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Preparation of Quenchbodies by protein transamination reaction

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Quenchbody (Q-body) is an antibody fragment labeled with fluorescent dye(s), which functions as a biosensor via the antigen-dependent removal of the quenching effect on fluorophores. It is based on the principle that the fluorescence of the dye(s) attached to the antibody N-terminal region is quenched primarily by the tryptophan residues present in the variable regions, and this quenching is released when the antigen binds to the antibody, resulting in increased fluorescence intensity. Hence Q-body is utilized in various immunoassays for the rapid and sensitive detection of analytes. So far, Q-bodies have been prepared by using a cell-free translation system or by combining *Escherichia coli* expression and post-labeling steps. However, the above methods need antibody gene cloning, and are time-consuming. In this study, we report a novel approach to prepare Q-bodies by protein N-terminal transamination. We used the antigen-binding fragment (Fab) of an antibody against the Bone-Gla-Protein (BGP), a biomarker for bone diseases, which was expressed in *E. coli*. The purified Fab was treated with Rapoport's salt to convert the amino group at the N-terminus to a ketone group, which in turn was allowed to react with fluorescent probes that have aminooxy or hydrazide groups, to prepare a Q-body. The Q-body prepared by this method could detect the BGP-C7 antigen at concentrations as low as 10 nM. Since the approach can label the protein N-terminus directly, it could be applied for preparing Q-bodies from natural antibodies and for the rapid screening of high-performance Q-bodies.

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[**Key words:** Fluorescence labeling; Bioconjugation; Antibody; Homogeneous immunoassay; Biosensor; *N*-Methylpyridinium-4-carboxaldehyde]

Immunoassays employing the antigen–antibody reactions exhibit high specificities and sensitivities for detecting target substances. Unlike traditional analytical methods such as liquid chromatography and mass spectrometry, which require large and costly instruments, immunoassays are simple, convenient, can be performed quickly, and have high specificity and sensitivity. Consequently, immunoassays have broad application prospects (1). Many types of immunoassays such as sandwich enzyme-linked immunosorbent assay (ELISA), competitive ELISA, and open-sandwich (OS) ELISA (2,3) have been developed. The last approach was applied to a homogeneous OS fluoroimmunoassay (4,5), which led to the recent discovery of Quenchbody (Q-body) technology that allows the detection of various antigens within a short time (6–8). Q-body operates based on the principle of the antigen-dependent removal of the quenching effect on a fluorophore that had been quenched by tryptophan (Trp) residues in the antibody fragment, or in some cases, by another fluorophore. The use of Q-body-based immunoassays has increased due to its high sensitivity and applicability to an extensive range of antigens. An advantage of the Q-body method is its simplicity, which is straightforwardly performed by mixing a Q-body with an antigen subsequent to fluorescence measurement. It is complementary to traditional methods, which require several incubation and washing steps, including laborious manipulations.

Thus far, Q-bodies were prepared using either a cell-free translation system or a combination of expression in recombinant *Escherichia coli* and a post-labeling of fluorescent dye(s). In the cell-free system, fluorescent dyes are introduced into the N-terminal region of antibody fragments as a non-natural amino acid (9). However, the limited yield obtained with this approach results in an increase in the cost of the product. Another approach is the combination of antibody fragment expression using *E. coli* and labeling with fluorescent dyes via maleimide-thiol reaction. However, since vector construction, protein expression, and purification are necessary, both of the above methods are still time-consuming.

Transamination is a chemical reaction between an amino acid, which contains an amine group, and a keto acid, which contains a keto group. In transamination, the NH_2 group on one molecule is exchanged with the =O group on the other molecule. This reaction can be described as the nucleophilic substitution of an amine or amide anion on an amine or ammonium salt. Gilmore et al. (10) reported a site-specific transamination reaction that introduces a new ketone group at the N-terminus of proteins on incubation with pyridoxal 5′-phosphate. The carbonyl groups introduced by this reaction are not naturally occurring in proteins; therefore, they are used as unique points of attachment for synthetic groups, through the formation of hydrazone or stable oxime bonds (11). More recently, Witus et al. (12) reported an improved protein transamination reagent, *N*-methylpyridinium-4-carboxaldehyde benzenesulfonate salt (Rapoport's salt; RS), which requires less harsh reaction condition.

Human osteocalcin (also known as bone γ-carboxyglutamic acid (Gla)-protein or BGP) is a 49-amino acid peptide that is a major

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non-collagen bone protein. It is known to be a marker for bone metabolism and is speculated to be involved in insulin regulation (13) and male fertility (14). BGP level in the blood of healthy individuals is estimated to be 2.5–10 ng/ml. Lim et al. (16) have developed a practical OS ELISA to detect the C-terminal peptide of BGP with an antibody, KTM219 (Fig. 1A), which showed higher sensitivity than the corresponding competitive ELISA. Furthermore, the sensitivity of OS ELISA improved ~300-fold by phage-based affinity maturation (17). In this study, we chose a human osteocalcin peptide fragment for targeted detection. By labeling the antigen binding fragment (Fab) of antibody KTM219 by protein transamination, we prepared a new type of Q-body to detect the BGP-C7 peptide.

