Catalytic Features and Eradication Ability of Antibody Light-chain UA15-L against *Helicobacter pylori*†§

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We have successfully developed a catalytic antibody capable of degrading the active site of the urease of *Helicobacter pylori* and eradicating the bacterial infection in a mouse stomach. This monoclonal antibody UA15 was generated using a designed recombinant protein UreB, which contained the crucial region of the *H. pylori* urease β-subunit active site, for immunization. The light chain of this antibody (UA15-L) by itself showed a proteolytic activity to substantially degrade both UreB and the intact urease. Oral administration of UA15-L also significantly reduced the number of *H. pylori* in a mouse stomach. This is the first example of a monoclonal catalytic antibody capable of functioning in vivo, and such an antibody may have a therapeutic utility in the future.

One of the potential utilities of catalytic antibodies is to use them for therapeutics, especially against the infectious agents, through the specific destruction of the essential proteins in a virus or bacteria. Many catalytic antibodies degrading antigens such as VIP† (1), DNA (2), HIV gp41 (3), HIV gp120 (4), and factor VIII (5) etc. have been reported in the past decade. However, none of these catalytic antibodies have been developed into a therapeutic agent. *Helicobacter pylori*, a Gram-negative spiral bacteria (6) infecting ~50% of the world’s populations, is an etiologic agent in a variety of gastroduodenal diseases and is the only microorganism known to regularly inhabit the human stomach (7). *H. pylori* produces a large amount of urease, which is essential for the colonization of the stomach and pathogenicity. Although *H. pylori* infection can currently be treated with antibiotics (8, 9), its complete eradication from the stomach is often difficult, due to the adverse effect of the antibiotics and the presence of a resistant bacterium.

*H. pylori* urease enables the bacterium to colonize the human stomach by neutralizing the acidic condition, through the conversion of urea into ammonium (bicarbonate is also produced as a by-product). Therefore, a monoclonal catalytic antibody capable of destroying urease has a potential to be an effective therapeutic strategy to protect the stomach from the infection of *H. pylori*.

We have already prepared HpU-9-L catalytic antibody light chain (10), which was obtained by the immunization of whole molecules of the urease. One of the most important subjects in the study on catalytic antibody is whether or not we are able to prepare the catalytic antibody for the designated portion, which possesses the crucial function of the protein. Such catalytic antibody can erase the crucial function of targeting protein. In this study, a designed antigen (UreB), whose sequence is essential for the active site of the urease, was prepared and a catalytic antibody, UA15-L, was derived.

**EXPERIMENTAL PROCEDURES**

The urease of *H. pylori* is a multimeric enzyme, which consists of an α-subunit and a β-subunit. The active site resides in the latter subunit. The crucial region to exhibit the enzymatic activity of the urease is considered to locate from aa 201–338 in the sequence of β-subunit of the urease (see the underlining in Fig. 1a). The polypeptide, referred as UreB, having the 138 amino acids was prepared in the following method.

**Expression of Immunized Antigen (UreB)**

Genomic DNA extracted from *H. pylori* (ATCC 43504) was used as a template for PCR with oligonucleotide primers, in which the following primers were employed: forward primer (5′-AAAGATCCCGCTTCTAAGCATGCGACG-3′), which contains a BamHI site (underlined), and the reverse (5′-GGG-AATTCCCTTGAATCAGCGAACTG-3′), which contains an EcoRI site (underlined). An aliquot of the PCR mixture was analyzed by agarose gel electrophoresis.

To construct a plasmid for the expression of recombinant UreB fused with the GST protein, the PCR-amplified DNA fragment was ligated to the expression vector, pGEX-6P-1 (Amersham Biosciences). *Escherichia coli* BL21 was transformed and induced the recombinant UreB protein by an
addition of a final concentration of 1 mM isopropyl-
1-thio-β-D-galactopyranoside.

Production and Characterization of mAb

BALB/c mice were primed subcutaneously using 100 
µg/mouse of the recombinant UreB-GST emulsified with 
Freund’s complete adjuvant (Difco Laboratories, Detroit, MI). 
Two weeks after the first immunization, a booster dose com-
posed of an equal amount of the recombinant UreB-GST in 
Freund’s incomplete adjuvant was given. A final booster dose 
without adjuvant was administered 2 weeks after the second 
immunization. Three days after the final booster, splenocytes 
were fused with NS1/1.Ag4.1 or X63-Ag8.635 myeloma cells 
using polyethylene glycol 1500 (Boehringer GmbH, Mannheim, 
Germany), followed by hypoxanthine-aminopterine-thymidine 
selection and screening. Hybridomas were screened using the 
culture supernatant by enzyme-linked immunosorbent assay using
UreB-GST and GST as the coated antigens. The mAbs reacting 
with UreB-GST but not with GST were picked up. Positive hyb-
dromas were subcloned more than thrice by the limited-dilution 
method. The class and subclass of each mAb were determined 
with a mouse mAb isotyping kit (Amersham Biosciences).

