Electronic Supplementary Information

Fluorescent substrates for covalent protein labeling catalyzed by microbial transglutaminase

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1. Effect of fluorolabeling of NK6-AP on the enzymatic function

Effect of MTG-mediated labeling of NK6-AP on the catalytic activity was investigated. After the incubation of NK6-AP with FITC-β-Ala-QG in the presence or absence of MTG, the catalytic activity of AP was measured with *p*-nitrophenylphosphate (p-NPP) as a substrate. To 1 mL of 1 M Tris-HCl buffer (pH 8.0), the hydrolysis of p-NPP (1 mM) was initiated by the addition of the same amount of each reaction mixture at 25 °C. Initial activity was determined by detecting the increase in the absorbance at 410 nm (derived from *p*-nitrophenol) by a UV-vis spectrophotometer (Jasco V-570, Tokyo). The same protocol was applied to wild-type AP.

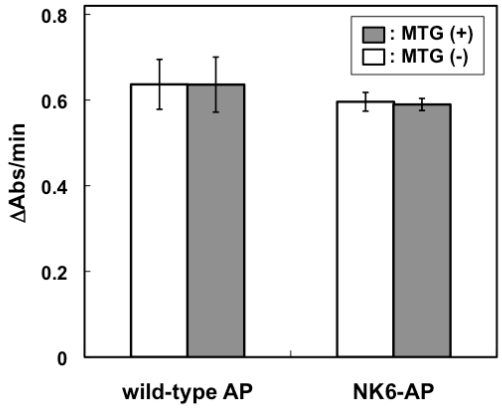

Fig. S1. Effect of MTG treatment on the catalytic activity of wild-type AP and NK6-AP with FITC-β-Ala-QG.

2. Time course of MTG-mediated labeling of NK6-AP with fluorescent substrates

The reaction mixture comprised NK6-AP (0.5 mg/mL) and each substrate (0.1 mM) in 100 mM Tris-HCl buffer (pH 8.0) containing 5 vol% of DMSO. The protein labeling reaction was initiated by

the addition of MTG (1 U/mL) at 4˚C. A small aliquot of the reaction mixture was periodically removed and mixed with the standard sample buffer for SDS-PAGE analysis (12 vol% 2-mercaptoethanol, 4 wt% SDS, 20 vol% glycerol in 0.1 M Tris-HCl, pH 6.8) to terminate the MTG reaction. The reaction was followed by the increase in the fluorescence of protein bands in the fluorescent image of the SDS-PAGE gel as described in the experimental section of the manuscript. For Dns-ε-Acp-QG, the gel image obtained was analyzed by a CS Analyzer (ATTO Co., Japan).

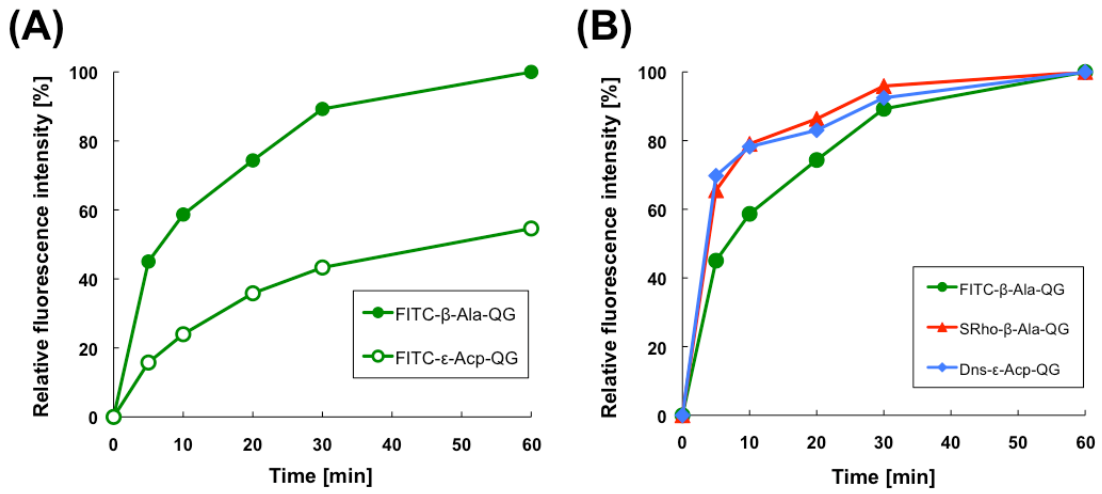

Fig. S2. Time courses of MTG-mediated labeling of NK6-AP with new fluorescent substrates. (A) Comparison of FITC-derivatized substrates. Fluorescent intensity derived from NK6-AP labeled with FITC-β-Ala-QG at 60 min was defined as a unit. (B) Fluorescent intensity derived from a labeled NK6-AP at 60 min was defined as a unit for each substrate.