MATERIALS AND METHODS

Materials *E. coli* strains XL10-Gold (Stratagene, La Jolla, CA, USA) and SHuffle T7 Express lysY (New England Biolabs, Ipswich, MA, USA) were used for cloning and protein expression, respectively. Restriction and modification enzymes were purchased from Takara Bio (Otsu, Shiga, Japan), Toyobo (Osaka, Japan), Roche Diagnostics (Tokyo, Japan), or New England Biolabs. BGP-C7 peptide (NH_2-RRFYGPV-COOH; molecular weight: 893) was synthesized by LifeTein (Somerset, NJ, USA), and oligonucleotides were synthesized by Eurofins Genomics (Tokyo, Japan). Other chemicals, reagents, and antibodies, unless otherwise indicated, were purchased from Sigma (St. Louis, MO, USA) or Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Construction of vector Previously, we constructed a vector pUQ1H-KTM219 to express the antigen-binding fragment (Fab) of the anti-osteocalcin antibody KTM219, with an additional cysteine at the N-terminal of heavy chain variable region (V_H) (7). To express the Fab without this N-terminal tag, we constructed pET-Fab(KTM219) (Fig. 1B), by removing the N-terminal tag sequence including cysteine. Briefly, the gene for V_H of KTM219 was PCR-amplified using the plasmid pUQ1H-KTM219 as the template, and the following primers: Nde-KTM219 (5′-TATACATATGGCTACCGGTCAAGTAAAGCT-3′) and VL219NcoSalBack (5′-GCTCGAGACGGTGACCGTGGTCCCTT-3′). The purified PCR products were treated with *Nde*I/*Xho*I. Furthermore, the plasmid pUQ1H-KTM219 (7) was also treated with *Nde*I/*Xho*I; the longer fragment was purified and ligated with the treated PCR products by using Ligation high ver. 2 (Toyobo). *E. coli* XL10-Gold cells transformed with the above mixture were cultivated overnight at 37°C on LB plates (1.0% tryptone, 0.5% yeast extract, 0.5% NaCl, 1.5% agarose) with 100 μg/ml ampicillin. Positive clones were identified by colony PCR screening, using the T7 promoter primer (5′-TAATACGACTCACTATAGG-3′) and T7 terminator primer (5′-ATGCTAGTTATTGCTCAGCGG-3′), and cultivated for the preparation of plasmids. The nucleic acid sequences were analyzed by the Biomaterial Analysis Center, Technical Department, Tokyo Institute of Technology.

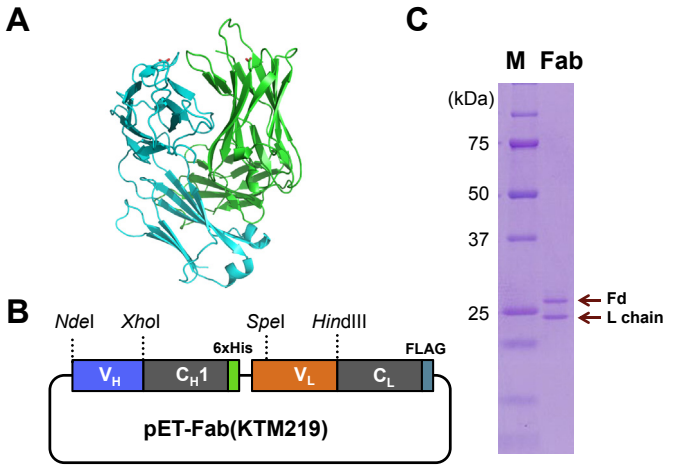

FIG. 1. Construction of KTM219 antigen-binding fragment (Fab). (A) Structure of KTM219 Fab based on the X-ray crystal structure (15). (B) Vector for expression of KTM219 Fab. (C) SDS-PAGE analysis of purified KTM219 Fab. Lane M, Precision Plus Protein Unstained Standards; V_H, variable region of heavy chain; C_H1, constant region 1 of heavy chain; V_L, variable region of light chain; C_L, constant region of light chain; 6× His, 6 histidine tag; Fd, V_H–C_H1 of KTM219 Fab.