Production of pAb against UreB

For obtaining the polyclonal antibodies, rabbits were immu-
nized subcutaneously as follows. One milligram of the UreB 
was dissolved in 1 ml of PBS, which was emulsified with 1 ml of 
Freund’s complete adjuvant. The emulsified mixture (totally 2 
ml) was immunized into one rabbit. The rabbits were given 
boosters at 2-week intervals in the same manner except 
Freund’s incomplete adjuvant instead of Freund’s complete 
adjuvant. The immunized rabbit was bled after the fourth 
booster immunization. The resultant antiserum was purified 
using affinity chromatography (Sepharose 4B coupled with H. pylori urease) for the experiment of immunohistochemical 
staining.

Sequencing and Molecular Modeling

Messenger RNA was isolated from the hybridoma secreting 
UA15 mAb using an mRNA purification kit (Amersham Bio-
sciences). The cDNA of the light and heavy chain were synthe-
sized by a first-strand cDNA Synthesis Kit (Life Science Inc.). 
The VH and VL fragments were amplified directly by adding 
them to a mixture containing PCR components and Mouse Ig 
primers specific for IgG (Mouse Ig primer kit, Novagen, Darm-
stadt, Germany). The amplified DNA was visualized on 2.0% 
agarose gel containing 0.5 µg/ml ethidium bromide. A band of 
~450 bp was observed, which corresponds to the size of the 
variable fragment of the antibody gene with little or no extra-
nearous product. The PCR product was cloned into a pGEM-T easy vector (Promega, Madison, WI). Sequencing was con-
ducted using the Auto Read Sequencing Kit (Amersham Bio-
sciences) and an automated DNA sequencer (OpenGene Sys-
tem, Long-Read Tower, Amersham Biosciences).

Computational analysis of the antibody structures was per-
formed using the deduced VL and VH amino acid sequences by 
a workstation (Octane 2, Silicon Graphics Inc., PA) running 
AbM software (Oxford Molecular Ltd., Oxford, UK), which is

used for building models of three-dimensional molecules. The
resulting Protein Data Bank data were applied to minimize the
total energy by using DS-Modeling (Accelrys Software Inc., San 
Diego, CA). This software uses the CHARMM algorithm for 
minimizing the energy of a molecule (11). Protein Adviser v. 3.5
(FQS Ltd., Fukuoka, Japan) was employed to visualize, analyze, 
and draw the structures.

Immunohistochemical Staining of Gastric Biopsy Specimens

Formalin-fixed/paraffin-embedded human gastric tissues 
infected with H. pylori were used. The sections (2 µm thick) 
were deparaffinized in xylene and dehydrated in graded etha-
nol, and then endogenous peroxidase activity was blocked by
3% H2O2. After rinsing in PBS, the sections were incubated 
with the antiserum (which was purified by affinity chromatography
using Sepharose 4B coupled with H. pylori urease) at a concen-
tration of 10 µg/ml in 1% bovine serum albumin/PBS at room
temperature for 60 min, and then washed three times with PBS 
for 5 min each. The slides were incubated with EnVision+
(horseradish peroxidase rabbit) reagent (Dako Japan Co. Ltd., 
Kyoto, Japan) for 60 min at room temperature and washed 
three times with PBS. Staining was performed using a 3,3’-
diaminobenzidine substrate kit (Nichirei Co., Tokyo, Japan).

Gimenez staining was performed as follows. The gastric
biopsy specimens were deparaffinized and hydrated in distilled 
water. The sections were filtered in a carbol fuchsin solution for
1 min. After washing in water, the slides were stained with 1%
malachite green for 5 s, then washed again in water and dried in
air.

Purification of the Antibody and the Isolation of
the Light Chain

UA15 mAb (IgG1(κ)) was purified according to the purifica-
tion protocol from Bio-Rad Protein A MAPS-II kit (Nippon
Bio-Rad, Tokyo, Japan) according to the purification protocol 
recommended in the Bio-Rad Protein A MAPS-II kit (Nippon
Bio-Rad). Detailed procedures were described in the reference
(10, 12–14).

Cleavage Assays

To avoid contamination, most glassware, plastic ware, and 
buffer solutions used in this experiment were sterilized by heat-
ing (180 °C, 2 h), autoclaving (121 °C, 20 min), or filtration
through a 0.20-µm sterilized filter as far as possible. The exper-
iments were mostly performed in a biological safety cabinet to
avoid air-borne contamination.

