their specific binding to target antigens and exerts a specific biological effect. However, the application of intrabodies that are bacterially expressed and isolated is usually limited by their poor stability and/or solubility. It is known that the over-expression of mammalian proteins, including intrabodies in *E. coli*, frequently results in the accumulation of inclusion bodies.

In this study, the intrabody 2H9-L, which functions as an anti-HCV inhibitor in human hepatic cells, was expressed in *E. coli* and silkworm pupae and was successfully purified as a soluble form, which has binding activity to the HCV core protein in vitro. The structural basis of HCV core-intrabody interfaces would allow a novel strategy to design and generate chemical drugs with antiviral activities. It has been shown that compared to the heavy chains, the light chains of the immunoglobulin molecules can easily be expressed in soluble form [15, 16], especially in *E. coli* [16]. In this study, 171 μg of purified intrabody was obtained from 100 ml of *E. coli* culture, and its yield was comparable to that from 10 pupae (132 μg). Solubility is advantageous for an intrabody,

which functions intracellularly to bind the HCV core protein during HCV amplification. In addition, the HCV core protein has hydrophobic domains; the recombinant HCV core protein expressed in *E. coli* was purified with a denaturing reagent in previous papers [17], and it was prone to aggregation in this study (data not shown). To analyze the HCV core protein in detail, this intrabody can be used to keep the HCV core protein soluble, even when its concentration is high.

In this study, HCV core protein variants were also expressed in *E. coli* and purified. Kunkel et al. [17] expressed and purified HCV C-124 and HCV C-179, which are composed of 1–123 amino acid residues and 1–179 amino acid residues of HCV core protein, respectively, and obtained yields of 10 and 5 mg from 1 liter of *E. coli* culture. These yields are higher than those obtained for C123, C152, C177 and C191 in this study. However, the HCV C-124 and HCV C-179 were in an insoluble form and solubilized completely by 8 M urea, followed by purification and refolding. In contrast, in this study, all of the HCV core protein variants were purified from *E. coli* without any solubilizing or refolding steps. This finding indicates that the pCold vector is more suitable for the soluble HCV core protein expression in *E. coli* than the pET vector is. In addition, HCV C-179 purified from *E. coli* was digested by chymotrypsin to HCV C-N-36, which lacks 35 amino acids at its N-terminus, and by trypsin to several variants [18]. This arrangement suggests that several of the HCV bands detected in this study might have been caused by the proteolytic cleavage of each of the HCV core protein variants expressed in *E. coli*.

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Table 1 Primers used

Name	5’ → 3’
IB-E-F	GAATCACAAAGTGCATATGGAGACAGACACACTC
IB-E-R	CCGGAATTCCTACTTGTCATCGTCATCCTTGTAGTCCCGTTTCAGCTCCAG
IB-Bm-F	CACCATGGAGACAGACACACTCCTGTTATGG
IB-Bm-R	CTACTTGTCATCGTCATCCTTGTAGTCCCGTTTCAGCTCCAG
HCV-core-F	TCATCATCATCATCATATGAGCACAAATCCT
C123-R	CCGGAATTCCTAGATGACTTTACCCACG
C152-R	CGACAAGCTTGAATTCCTACGCGACAGCTCTGGC
C177-R	CCGGAATTCCTAGAAGATAGAAAAGGGG
C191-R	CAAGAATTCCTAAGCAGAGACCGGAACG

Figure legends

Fig. 1. Construction of the intrabody (A) and HCV core protein variants (B) expressed in this study. The intrabodies expressed in *E. coli* and silkworm pupae have a native signal sequence and FLAG tag sequence at the N- and C-terminus, respectively. Each HCV core protein expressed in *E. coli* has a 6 × His tag sequence at its N-terminus.