Protein expression and purification *E. coli* SHuffle T7 Express lysY cells were transformed with the constructed plasmid and cultured overnight at 30°C in LB agar containing 100 μg/ml ampicillin and 30 μg/ml chloramphenicol (LBAC). The cells picked from a single colony were grown in liquid LBAC medium, and when the absorbance at 600 nm (OD_{600}) reached 0.5, 0.4 mM isopropylthio-β-galactopyranoside (IPTG) was added to induce protein expression. The culture was further incubated for 16 h at 16°C with shaking at 200 rpm. Intracellular soluble Fab proteins were extracted by sonication and purified using TALON immobilized metal affinity resin (Clontech, Takara Bio). To confirm the amount and purity of the protein, 4 μl of each sample was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and stained with a Quick-CBB staining kit (Wako Pure Chemicals).

Preparation of TAMRA-C5-peptide-hydrazide The following two fluorescent probes were used to label the Fab fragment: Aminooxy-5(6)-TAMRA, purchased from Biotium Inc., and TAMRA-C5-peptide-hydrazide (Fig. 2B). The latter was prepared as shown in Fig. S2. In brief, the peptide NH_2-CSNETGGGSGGGS-hydrazide with a cysteine at the N-terminus and a hydrazide group at the C terminus was synthesized by AnaSpec Inc. (Fremont, CA, USA), and equilibrated with phosphate buffer (50 mM sodium phosphate, 100 mM NaCl, 10 mM EDTA; pH 7). Equal volumes of the peptide and immobilized Tris(2-carboxyethyl) phosphine hydrochloride (TCEP-HCl) disulfide reducing gel slurry (Thermo), were added to a microtube and centrifuged at 500 ×*g* for 1 min. Equal amounts of the collected supernatant and TAMRA-C5-maleimide were mixed, and incubated for 1 h at room temperature, on a rotating wheel. After centrifuging for 1 min, 1 mM cysteine was added to the mixture to block the unreacted maleimide groups. The molar ratio of peptide to TAMRA-C5-maleimide was 1:1.

Labeling of Fab by protein transamination Scheme of the labeling reactions is shown in Fig. 2A. Equal volumes of stock solutions of Fab fragments and RS, each prepared at twice the desired final concentration (2×), were mixed in a 1.5 ml tube. The final volume of each reaction was 100 μl. The 2× Fab stock solutions were prepared at a concentration of 1 mg/ml in 25 mM phosphate buffer at pH 6.5. The 2× RS stock solution (200 mM) was freshly prepared before each reaction, in 25 mM phosphate buffer (with 0.02% NaN_3) at pH 6.5. The reaction mixture was briefly agitated to ensure mixing and then incubated at 37°C for 1 h without further agitation. Following the reaction, the excess aldehyde was removed using a Zebra Desalting column (Pierce, USA). The resulting keto-Fab solution was exchanged for the buffer using a Nanosep Centrifugal-10 k Ultrafiltration Device (Millipore, Billerica, MA, USA). The buffer exchange involved the dilution of each sample to 500 μl, with 25 mM phosphate (pH 6.5). Then, each sample was concentrated to 100 μl, and this process was repeated thrice. The resulting keto-Fab solution was then treated with the stock solution of the desired fluorescent probe (10-fold molar of protein in dimethylsulfoxide) and 1 mM *p*-phenylenediamine, in a 1.5-ml tube and incubated at room temperature for 2 h. After oxime formation, the product was subsequently incubated with 20 μl Flag M2 affinity gel for 2 h at room temperature. After incubation, the column was washed thrice with wash buffer (20 mM phosphate, 0.5 M NaCl, 0.1% polyoxyethylene(23)lauryl ether (Brij-35); pH 7.4). The bound Q-bodies were subsequently eluted with 50 μl wash buffer containing 150 μg/ml Flag peptide. The Q-body concentration was determined by comparing the fluorescence intensities of a known concentration of fluorescent dye with that of the Q-body under the denaturing conditions of 7 M guanidinium hydrochloride (GdnHCl) with 100 mM dithiothreitol (DTT).

SDS-PAGE analysis SDS-PAGE was performed on a SuperSep Ace gel (Wako Pure Chemicals). After electrophoresis, the gel was visualized using a fluorescence imaging system to confirm the presence of the label in the protein, followed by staining with a Quick-CBB staining kit (Wako). Precision Plus Protein Dual Color Standards (Bio-Rad, Heracures, CA, USA) were used as protein standards. To separate Fd and L chains, samples were loaded without dithiothreitol (DTT).