In the cleavage assay for UreB, the expressed UreB-GST pro-
tein was digested by trypsin for the purpose of recovery of UreB. 
Then the UreB was purified using UA-15 mAb-fixed affinity
chromatography. The purity was over 99% in SDS-PAGE anal-
ysis. The whole antibody of UA15 does not have a catalytic
activity. Hence, the purification could be performed without
trouble (see under “Results”).

Prior to the cleavage test for UreB protein and the purified
urease of H. pylori, a peptide, TPRGDPREGIEEGGERD
(TP41-1), which has mostly been used for monitoring the cat-
lytic activity of the antibody and/or its subunits (3, 10, 12–14),
was completely digested by the catalytic reaction of UA15-L. By
this reaction, the catalytic activity of UA15-L was held constant, showing no induction time (3, 12–15). In the cleavage assay of UreB and the urease, the UA15-L mentioned above was used. Cleavages of recombinant UreB (10.7 μM) and purified H. pylori urease (57 nM) were carried out using UA15-L in a 15 mM phosphate buffer (pH 6.5) at 25 °C. The reactions were measured with Coomassie Brilliant Blue and silver-stained SDS-PAGE for UreB and urease, respectively, under non-reduced conditions. For the cleavage assay using living H. pylori cells, the surviving number of H. pylori cells was first counted after 0 to 48 h in phosphate buffer. The number of the cells (10^8 cells were prepared) remained almost constant during the incubation without the catalytic antibody. The cleavage reaction was monitored by Western blot analysis using HpU-17 (16) labeled with POD.

**Analysis of the N-terminal Sequence**

For UreB and the urease, the reaction solution at 7–8 h of incubation was recovered and concentrated up to 15- and 10-fold, respectively, using a ultrafiltration membrane (Amicon Ultra-4 5000MWCO, Millipore Corp., Bedford, MA). Then the samples were submitted to a 12% gel SDS-PAGE (reduced condition for UreB and non-reduced for the urease). The bands were transferred for 1 h at 112 mA onto an Immobilon-PVS PVDF membrane (Millipore Corp.) in 0.1 M Tris-HCl, 0.19 M glycine, 5% methanol at pH 8.7. After being stained with Coomassie Brilliant Blue, visible bands were cut and subjected to the N-terminal sequence analysis (Automated Protein Sequencer, Prosize 494 HT, Applied Biosystems, Foster City, CA) with the amount of protein used ranging from 2 to 40 pmol. For 0.5–2 pmol of the fragment, an automatic protein microsequencer Prosize 494 LC (Applied Biosystems) was used.

**Immunoblot Analysis**

After SDS-PAGE was carried out without staining, the proteins were transferred from the gel onto an Immobilon-P PVDF membrane. The PVDF membrane was blocked with TBS containing 3% skim milk and 0.05% Tween 20. After washing with TBS containing 0.05% Tween 20 (TBS-T), it was incubated with peroxidase for 1 h at room temperature. After washing with TBS-T, the color development was performed by using 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (Kirkegaard & Perry Laboratories). For H. pylori cells, 7% SDS-PAGE at 20 mA in a non-reducing condition was employed for detection of the fragments of H. pylori urease.

**Preparation of H. pylori Urease**

H. pylori of the Sydney strain (SS1) was cultured on a Brucella broth (BBL, Cockeysville, MD) agar medium containing 10% fetal bovine serum at 37 °C for 4 days under microaerophilic conditions. Detailed purification methods of the H. pylori urease from the harvested pellet were described in the literatures (16).

**In Vivo Assay**

**Animals**—Specific pathogen-free, 6-week-old female C57BL/6 mice were purchased from Shimizu Laboratory Supplies Co., Ltd. (Kyoto, Japan). Mice were housed under specific pathogen-free conditions and were allowed free access to food and water. Experiments were performed according to the guideline of the Ethical Committee for Animal Experiments at the Faculty of Medicine of Oita University (Oita, Japan) and Prefectural University of Hiroshima (Hiroshima, Japan).

**Infection**—Mice were orally challenged two times at 1-day intervals with 0.5 ml of H. pylori SS1 (1 × 10^9 colony-forming units (CFU)/ml). Seventeen days after the last inoculation, 2 of 29 mice were sacrificed to confirm the colonization of H. pylori in the stomach.