Fig. 2. Expression of the intrabody in *E. coli* and silkworm pupae. (A) Western blotting of the intrabody expressed in *E. coli* and silkworm pupae. Western blotting was conducted by using the anti-DYKDDDDK tag monoclonal antibody as a primary antibody. **(B)** Purification of the intrabody from the extract from *E. coli* and silkworm pupae using DDDDK-tagged Protein PURIFICATION GEL and anti-FLAG M2 antibody agarose. To purify the intrabody from the silkworm pupae, anti-FLAG M2 antibody agarose chromatography was conducted 2 times.

Fig. 3. Expression of each HCV core protein in *E. coli*. (A) Western blotting of each HCV core protein expressed in *E. coli*. Western blotting was conducted using anti-polyhistidine-peroxidase antibody and anti-HCV core protein monoclonal antibody as a primary antibody. **(B)** Purification of each HCV core protein from *E. coli* using TALON metal affinity resin. The purification was performed in the presence of DDM.

Fig. 4. ELISA using purified intrabodies and HCV core protein variants. The intrabody from *E. coli* **(A)** and from pupae **(B)** was immobilized onto the wells of an ELISA plate, followed by the binding of each purified HCV core protein after its blocking. The binding of the intrabody to each HCV core protein was detected using anti-polyhistidine monoclonal antibody-HRP conjugate. Gray and white bars in (A) and

(B) denote intrabody and BSA, respectively.

Fig. 5. ELISA using purified intrabodies and HCV core protein variant (C152) on a glass plate. Purified C152 was immobilized onto a SuperEpoxy2 glass slide, followed by the binding of the intrabody from *E. coli* **(A)** and from pupae **(B)** after its blocking. The binding of the intrabody to each HCV core protein was detected using anti-DYKDDDDK tag monoclonal antibody and Fluorescein (FITC) AffiniPure anti-Mouse IgG (H+L).