Enzyme linked immunosorbent assay The antigen-binding activity of the Fab fragments and the Q-bodies were tested using enzyme linked immunosorbent assay (ELISA) (Fig. 3A). A 96-well microplate (Greiner, Tokyo, Japan) was coated overnight with 100 μl streptavidin (10 μg/ml in PBS) per well, at 16°C. Some of wells were washed, added with biotinylated BGP-C11 and incubated for 30 min. Then, the plate was blocked with 20% ImmunoBlock (DS Pharma Biomedical Co., Ltd.) at 25°C for 2 h, washed thrice with PBST (0.2% Tween-20 in PBS), and incubated with 100 μl/well of 5 μg/ml Fab or Q-bodies (in PBS), at 25°C for 1 h. The plate was washed thrice with PBST, and incubated with 100 μl/well of HRP-conjugated anti-His monoclonal antibody (Wako Pure Chemicals) diluted 1:5000 in PBS, at 25°C for 1 h. The plate was then washed thrice with PBST again and developed with 100 μl/well TMBZ solution [100 μg/ml 3,3′,5,5′-tetramethylbenzidine (Sigma) and 0.04 μl/ml H_2O_2, in 100 mM NaOAc; pH 6.0]. After incubating for 5–30 min, the reaction was terminated by adding 50 μl/well of 10% sulfuric acid, and the absorbance at 450 nm was read using a model 680 microplate reader (Bio-Rad, Tokyo, Japan). The absorbance at 655 nm was taken as the control.

Fluorescence measurement Purified Q-body (25 ng) in 250 μl of PBST containing 1% BSA was added into a 5 mm × 5 mm quartz cell (Starna Scientific,

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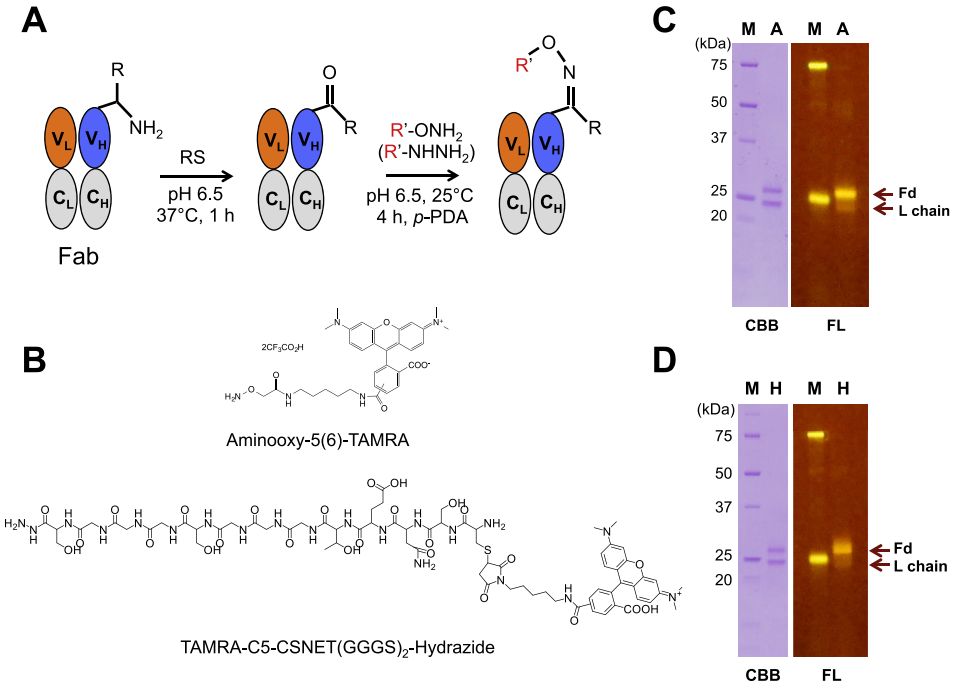

FIG. 2. Preparation of Q-bodies by transamination. (A) Scheme of the reactions. RS, Rapoport's salt (*N*-methylpyridinium-4-carboxaldehyde benzenesulfonate salt); *p*-PDA, *p*-phenylenediamine. (B) Structure of Aminooxy-5(6)-TAMRA and TAMRA-C5-peptide-hydrazide. (C and D) SDS-PAGE analysis of AoQ-body (C) and HzQ-body (D) by Coomassie Brilliant Blue (CBB) staining and observing fluorescence (FL). Lane M, Precision Plus Protein Dual Color Standards; lane A, AoQ-body; lane H, HzQ-body.

Hainault, UK), and 2.5 μl BGP-C7 peptide at varied concentrations were added by titration. After each addition, the solution was incubated at 25°C for 2 min prior to the spectral measurements. As a control, PBS instead of the peptide solution was added into quartz cells containing the same volume and amount of Q-body as in the experimental cells. The fluorescence emission spectra were obtained at 25°C, using a fluorescence spectrophotometer Model FP-8500 (JASCO, Tokyo, Japan), with excitation at 546 nm. The excitation and emission slit widths were set to 5.0 nm. Dose-response curves were fitted to a 4-parameter logistic equation at the maximum emission wavelength, using KaleidaGraph 4.1 (Synergy Software, Reading, PA, USA), and the EC_{50} value was calculated from the curve. The limit of detection (LOD) was obtained as the estimated antigen concentration that shows the mean blank value plus 3 SDs.