**Oral Administration of UA15-L**—Each mouse (n = 9) was orally administered with a 0.5 ml of UA15-L (20 μg/ml) containing solution in which Meyron (7% sodium bicarbonate, 833.2 mM, Otsuka Pharmaceuticals, Osaka, Japan) was added with one-tenth of the volume in order to neutralize the gastric acidity. In this study, nine mice were used for the administration. The nine mice were used for the control experiment in which the same solution containing Meyron without UA15-L was orally delivered. The administered UA15-L was prepared as described in the section of cleavage assay under “Experimental Procedures.”

**Assessment of Eradication of H. pylori**—All mice were sacrificed, and the stomach of each mouse was isolated a day after the oral administration for bacterial and histological examination. The stomach was washed in sterile 0.8% NaCl and cut longitudinally into two pieces. One half was homogenized in 500 μl of Brucella broth by using a glass homogenizer (Iwaki Glass Co. Ltd., Tokyo, Japan). Fifty microliters of gastric homogenate was serially diluted with Brucella broth and inoculated onto Helicobacter-selective agar plates (NISSUI pharmaceutical Co., Ltd., Tokyo, Japan) at 37 °C for 4 days under microaerobic conditions. Colonies were counted, and expressed as CFU/gm stomach tissue. The numbers of bacterial colony were analyzed and compared by the t test. p values of <0.05 were considered to indicate a significant difference.

The other half of the stomach was used for the histological examination. Histological examination of gastric mucosa included longitudinal sections of the stomach, from the esophagael-cardiac junction through the duodenum, which were fixed in neutral-buffered 10% formalin and embedded in paraffin. Sections 5 μm in length were stained with hematoxylin & eosin (gastric sections were scored and evaluated by the prescence of inflammation characterized by the intensity of neutrophilic and/or lymphocytic infiltration in blind fashion by two independent examiners.)

**RESULTS**

For the accuracy, some data were confirmed by repeated experiments.

**Design of the Immunizing Antigen UreB—H. pylori urease** is a hexamer consisting of α- and β-subunits, with molecular sizes of 26.4 and 61.6 kDa, respectively (17). In the β-subunit, the amino acid residues number (aa) from aa 201 to 338 are essential for its activity. Cys321 (violet colored character in Fig. 1a) strongly interacts with two nickel ions (bi-nickel center) and His221, His248, and His274 also coordinate the ions (18, 19). In addition to these, other amino acid residues including His271,
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Expression of UreB and the Production of the mAb—UreB was expressed in E. coli (BL21) as a fusion protein with GST (UreB-GST). After the immunization of UreB-GST into BALB/c mice, a monoclonal antibody (mAb; UA15) was established using the conventional hybridoma generation technique (16). The mAb obtained specifically reacted with UreB and the intact H. pylori urease but not with GST, bovine serum albumin, human serum albumin, and human γ-globulin. However, it slightly cross-reacted with Jack bean urease.

Molecular Modeling—Sequencing of cDNAs of variable region of the light (UA15-L) and heavy chain of UA15 mAb was conducted, and the amino acid sequences were deduced. Five aa residues at the N terminus of UA15-L were determined, and the sequence (Asp⁵, Ser¹⁷⁴, and His⁹⁵) locate in a close position, which are assumed to form a catalytic triad in such catalytic antibody light chains as VIPase (vaso-intestinal peptide) (20) and i41SL1-2 (15) (these belong to the same germ line gene bd2).

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reacted to the surface urease of the commercially available pAb (DAKO Japan Co. Ltd., Kyoto, Japan) as shown in Fig. 3 (lanes 1 and 2, SDS-PAGE for purified urease and lysate of the bacterium, respectively; lane 3 and 4, Western blots by UA15 mAb for purified urease and lysate of the bacterium, respectively; lanes 5 and 6, Western blots by UA15-L for purified urease and lysate of the bacterium, respectively; M, marker. SDS-PAGE: 12% gel under non-reduced. Urease: 0.2 μg/ml (57 nM). H. pylori; 2 × 10^8 cells/ml. Western blots: immunoreaction: 1 h at room temperature. UA15 mAb: 1 μg/ml. UA15-L: 77 μg/ml. Both antibodies, UA-15 mAb and its light chain (UA15-L), reacted with the β-subunit of the urease of H. pylori (SS1 strain). This result was confirmed by repeated experiments.

Fig. 3 shows the results of the immunohistochemical staining of the formalin-fixed section of the human gastric tissues infected with H. pylori. As previously reported (19), an anti-UreB polyclonal antibody (pAb) produced by the immunization of UreB into rabbit specifically reacted with H. pylori, and the infected sites were clearly observed on the mucosal surface of human stomach (Fig. 3a). Fig. 3b shows the result using a commercially available pAb (DAKO Japan Co. Ltd., Kyoto, Japan) as a positive control. It is considered that the antibodies mainly reacted to the surface urease of H. pylori. Interestingly, the UA-15 mAb reacted similarly with H. pylori (a different specimen from the experiment using pAb) as shown in Fig. 3 (c and d), although it had been known that monoclonal antibodies tend not to react with the formalin fixed specimens.