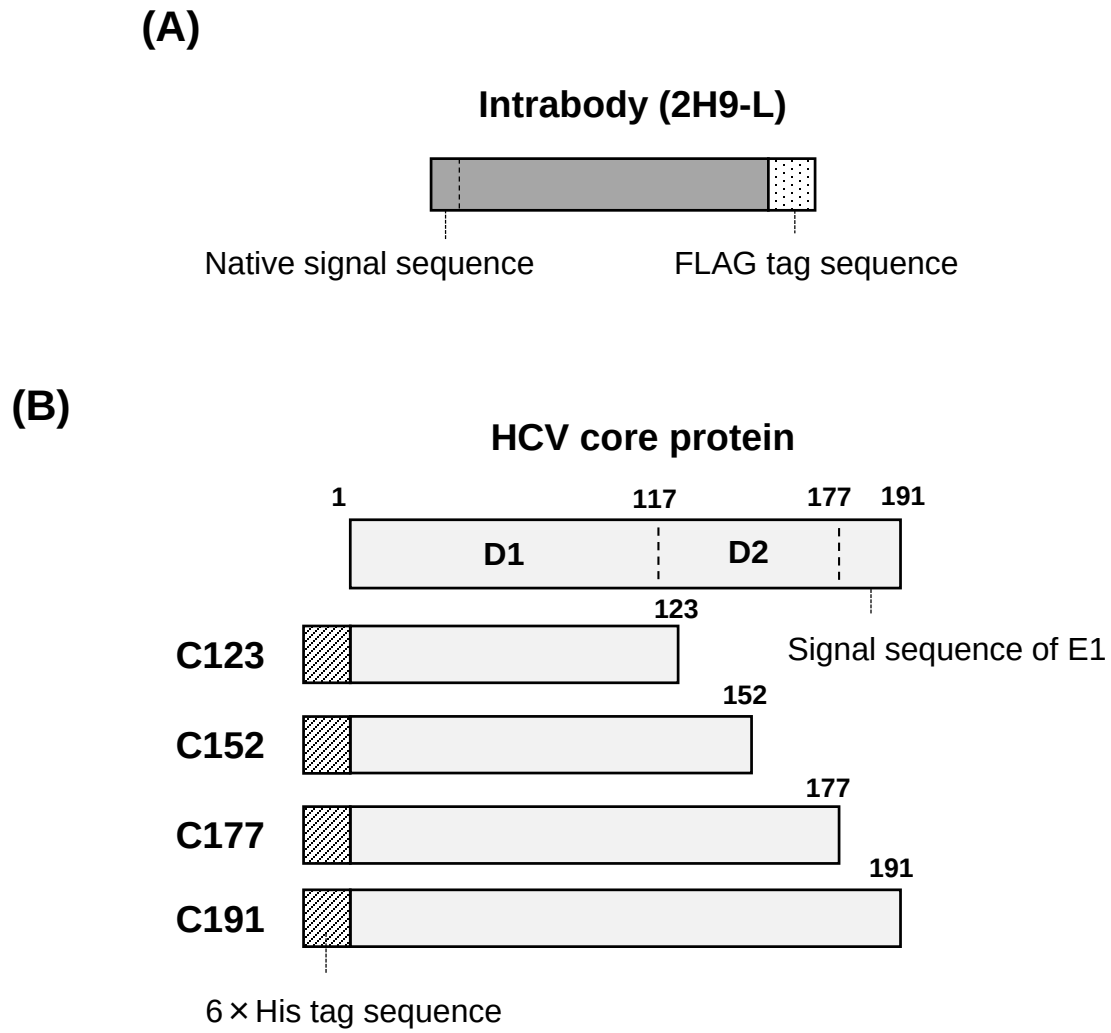

(A)
Intrabody (2H9-L)
Native signal sequence
FLAG tag sequence
(B)
HCV core protein
1
117
177
191
D1
D2
123
C123
Signal sequence of E1
152
C152
177
C177
191
C191
6 × His tag sequence

Fig. 2, Kato et al.