RESULTS

Expression and purification of KTM219 Fab Fab fragments were expressed in the oxidative cytoplasm of *E. coli* SHuffle T7 express lysY strain, and the correctly assembled protein with C-terminal His- and Flag-tags was purified using TALON resin and anti-FLAG M2 beads. As shown in Fig. 1C, two protein bands were clearly observed. Based on the MWs, the upper and lower bands were supposed to be Fd (V_H–C_H1; MW 26 kDa), and L chain (MW ~25 kDa), respectively. In this experiment, the protein sample for SDS-PAGE was not treated with dithiothreitol (DTT); thus, the positions of the bands may not reflect the actual protein sizes. We also analyzed the Fab fragments by SDS-PAGE after DTT treatment; in this case, two bands were observed at the same position (Fig. S3). These results indicated that the Fab fragments were well purified, and 128 μg protein was obtained from a 100-ml culture of *E. coli*. The N-terminal amino acids were found to be ATGQVKL in Fd, and SDIELTQ in the L chain, which is consistent with the amino acid sequence encoded by the plasmid (Fig. S1). Moreover, the removal of the amino-terminal methionine by methionine aminopeptidase, a reportedly routine process in prokaryotic translation, was also confirmed (18).

Labeling of Fab with fluorescent probes by transamination The Q-bodies prepared with Aminooxy-5(6)-TAMRA (AoQ-bodies) are shown in Fig. 2C. Aminooxy group can react with ketone group generated by the treatment with RS, in the presence of *p*-phenylenediamine that significantly improves the reaction rate (19). The Fd and the L chain of AoQ-bodies appeared as two clear bands upon CBB staining. During fluorescent imaging, the Fd band showed very strong fluorescence, whereas the L chain band showed very weak fluorescence. Using this method, 22.7 μg AoQ-bodies were prepared. The labeling efficiency in Fd, calculated by dividing the number of fluorescent dye molecules by that of Fab molecules, was determined to be 51.5%.

Another fluorescent probe, TAMRA-C5-peptide-Hydrazide (HzQ-body), was prepared using the scheme shown in Fig. S2, and

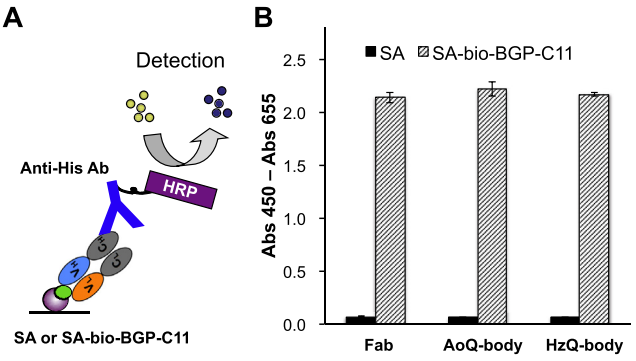

FIG. 3. Antigen-binding activity of KTM219 Fab, AoQ-body and HzQ-body. (A) Schematic of ELISA. (B) Antigen-binding activity. SA, streptavidin; bio-BGP-C11, biotinylated C-terminal peptide of BGP.

used to label Fab by the same procedure as that used to make the AoQ-bodies. Fig. 2D shows the SDS-PAGE analysis of the BGP HzQ-bodies. Two clear protein bands were identified upon CBB staining, which represent the Fd and L chains of the Fab. During fluorescent imaging, the Fd band showed much stronger fluorescence than did the L chain band. The labeling efficiency in Fd was determined to be 61%.

The effect of treatment with RS was also investigated. Figs. S3A and S3B show the SDS-PAGE analysis of the AoQ-body and the HzQ-body, respectively. In both the images, lane 1 represents the Fab before labeling, lane 2 represents the labeled Fab after RS treatment, and lane 3 shows the labeled Fab with fluorescent probes, without RS treatment. The fluorescence of Fab was clearly negligible without RS treatments, suggesting that RS treatment is essential for labeling.

Antigen-binding activity of Q-bodies Antigen-binding activities of BGP AoQ-body and HzQ-body were analyzed using ELISA. Fig. 3B shows the results of ELISA of the Fab fragment, AoQ-body, and HzQ-body. Although signals were detected when the antigen bio-BGP-C11 was immobilized through streptavidin, no signals were detected from samples with streptavidin only. These results suggest that the Q-bodies and Fab fragments were captured on the microplate by binding to immobilized bio-BGP-C11, but not by the streptavidin. The prepared Q-bodies retain the antigen-binding activity of the parental antibody Fab fragments at similar levels.