Cleavage Activity against UreB—Because the epitope of UA15 mAb is not determined, a peptidase activity of UA15-L was examined by using a TP41-1 peptide (TPRGPDPEGIEEGGERDRD), which has been used to monitor the peptidase activity of the catalytic antibody or its subunits (3, 12, 13, 15, 16). The degradation of TP41-1 peptide by UA15-L followed the Michaelis-Menten equation, and its kinetic parameters were obtained as k_cat = 0.24 min^-1 and K_m = 6.2 × 10^{-5} M (see supplemental Appendix S1). These values were similar to those of other catalytic antibodies reported so far (3, 10, 14, 16). The heavy chain by itself or the whole antibody (UA15 mAb) did not show any catalytic activity in this assay. In addition, the light and heavy chains of UA11 mAb, which specifically binds to UreB, hardly showed the catalytic activity when they were monitored up to 200-h incubation.

According to the experimental protocol under “Experimental Procedures,” highly purified UreB (>99%; 10.7 μM) was mixed with UA15-L (0.6 μM) in phosphate buffer (pH 6.5) at 25°C, and the degradation of UreB was monitored by SDS-PAGE under reduced condition with Coomassie Brilliant Blue staining, as shown in Fig. 4a. The band of UreB (19 ± 0.4 kDa) became gradually faint with an increased incubation time, with its complete disappearance after 24 h. At 4- and 8-h time points, a novel fragment with a size of 14.4 (±0.2) kDa was observed, which became faint with further incubation. At 24-h incubation, this 14.4-kDa band disappeared as well as that of UreB, suggesting that the consecutive degradations took place. No other fragment was visible. UreB was not degraded without UA15-L (Fig. 4b).

We characterized the cleavage sites of UreB by N-terminal amino acid sequencing. From the 14.4-kDa fragment, a sequence of DVQVA was detected (the detection intensity = 33 pmol), indicating that the bond between Tyr46 and Asp47 was cleaved. The sequence of this scissile bond is indicated with a red arrow in Fig. 1a.

Cleavage of Purified Urease—The cleavage of H. pylori urease (57 nM) by UA15-L (0.4 μM) was performed under the same reaction condition. The reaction was monitored by SDS-PAGE under a non-reduced condition with silver staining at 0.5, 5, 10, and 24 h of incubation (Fig. 4c). H. pylori urease is a heterohexamer of the α- and the β-subunits with a size of 528 kDa (26.4 kDa × 6) + (61.6 kDa × 6). In a control experiment with the SDS-PAGE under non-reduced condition, we detected the monomers and the multimers of the urease subunits (Fig. 4d). The bands at 31.0 (±0.5) kDa and at 66.0 (±2.8) kDa appeared...
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The N-terminal amino acid sequences of the urease-derived fragments were determined by a procedure similar to the one used for the UreB-derived fragments. After concentrating the samples by up to 15-fold through ultrafiltration, we were able to analyze six bands as summarized in Table 1. Band 1 was derived from the cleavage of the bond at Glu124, Gly125 of the β-subunit (presumably, Gly125 to the C terminus of the β-subunit). From band 2, the cleavages at Tyr241, Asp242 and Met262-Ala263 were identified. Band 3 was identified as the monomeric form of the α-subunit. Band 4 included three fragments. The strongest intensity was QAMGR. Two other were SQAMG and DSQAM corresponding to the cleavages at Asp362-Ser363 and Ser361-Ala362, respectively. Band 5 had a sequence of M KKIS matching with the β-subunit N terminus. Judging from its size, this fragment was likely to be a fragmented portion of the β-subunit, Met1 to Glu124 generated by a cleavage at Glu124, Gly125. A minor sequence GLIVT was also detected in band 5, which was likely to be generated through the successive cleavage of band 1. From band 6, three N-terminal sequences, GLIVT, MKKIS, and MKLTP, were identified. The GLIVT and MKKIS were present in the β-subunit sequence and likely were the products of successive cleavages of bands 1 and 3. The MKLTP sequence matched the N terminus of the α-subunit. Thus UA15-L appeared to have cleaved the α-subunit slightly too. All the detected bands were derived from the urease or its fragments. Thus UA15-L was capable of consecutive cleavages of urease. The enzymatic activity of urease decreased to ~3% of the original activity with the advancement of the degradation.