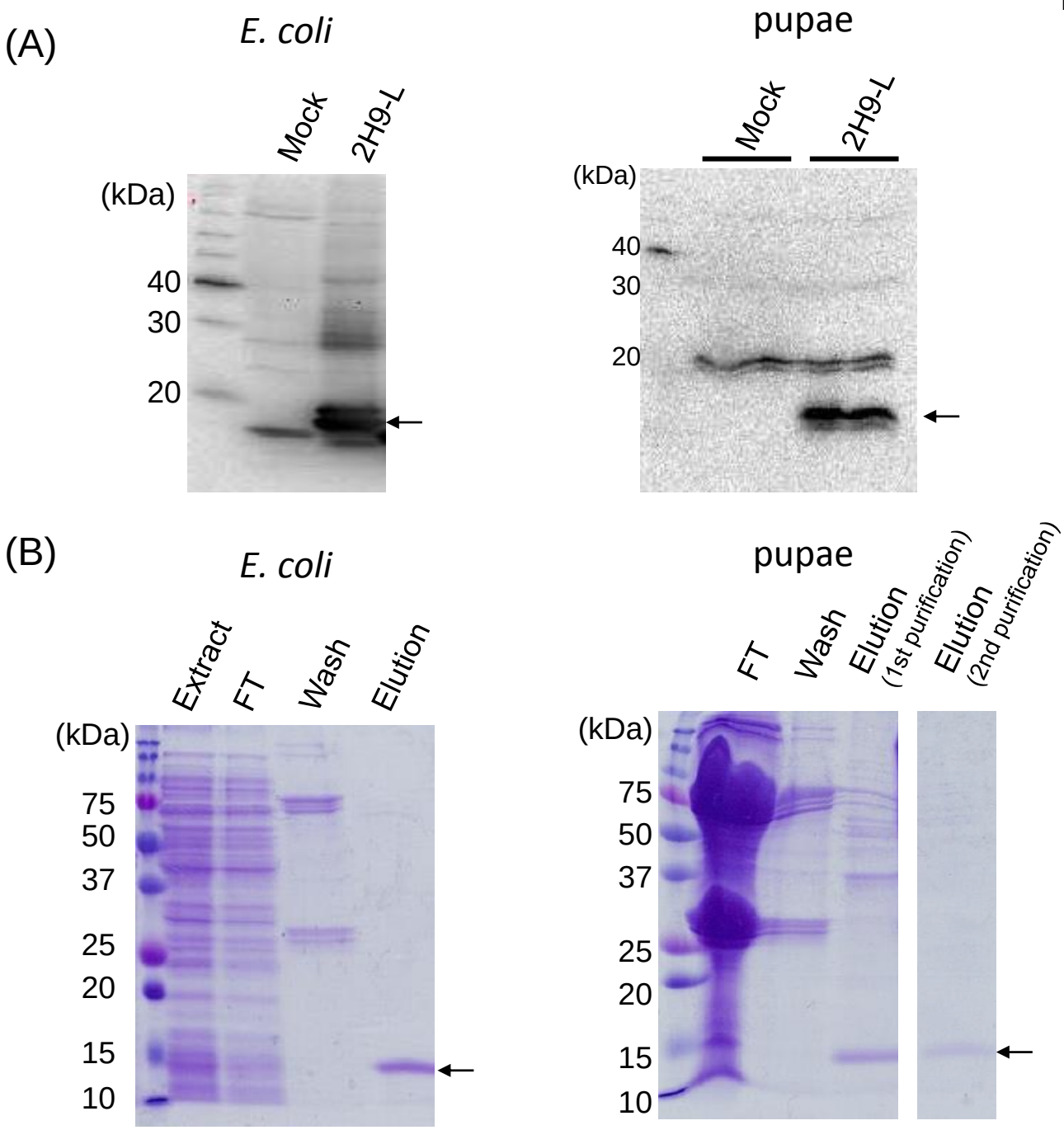