De-quenching of Q-bodies To evaluate the degree of initial quenching, Q-bodies prepared with Aminooxy-5(6)-TAMRA and TAMRA-C5-peptide-hydrazide were denatured under the denaturing conditions of 7 M guanidinium hydrochloride (GdnHCl) along with 100 mM DTT, and their fluorescence intensities were compared. As shown in Fig. 4, the fluorescence intensity of the BGP AoQ-body increased 1.95-fold, while that of the BGP HzQ-body increased 3.5-fold, when they were completely denatured.

Antigen response of Q-bodies Fluorescence spectra of Q-bodies upon addition of BGP-C7 were measured. Fig. 5A shows the spectra of BGP HzQ-body with different concentrations of BGP-C7; the fluorescence intensity increased with increasing concentration of BGP-C7. Based on the spectra, dose–response curve was prepared, as shown in Fig. 5B. The signal increased 2.75-fold and the LOD was calculated to be around 10 nM. Fig. S4A shows the spectra of BGP AoQ-body with increasing concentration of BGP-C7. Based on the spectra, a dose–response curve was plotted (Fig. S4B); the calculated LOD for the AoQ-body was similar to that for the HzQ-body; however, the maximum increase in the signal was 1.70-fold.

DISCUSSION

Chemical modification of proteins is important for a wide range of fields, including cell biology research (20–22), the construction of new biomaterials (23), and the development of novel therapeutics (24,25). In this study, we successfully prepared Q-bodies, a recently developed novel type of biosensor, by protein transamination.

Two fluorescent probes were used to make Q-bodies: aminooxy-5(6)-TAMRA, which is commercially available, and TAMRA-C5-peptide-hydrazide, which was prepared in-house. The two types of Q-bodies showed different behaviors. In guanidinium hydrochloride (GdnHCl) denaturation experiments, the fluorescence intensity of AoQ-bodies and HzQ-bodies increased 1.95-fold and 3.5-fold, respectively. Considering their similar Fd-/L-chain labeling ratios, this difference might be due to the distance between the dye and the labeled site. TAMRA-C5-peptide-hydrazide is much longer than Aminooxy-5(6)-TAMRA, owing to which the dye molecules in the former probe are better positioned for quenching than are those in the latter probe.

As a traditional immunoassay, ELISA is applied in many fields including clinical diagnostics and environmental monitoring. However since multiple reaction and washing steps are essential, it is still time-consuming and laborious. Compared with ELISA, Q-body assay is a homogeneous assay that only needs addition of analyte and fluorescence measurement after a short incubation period. In this study, we could detect BGP C-terminal peptide with a LOD of 10 nM with a Q-body prepared by protein transamination. The LOD is similar or superior to that of competitive ELISA using the same antibody fragment (20 nM) (16). This suggested the Q-body assay attained by the reported method could be an alternative of ELISA, with additional practical utility.

The N-terminal amino acid sequence is also an important factor in a transamination reaction. Some amino acid sequences such as EES, MEE, and AKT have been reported to be preferable for this reaction, while other sequences, such as AEE may not get efficiently modified to a ketone group (26). In this study, we expressed the Fab and analyzed the N-terminal amino sequence of Fd to be ATG and that of the L chain to be SDI. We found that ATG is a preferable

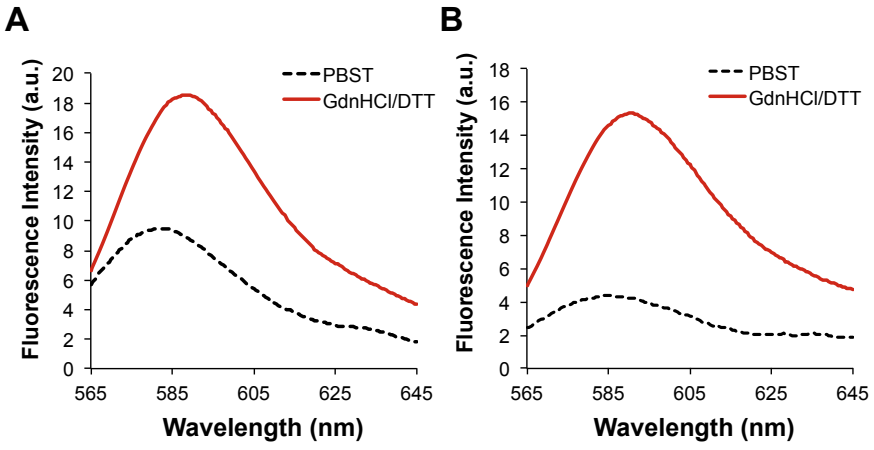

FIG. 4. Effect of denaturant on the fluorescence spectra of AoQ-body (A) and HzQ-body (B). PBST, PBS buffer containing 0.05% of Tween 20; GdnHCl/DTT, 7 M guanidinium hydrochloride (GdnHCl) with 100 mM dithiothreitol (DTT).