3. Reactivity of a new fluorescent substrate with a transglutaminase of different origin

The reactivity of FITC-β-Ala-QG as a substrate for guinea pig liver transglutaminase (GTG) was examined using β-casein as an acyl-acceptor proteinaceous substrate. The reaction mixture comprised β-casein (0.5 mg/mL) and FITC-β-Ala-QG (1 mM) in Tris buffered saline (pH 7.4) containing 5 vol% of DMSO. The protein labeling reaction was initiated by the addition of MTG or GTG (1 U/mL) at 4 or 27 ˚C. In the case of GTG, 5 mM $CaCl_2$ was added to the reaction mixture. After incubation for 16 h, the reaction products were analyzed by SDS-PAGE. The fluorescent image of the gels was obtained as described in the experimental section of the manuscript. GTG was purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan).

As shown in the top column, β-casein was readily cross-linked by GTG and MTG, and polymeric products (indicated as polymerized β-casein) was obtained (lanes 3, 4, 6 and 7 in Fig. S3A). When the reaction was conducted with FITC-β-Ala-QG, the fluorescent substrate could be randomly involved in the cross-linking process. This should result in the formation of fluorescent products

with a wide range of molecular weight. As shown in lane 7 of the fluorescent images (Figs. S3A and B), smear protein bands were found at both temperatures when cross-linking reaction was conducted with MTG. By contrast, only at 27 ˚C, fluorescence was observed from GTG-treated products (lane 4 in the fluorescent image of Fig. S3B), although the fluorescent intensity was much weaker than that of MTG-treated products.

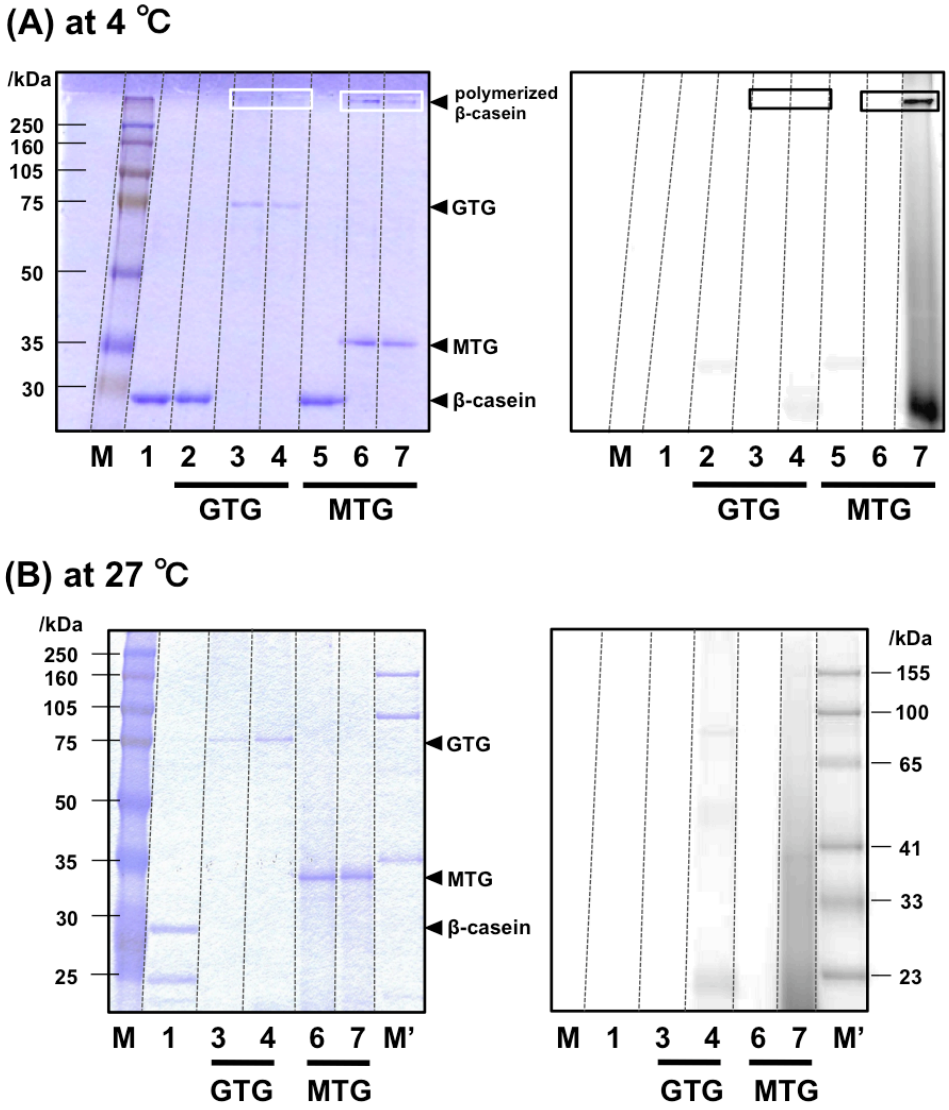

Fig. S3. MTG- or GTG-mediated labeling of β-casein with FITC-β-Ala-QG at different temperature. (Left) an example of SDS-PAGE gel stained with CBB. (Right) a fluorescent image of the corresponding gel before CBB staining. M: molecular weight marker; M': fluorescent molecular weight marker; Lane 1: β-casein; Lane 2: β-casein + FITC-β-Ala-QG; Lane 3: β-casein + GTG; Lane 4: β-casein + FITC-β-Ala-QG + GTG; Lane 5: β-casein + FITC-β-Ala-QG; Lane 6: β-casein + MTG; Lane 7: β-casein + FITC-β-Ala-QG + MTG.