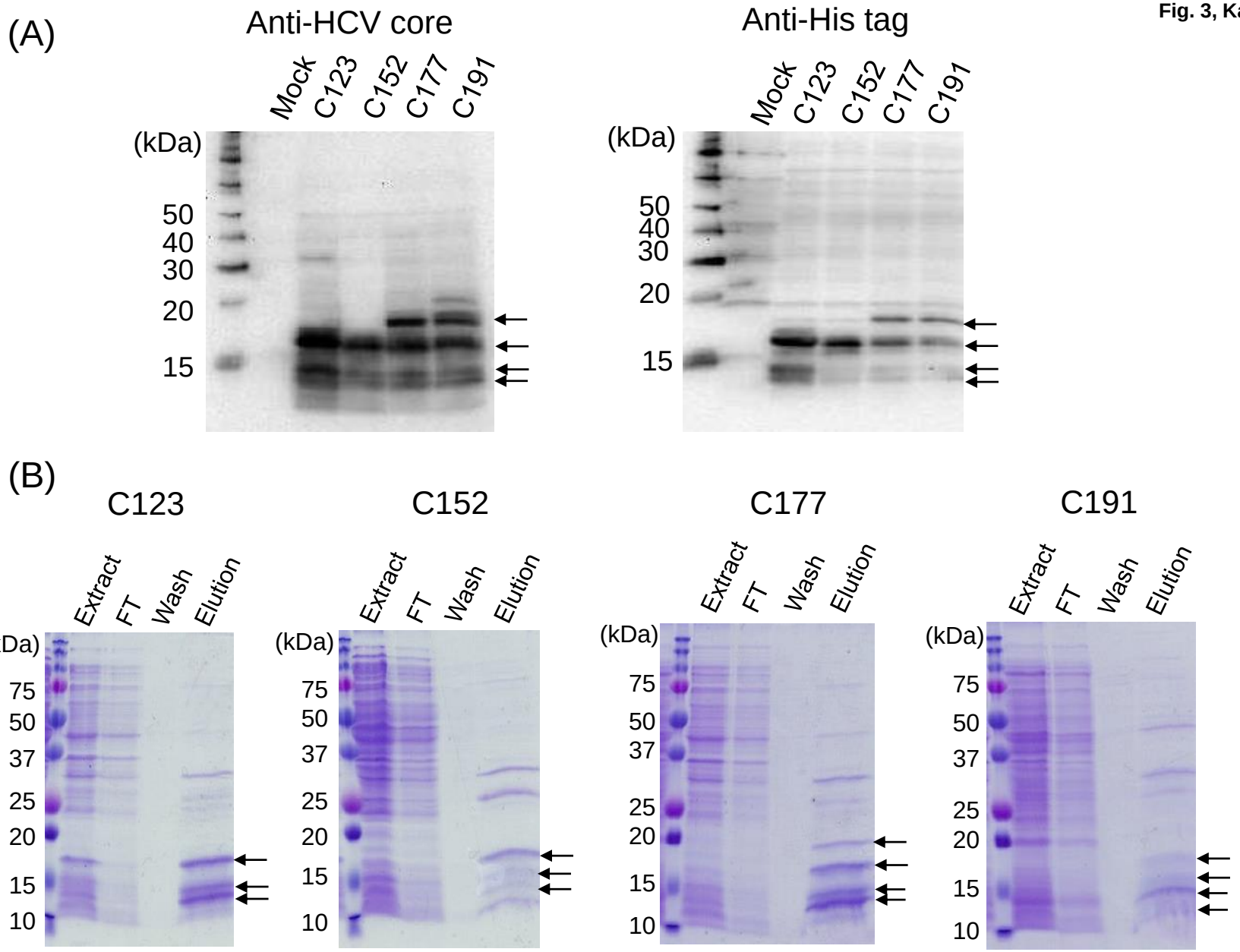