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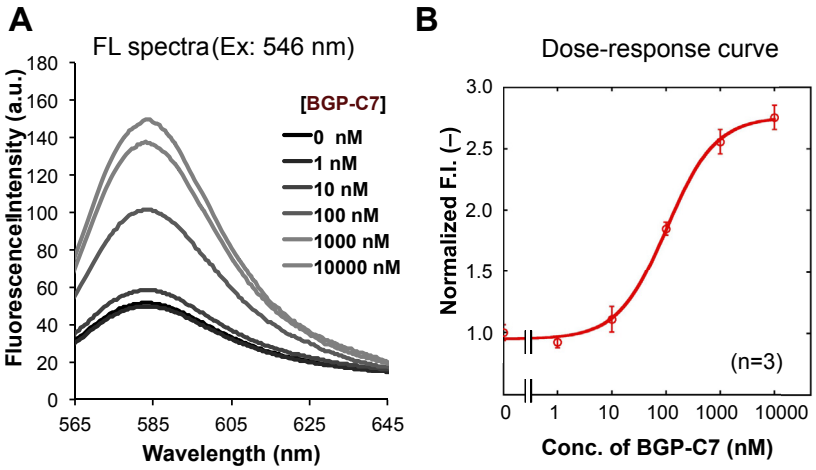

FIG. 5. Spectra of BGP HzQ-body with the addition of BGP-C7 at different concentrations (A) and dose–response curve at maximum emission wavelength (B).

sequence for transamination, whereas SDI is not. This finding may be useful for labeling not only antibodies but also other proteins. For preparation of Q-bodies, sometimes single-labeled Q-bodies perform better than double-labeled Q-bodies, especially when TAMRA or other dimer-prone rhodamine dyes are used (6). In such case, using a pair of ATG and SDI at the N-terminal sequences might be a fast and smart design. Recently, another labeling method of N-terminal Ser by mild oxidation to aldehyde is reported (27). Combination of these two methods will enable hetero-double labeling by two dyes, with enhanced quenching by fluorescence resonance energy transfer between them.

In this study, we have labeled Fab fragments without Cys-containing tag sequence; in theory this novel approach is applicable to natural antibodies. Direct labeling of full-length antibodies might make Q-body screening faster and will promote further research on Q-bodies.

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jbiosc.2015.12.010.