No Bovine Serum Albumin Cleavage by UA15-L—To examine the substrate specificity, UA15-L (0.4 μM) was incubated with bovine serum albumin (0.58 μM), under the conditions identical to those employed for H. pylori urease. No cleavage of bovine serum albumin was detected after 24 h of incubation (data not shown).

Reaction with Intact H. pylori Cells—UA15-L (0.8 μM) similarly prepared as the case of previous experiments was mixed with living H. pylori cells (5 × 10^9 cells/ml) in 15 mM phosphate buffer (pH 6.5) followed by an incubation at 25 °C with shaking. In this experiment, the reaction was monitored by a Western blot analysis (7% gel was used in SDS-PAGE) using POD-labeled HpU-17 mAb (16), which specifically recognizes the β-subunit. As shown in Fig. 5a, when UA15-L and the bacterium were mixed, the urease β-subunit was gradually degraded with incubation. The degradation time course of the β-subunit is presented in Fig. 5c. It was also observed that the αβn bands gradually became faint with a prolonged incubation. At 0.3 h, a new band appeared at <50 kDa, whose molecular size was correctly estimated to be 30 kDa using 14% gel in SDS-PAGE. The band strength increased with the incubation time. This band is likely to be generated through the successive cleavage of the β-subunit. In contrast, the α-subunit of the urease was barely degraded (data not shown).

In control experiment (Fig. 5b), the β-subunit was clearly visible at 66 kDa, and αβn also remained. These bands were not affected by an incubation up to 24 h without UA15-L.

In Vivo Assay—The most appropriate schedule, which was found by many preliminary experiments, for H. pylori infection

FIGURE 4. Cleavage assays for UreB and purified urease by UA15-L. a, cleavage for UreB with UA15-L (UreB, 10.7 μM; UA15-L, 0.6 μM); b, control (without UA15-L). The SDS-PAGE (12% gel) was performed under reduced condition with Coomassie Brilliant Blue staining. The catalytic reaction was carried out in 15 mmoles phosphate buffer (pH = 6.5) at 25 °C. The band of UreB became faint with an increase of the reaction time. At 4 h of incubation, a new band at 14.4 kDa appeared, and it completely disappeared at 24 h of incubation, suggesting that the consecutive degradation took place. In the control experiment, UreB was unchanged. c, cleavage of purified urease by UA15-L (purified urease: 57 nM; UA15-L: 0.4 μM); d, control experiment without UA15-L. SDS-PAGE (12% gel) was performed with silver staining under non-reduced condition. The reaction at 25 °C was carried out under similar conditions as employed in a and b. There were many fragmented bands observed (indicate with open triangle arrows). UA15-L could completely decompose the β-subunit of the urease in a time-dependent manner. In the control experiment, no degradation of the β-subunit (and α-subunit) was observed. The cleavage assay was performed six times. All experiments showed similar results.
through oral administration is shown in Fig. 6a. First, C57BL/6J mice were orally inoculated two times at 1-day intervals with \( H. \) \textit{pylori} (SS1: \( 50 \times 10^6 \) CFU for each mouse). To confirm the infection of the bacteria in the mice stomach, 2 of 29 mice were sacrificed and were examined for the number of colonized \( H. \) \textit{pylori}. At 17 days after the last inoculation, \( 15.9 \times 10^6 \) CFU of bacteria per 1.0 g of stomach tissue were recovered. At 24 days after the confirmation of infection, 0.5 ml of UA15-L (0.8 \( \mu \)M), UA15 mAb (0.4 \( \mu \)M), or phosphate buffer (control) were orally administered to each C57BL/6J mouse. All the administered solution contained 10% Meyron (7% sodium bicarbonate, 833.2 mM) for the neutralization of the gastric acidity in the stomach.

For each group, nine or eight mice were used. No mucosal adjuvant such as cholera toxin, which is mostly used for the oral vaccination, was employed in this experiment.

After the next day of the administration, all mice were sacrificed, and the stomach was isolated for the assessment of eradication of \( H. \) \textit{pylori} and the histological examination of the gastric mucosa. One half of the specimen was homogenized for the bacterial examination to assess the number of infected \( H. \) \textit{pylori}. The other was used for the histological examination. The results of the bacterial examination are shown in Fig. 6b. The mean bacteria number calculated for the UA15-L administered mice with was \( 1.71 \times 10^6 \) CFU/g of stomach tissue. The value for whole antibody UA15 mAb was \( 2.82 \times 10^6 \) CFU/g of stomach tissue. On the other hand, the control mice showed the mean value of \( 4.93 \times 10^6 \) CFU/g of stomach tissue. Thus the number of bacteria colonizing the stomach was reduced to one-third less with the administration of UA15-L, compared with the control group (\( p < 0.05 \)). The decrease of \( 15.9 \times 10^6 \) CFU of the sacrificed mice to \( 4.93 \times 10^6 \) CFU of the control mice may be due to the interval of 24 days by the administration of UA15-L, because the number of colonies has a tendency to decline along with the time after infection. Whole antibody, UA15 mAb, did not show a statistically significant difference from the control group.