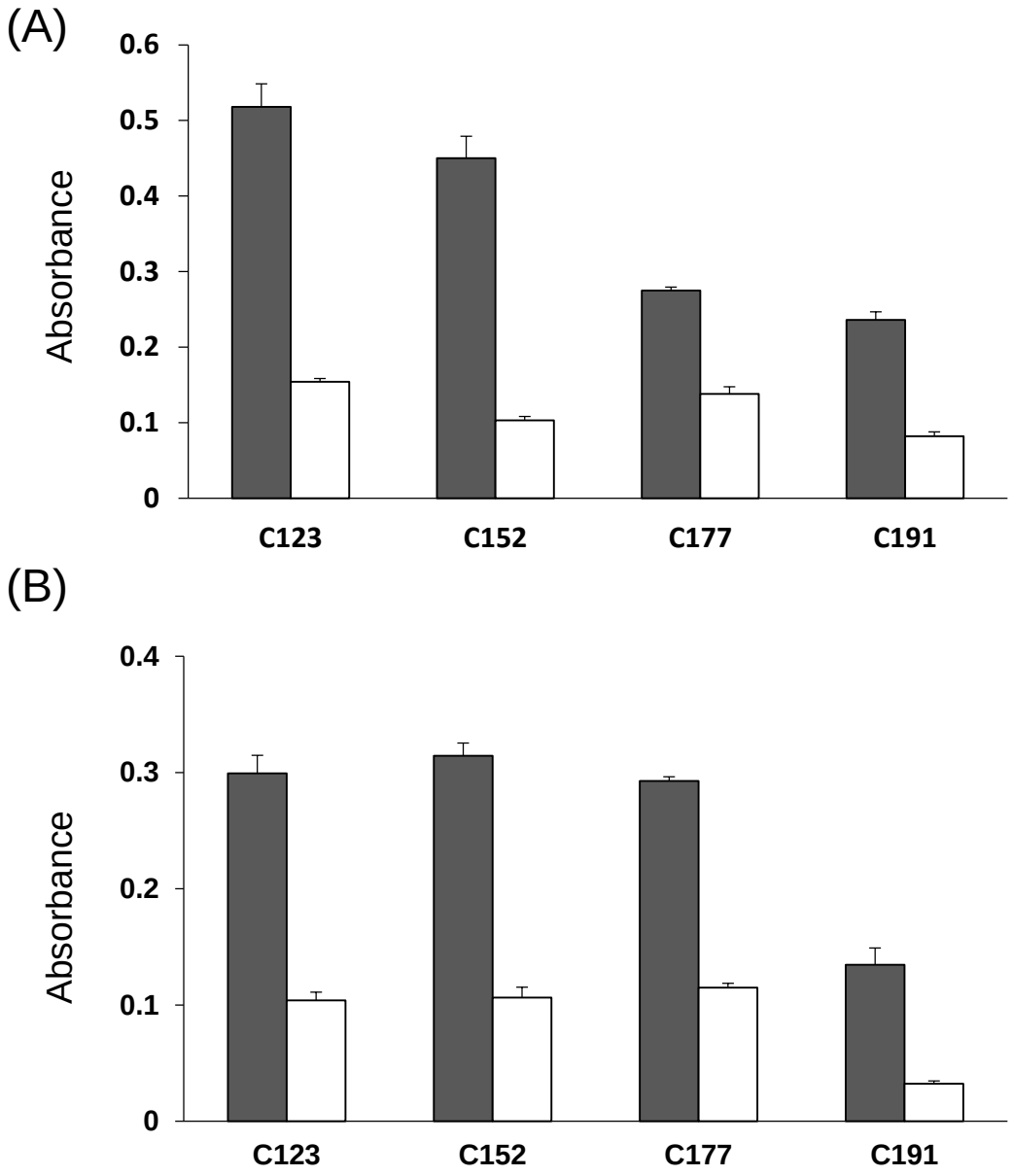
