Position-Specific Incorporation of Highly Durable and Blue-Laser Excitable Fluorescent Amino Acid into Proteins for Fluorescence Sensing

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Abstract

A new fluorescent amino acid, L-2-acridonylalanine (acdAla), was incorporated into proteins at their specific positions using 4-base codon/anticodon strategy. AcdAla was highly fluorescent when it was excited at the wavelengths of blue lasers (415 nm) and highly durable compared with conventional fluorophores often used for biological analyses. The amino acid was incorporated into various positions of streptavidin (SA) or camel single-chain antibody (cAb). The efficiency of the incorporation of acdAla was high enough to obtain a large quantitiy of the protein (Maximun incorporation efficiency was about 70 %). The mutant proteins were found to work as fluorescence probes for biotin and antigenic lysozyme binding.

Experimental

Protein biosynthesis was carried out as follows. The pdCpA-acdAla was chemically synthesized then coupled with the $tRNA_{CGGG}$(-CA) with T4 RNA ligase. The $tRNA_{CGGG}$ carring the acdAla was mixed with the in vitro biosynthesizing system of E. coli S30 lysate together with the mRNA of the mutant protein. The synthesis of the full-length mutant protein was confirmed by Western blot analysis using anti-T7 tag antibody and alkaline phosphatase-labeled anti-mouse IgG. Biotin binding activity was tested by a dot blot method using alkaline phosphatase-labeled biotin. The antigen binding activity was also confirmed by the change of fluorescence polarization of FITC-labeled antigen with the addition of the mutant cAb.

Results and Discussion

As shown in **Fig. 1**, full-length proteins were synthesized only in the presence of the $tRNA_{CGGG}$-acdAla. The expression of full-length protein indicates that the nonnatural amino acid was successfully incorporated into the specified position by using four-base codon/anticodon pair. As shown in **Fig. 2** and **Fig. 3**, the fluorescence polarization of FITC-antigen gradually increased by the addition of each mutant antibody and fluorescence intensities were varied according to the positions of the nonnatural amino acid by the addition of the antigen.

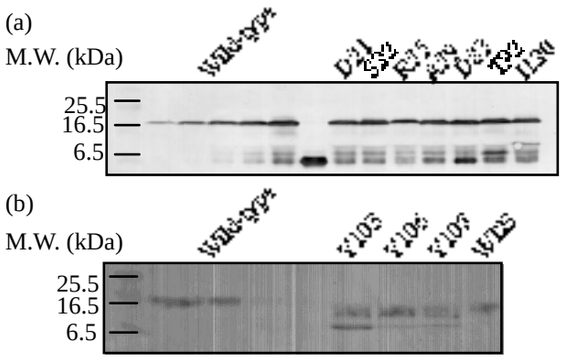

Fig. 1 Western blot analysis of the translation products of various mRNAs. (a) : mutated SA, (b) : mutated cAb.

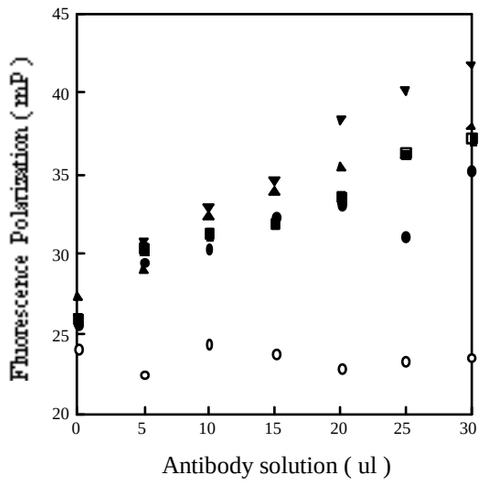

Fig. 2 Fluorescence polarization of FITC-antigen in the presence of various amounts of proteins. [FITC-antigen] = 2 nM, λ ex = 490 nm in 20 mM Tris-HCl buffer, 100 mM NaCl, pH = 7.0 at 25 ℃. (▲ : Y103-acdAla, ● : Y106-acdAla , ■ : Y107-acdAla, ▼ : W123-acdAla, ○ : control)

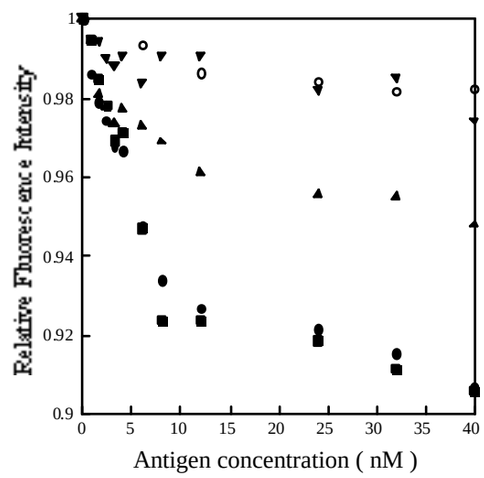

Fig. 3 Fluorescence measurement of acdAla single mutant cAb in the presence of various amounts of antigen. λ ex = 385 nm in 20 mM Tris-HCl buffer, 100 mM NaCl, pH = 7.0 at 25 ℃.