Histological examination was performed by hematoxylin & eosin staining using the \( H. \) \textit{pylori}-infected stomach. Although \( H. \) \textit{pylori} was partially eradicated by the administration of UA15-L, the gastritis scores of the stomach among UA15-L-, UA15 mAb-, and PB-administered groups were not different. These results might be due to the fact that the histological analysis was carried out the next day of the administration of UA15-L. The effect on gastric scores might have needed a longer time to take place.

**DISCUSSION**

A monoclonal antibody UA15 was produced by immunization using a recombinant protein UreB, which contained the crucial part of the \( H. \) \textit{pylori} urease (19) active site. UA15 mAb could bind to UreB as well as the urease \( \beta \) subunit. Interestingly, its light chain, UA15-L by itself showed the same binding feature as that of UA-15.

Erhan \textit{et al.} (22) suggested that the light chain of the antibody could function as a peptidase/protease by itself based on the homology between antibody light chains and serine proteases. It has been reported that the active site composed of catalytic dyad or triad of the catalytic antibody can function to hydrolyze the antigens. Kolesnikov \textit{et al.} (23) reported that the antibody possessing the catalytic dyad (His\textsuperscript{35} and Ser\textsuperscript{99}) in the heavy chain is the active site that hydrolyzes the acetylthiocholine molecule. In addition, Paul \textit{et al.} (20) also revealed that the catalytic triad composed of Asp\textsuperscript{1}, Ser\textsuperscript{277}, and His\textsuperscript{93} in the light chain (whose germ line belongs to \( bd2 \)) could catalytically hydrolyze the antigen VIP. In our case, UA15-L belonged to \( bd2 \) germ line (DDBJ; accession No. AB286872) and possess the identical aa residues to the catalytic triad of VIPase (20), ECL2B-L (13, 21), and i41SL2-1-L (15, 21). Thus, it was predicted that UA15-L could hydrolyze the antigen UreB. In contrast, we could not see any possible catalytic triad structure in the heavy chain because of the lack of His in the variable region.

As the result of the cleavage assay, UA15-L decomposed the peptide bond at Tyr\textsuperscript{46}–Asp\textsuperscript{57} of UreB. In addition, the light chain could cleave the same peptide bond in the urease \( \beta \) subunit. Hence, it appeared that UA15-L first bound to the UreB region and cleaved the peptide bonds at Tyr\textsuperscript{241}–Asp\textsuperscript{242} or Met\textsuperscript{262}–Ala\textsuperscript{263}. Successively, peptide bonds at Ser\textsuperscript{362}–Asp\textsuperscript{362}, Asp\textsuperscript{362}–Ser\textsuperscript{363}, and Ser\textsuperscript{363}–Gln\textsuperscript{364} were cleaved. The position Glu\textsuperscript{124}–Gly\textsuperscript{125} was also cleaved, generating two bands corresponding to Met\textsuperscript{1}–Glu\textsuperscript{125} and Gly\textsuperscript{125}–Asn\textsuperscript{200}. There have been reports of natural antibodies capable of cleaving several peptide bonds. Kaveri \textit{et al.} reported that several peptide bonds were cleaved in their natural catalytic antibody for factor VIII (5). Paul \textit{et al.} also reported multiple cleavage sites in HIVgp120 by a catalytic IgM monoclonal antibody (4). The UA15-L also was capable of peptide bond cleavage at multiple sites. For the cleav-

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**TABLE 1**

Results of N-terminal amino acid sequence analysis for fragmented polypeptides from the urease of \( H. \) \textit{pylori}

| Fragmented band | Size (kDa) | aa residues of N-terminal (5 residues) | Cleavage site |
|-----------------|------------|---------------------------------------|--------------|
| Band 1          | 51.4       | Gly (0.73), Leu (0.72), Ile (0.73), Val (0.59), and Thr (0.37) | Glu\textsuperscript{124}–Gly\textsuperscript{125} of \( \beta \) subunit |
| Band 2          | 51.4       | Asp (0.21), Val (0.30), Gln (0.24), and Val (0.34) | Tyr\textsuperscript{241}–Asp\textsuperscript{242} of \( \beta \) subunit |
| Band 3          | 31.1       | Ala (1.17), Ala (1.34), Ile (0.89), Ala (1.02), and Gly (0.52) | Met\textsuperscript{262}–Ala\textsuperscript{263} of \( \beta \) subunit |
| Band 4          | 28.8       | Met (11.3), Lys (10.2), Leu (10.0), Thr (8.4), and Pro (4.8) | N-terminal of \( \alpha \) subunit |
| Band 5          | 17.5       | Gln (6.3), Ala (7.8), Met (4.8), Gly (5.0), and Arg (1.7) | Ser\textsuperscript{362}–Gln\textsuperscript{364} of \( \beta \) subunit |
| Band 6          | 15.3       | Gln (6.3), Ala (6.2), Met (3.5), and Gly (4.3) | Asp\textsuperscript{362}–Ser\textsuperscript{363} of \( \beta \) subunit |