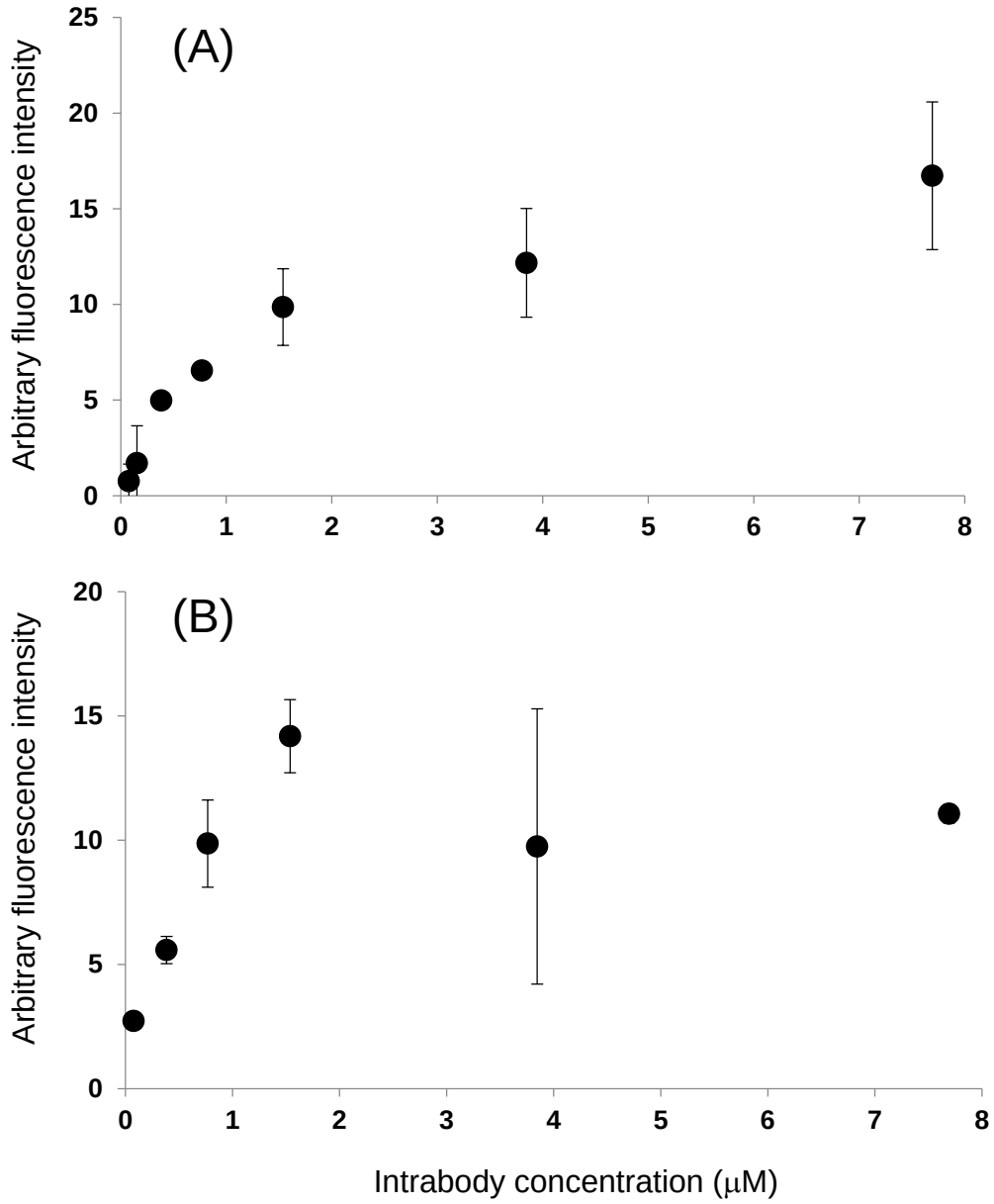