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References

1. **Ekins, R.:** More sensitive immunoassays, Nature, **284**, 14–15 (1980).
2. **Ueda, H., Tsumoto, K., Kubota, K., Suzuki, E., Nagamune, T., Nishimura, H., Schueler, P. A., Winter, G., Kumagai, I., and Mohoney, W. C.:** Open sandwich ELISA: a novel immunoassay based on the interchain interaction of antibody variable region, Nat. Biotechnol., **14**, 1714–1718 (1996).
3. **Dong, J., Ihara, M., and Ueda, H.:** Antibody Fab display system that can perform open-sandwich ELISA, Anal. Biochem., **386**, 36–44 (2009).
4. **Ueda, H., Kubota, K., Wang, Y., Tsumoto, K., Mahoney, W., Kumagai, I., and Nagamune, T.:** Homogeneous non-competitive immunoassay based on the energy transfer between fluorolabeled antibody variable domains (Open sandwich FIA), Biotechniques, **27**, 738–742 (1999).
5. **Sasajima, Y., Aburatani, T., Sakamoto, K., and Ueda, H.:** Detection of protein tyrosine phosphorylation by open sandwich fluoroimmunoassay, Biotechnol. Prog., **22**, 968–973 (2006).
6. **Abe, R., Ohashi, H., Iijima, I., Ihara, M., Takagi, H., Hohsaka, T., and Ueda, H.:** "Quenchbodies": quench-based antibody probes that show antigen-dependent fluorescence, J. Am. Chem. Soc., **133**, 17386–17394 (2011).
7. **Abe, R., Jeong, H. J., Arakawa, D., Dong, J., Ohashi, H., Kaigome, R., Saiki, F., Yamane, K., Takagi, H., and Ueda, H.:** Ultra Q-bodies: quench-based antibody probes that utilize dye-dye interactions with enhanced antigen-dependent fluorescence, Sci. Rep., **4**, 4640 (2014).
8. **Ueda, H. and Dong, J.:** From fluorescence polarization to quenchbody : recent progress in fluorescent reagentless biosensors based on antibody and other binding proteins, Biochim. Biophys. Acta – Proteins Proteomics, **1844**, 1951–1959 (2014).
9. **Hohsaka, T., Ashizuka, Y., and Sisido, M.:** Incorporation of two nonnatural amino acids into proteins through extension of the genetic code, Nucleic Acids Symp. Ser., **79–80** (1999).
10. **Gilmore, J. M., Scheck, R. A., Esser-Kahn, A. P., Joshi, N. S., and Francis, M. B.:** N-terminal protein modification through a biomimetic transamination reaction, Angew. Chem. Int. Ed. Engl., **45**, 5307–5311 (2006).
11. **Kalia, J. and Raines, R. T.:** Hydrolytic stability of hydrazones and oximes, Angew. Chem. Int. Ed. Engl., **47**, 7523–7526 (2008).
12. **Witus, L. S., Netirojjanakul, C., Palla, K. S., Muehl, E. M., Weng, C. H., Iavarone, A. T., and Francis, M. B.:** Site-specific protein transamination using *N*-methylpyridinium-4-carboxaldehyde, J. Am. Chem. Soc., **135**, 17223–17229 (2013).
13. **Lee, N. K., Sowa, H., Hinoi, E., Ferron, M., Ahn, J. D., Confavreux, C., Dacquin, R., Mee, P. J., McKee, M. D., Jung, D. Y., and other 5 authors:** Endocrine regulation of energy metabolism by the skeleton, Cell, **130**, 456–469 (2007).
14. **Oury, F., Sumara, G., Sumara, O., Ferron, M., Chang, H., Smith, C. E., Hermo, L., Suarez, S., Roth, B. L., Ducy, P., and Karsenty, G.:** Endocrine regulation of male fertility by the skeleton, Cell, **144**, 796–809 (2011).
15. **Komatsu, M., Dong, J., Ueda, H., and Arai, R.:** Crystal structure of Fab fragment of an anti-osteocalcin C-terminal peptide antibody KTM219, Phot. Fact. Act. Rep. 2014 Part B, 205, http://pfwww.kek.jp/acr/2014pdf/part_b/pf14b0205.pdf (2015).
16. **Lim, S. L., Ichinose, H., Shinoda, T., and Ueda, H.:** Noncompetitive detection of low molecular weight peptides by open sandwich immunoassay, Anal. Chem., **79**, 6193–6200 (2007).
17. **Iwai, H., Ozturk, B., Ihara, M., and Ueda, H.:** Antibody affinity maturation in vitro using unconjugated peptide antigen, Protein Eng. Des. Sel., **23**, 185–193 (2010).
18. **Ben-Bassat, A., Bauer, K., Chang, S. Y., Myambo, K., Boosman, A., and Chang, S.:** Processing of the initiation methionine from proteins: properties of the *Escherichia coli* methionine aminopeptidase and its gene structure, J. Bacteriol., **169**, 751–757 (1987).
19. **Wendeler, M., Grinberg, L., Wang, X., Dawson, P. E., and Baca, M.:** Enhanced catalysis of oxime-based bioconjugations by substituted anilines, Bioconjug. Chem., **25**, 93–101 (2014).
20. **O'Hare, H. M., Johnsson, K., and Gautier, A.:** Chemical probes shed light on protein function, Curr. Opin. Struct. Biol., **17**, 488–494 (2007).
21. **Griffin, B. A., Adams, S. R., and Tsien, R. Y.:** Specific covalent labeling of recombinant protein molecules inside live cells, Science, **281**, 269–272 (1998).
22. **Cravatt, B. F., Wright, A. T., and Kozarich, J. W.:** Activity-based protein profiling: from enzyme chemistry to proteomic chemistry, Annu. Rev. Biochem., **77**, 383–414 (2008).
23. **Witus, L. S. and Francis, M. B.:** Using synthetically modified proteins to make new materials, Acc. Chem. Res., **44**, 774–783 (2011).
24. **Zalipsky, S.:** Functionalized poly(ethylene glycol) for preparation of biologically relevant conjugates, Bioconjug Chem., **6**, 150–165 (1995).

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25. **Zalipsky, S., Puntambekar, B., Boulikas, P., Engbers, C. M., and Woodle, M. C.:** Peptide attachment to extremities of liposomal surface grafted PEG chains: preparation of the long-circulating form of laminin pentapeptide, YIGSR, Bioconjug Chem., **6**, 705–708 (1995).
26. **Palla, K. S., Witus, L. S., Mackenzie, K. J., Netirojjanakul, C., and Francis, M. B.:** Optimization and expansion of a site-selective N-methylpyridinium-4-carboxaldehyde-mediated transamination for bacterially expressed proteins, J. Am. Chem. Soc., **137**, 1123–1129 (2015).
27. **Thompson, P., Bezabeh, B., Fleming, R., Pruitt, M., Mao, S., Strout, P., Chen, C., Cho, S., Zhong, H., Wu, H., Gao, C., and Dimasi, N.:** Hydrolytically stable site-specific conjugation at the N-terminus of an engineered antibody, Bioconjug Chem., **26**, 2085–2096 (2015).

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