* These values were not highly accurate.
The degraded band at 30 kDa corresponds to band 4, suggesting that the degradation of urease of living bacteria was strongly observed, indicating that the degradation of urease of living bacteria was strongly degraded with the increase of the incubation time. During the degradation, a strong band below 50 kDa (whose molecular size was correctly estimated at Ct) was observed, suggesting that the degradation of urease of living H. pylori cells occurred.

Regarding the possession of catalytic activity of the light chain, we consider that the location of catalytic triad (Ser, His, and Asp) in the antibody structure may be crucial. If the triad is located close to the surface of the antibody, both light chain and the whole antibody may have a catalytic activity. However, when the triad locates at the interface between the light and the heavy chain, the whole antibody cannot exhibit the catalytic activity. Once the light chain is separated from the heavy chain, we are able to observe the catalytic activity. One more possibility is considered. The structure of whole antibody is rigid. In contrast, that of light chain is flexible, so that it can easily change the conformation. As a result, three amino acids (Ser, His, and Asp) can come into a close position so as to make a catalytic triad showing the catalytic activity. We have already reported that the conformational change of the light chain takes place in the induction period of the cleavage reaction (12). During the cleavage reaction, the enzymatic activity of urease declined to 3%, indicating that UA15-L destroyed not only the β-subunit but also the enzymatic function of the urease. As pointed out by Ha et al. (18), the enzyme sequence from Glu313 to Asp336 is the flap (helix-turn-helix) and flanking region (magenta dotted underline in Fig. 1a) of H. pylori urease. The region is highly conserved among the bacteria such as K. aerogenes and P. mirabilis, and the flap region is essential for these bacteria. UA15-L cleaved the peptide bonds of Tyr241-Asp242 and Met262-Ala263, located upstream of the flap region. It also digested the peptide bonds of Ser361-Asp362-Ser363-Gln364, located downstream of the flap region. The cleavages of these peptide bonds by UA15-L released this region from the β-subunit leading to the activity loss. Note that the urease was also degraded when the intact bacteria were treated with UA15-L. The degraded band at 30 kDa corresponds to band 4 in Table 1. Moreover, the antibody light chain could suppress the number of colonizing H. pylori in the stomach (p < 0.05) in vivo.

Recently, triple therapy, which typically consists of two antibi-otics with anti-acid drugs, has become “the gold standard” for...
treatment to eradicate *H. pylori*. However, adverse reactions, including allergy, liver dysfunction, and diarrhea, remain to be overcome. In addition, the emergence of antibiotic-resistant bacteria has complicated therapeutic strategies. A single-dose administration of antibiotics exhibited only a ~37% eradication rate (24). Therefore, the antibiotics are usually administered every 7 days. Although in our case because of the difficulty of the production of enough quantity of the catalytic antibody for in vivo assay, one dose was given. Nonetheless, colonizing bacteria in mouse stomach was reduced up to 70% compared with control. This should be a significant reduction as well as the administration of antibiotics. On the other hand, prophylactic and therapeutic vaccinations have been extensively studied as the alternatives to antimicrobial treatment. In these studies, repeated vaccinations (three to five repeats) using antigens such as a bacterial lysate of *H. pylori*, a recombinant urease protein, inactivated bacterial protein, and others were orally performed using mucosal adjuvant (*e.g.*, cholera toxin) (25–28). It was reported that the number of bacteria reduced to 10% of control even in the most effective case.

Taking together these data and results, the effect of the administration of the catalytic antibody might be comparable or superior to the antibiotics and the oral vaccinations. More detailed experiments should be conducted in the near future. Although the mechanism of this eradication of *H. pylori* living in the stomach is not clear at the present time, it is likely that *H. pylori* was killed by the strong acid in the stomach, because of the reduced urease activity due to this catalytic antibody.

Conclusively, this type of antibody could have utility as a therapeutic medicine after more rigorous testing of cleavage activity due to this catalytic antibody.

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**Antibody Light-chain UA15-L against H. pylori**