Fig. 3, Kato et al.




(A)
Absorbance
0.6
0.5
0.4
0.3
0.2
0.1
0
C123
C152
C177
C191
(B)
Absorbance
0.4
0.3
0.2
0.1
0
C123
C152
C177
C191


(A)
Arbitrary fluorescence intensity
25
20
15
10
5
0
0
1
2
3
4
5
6
7
8
(B)
Arbitrary fluorescence intensity
20
15
10
5
0
0
1
2
3
4
5
6
7
8
Intrabody concentration (μM)

Supplementary Information

Expression of a functional intrabody to hepatitis C virus core protein in *Escherichia coli* and silkworm pupae

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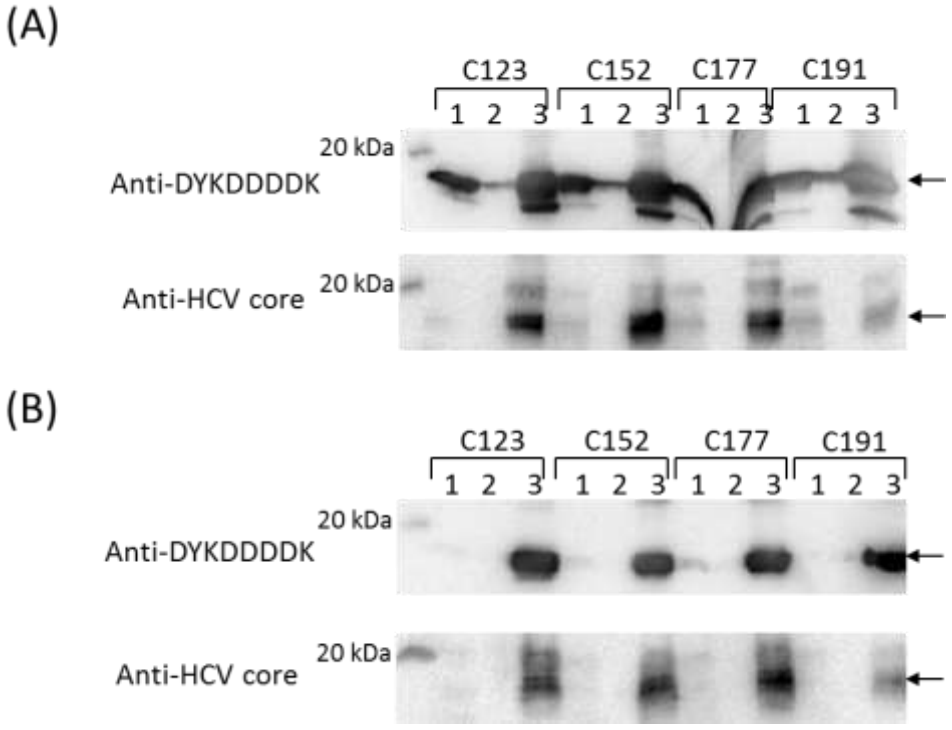
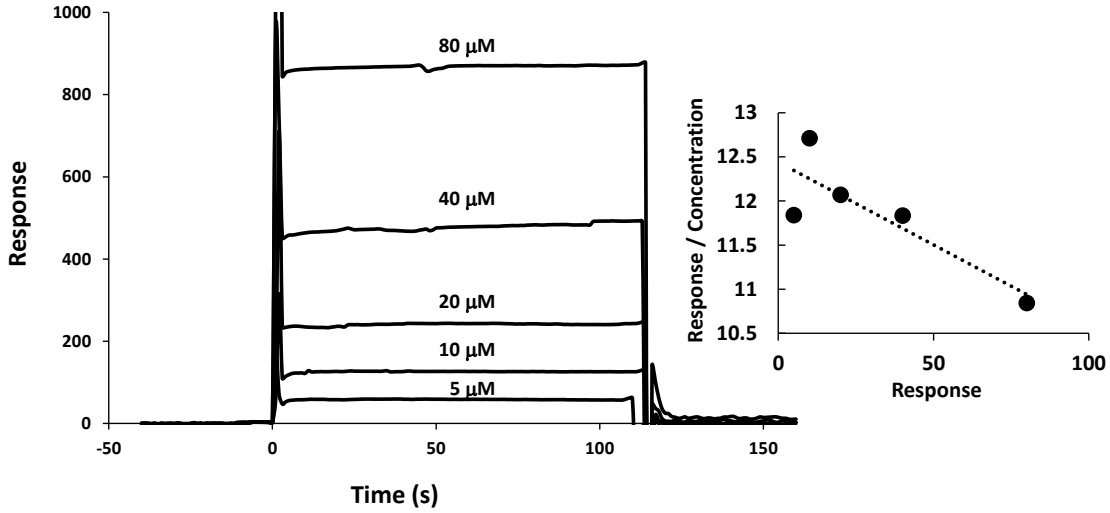


Supplementary Fig. 1. Immunoprecipitation using purified intrabodies and each HCV core protein. Immunoprecipitation was performed using DDDDK-tagged Protein PURIFICATION GEL. Twenty microgram of intrabody purified from *E.coli* **(A)** or silkworm pupae **(B)** and each core protein was mixed and incubated at 4°C for 5 h with gently stirring. DDDDK-tagged Protein PURIFICATION GEL was added into the mixture and incubated at 4°C for 1 h with gently stirring. The mixture was centrifuged at 8000 × g. The supernatant was removed and the gel was washed 3 times by PBS. Sample buffer for SDS-PAGE was added into the gel and boiled for 5 min. Each protein was detected by western blot. Lane 1: Supernatant after immunoprecipitation, Lane 2: Wash fraction of immunoprecipitation, Lane 3: Immunoprecipitation fraction.



Supplementary Fig. 2. Surface plasmon resonance analysis of the intrabody purified from silkworm pupae using Biacore. Surface plasmon resonance experiment was carried out using Biacore 2000 (GE Healthcare Japan, Tokyo, Japan) and HPS-EP buffer (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.005% Surfactant P-20 [GE Healthcare Japan], pH 7.4) as a running buffer. Purified HCV core protein (C152) was immobilized on a CM5 chip (GE Healthcare Japan) by amine coupling at pH 4.0 (1500 – 2000 RU). Purified intrabody was injected into the sensor chip at 30 μl/min. To regenerate the HCV core protein-immobilized chip, 100 mM glycine-HCl buffer (pH 3.5) was used. The dissociation constant (K_d) was calculated by equilibrium analysis.