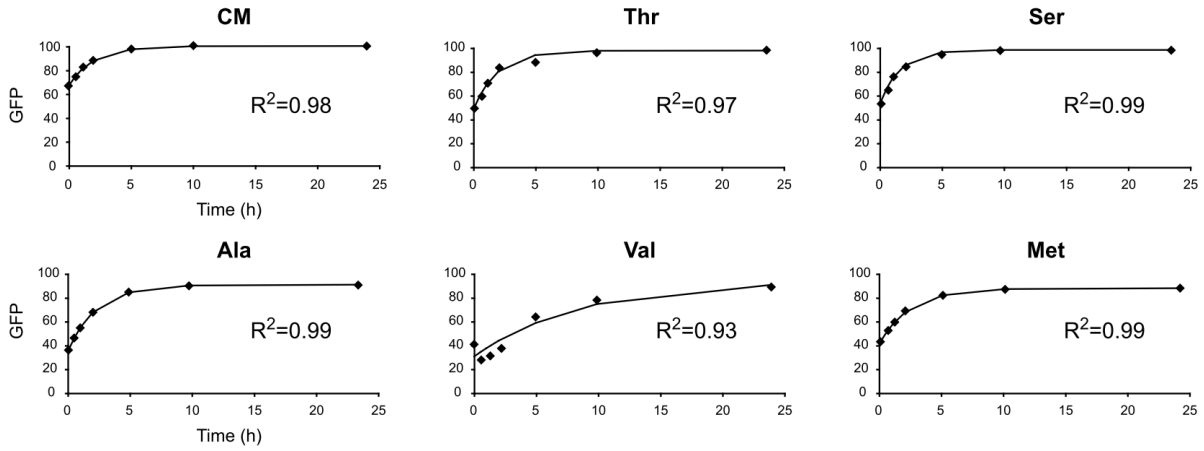

FIG. 1. Plot of data and model to derive rate constants in Figure 2C (cleavage mutant CM has Cys). Points are experimental measurements, whereas lines indicate the model used to generate the rate constants.

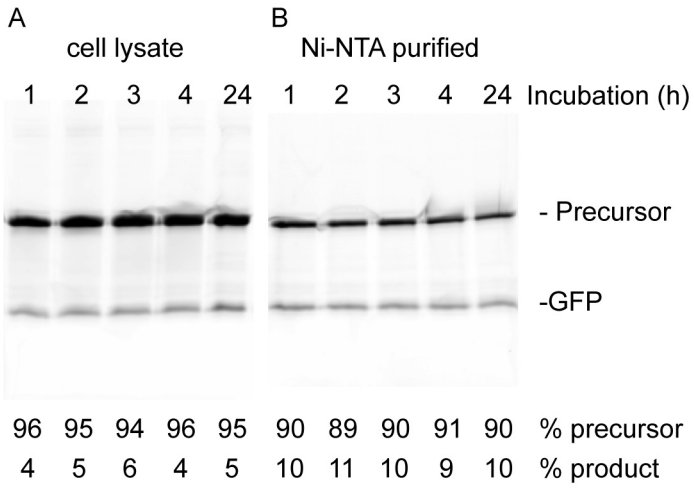

FIG. 2. Stability of the Cys+1/Val precursor *in vitro*. Gel analysis of incubation of the Cys+1/Val precursor (A) in cell lysate and (B) as a Ni-NTA-purified protein at 4° C is shown. The precursor and cleavage product were visualized by GFP fluorescence.

To demonstrate utilization of the Cys+1/Val mutant for protein purification, an intein-containing tripartite fusion was modified. The pET/CΔIaFGF-CM(C+1V) plasmid was constructed by mutating the Cys+1 to Val in the pET/CΔIaFGF-CM plasmid (generous gift of David Wood). The pET/CΔIaFGF-CM plasmid is a pET vector with self-cleaving chitin-binding domain (CBD) tag, separated by the (*Mtu*) RecA intein from human acidic fibroblast growth factor (aFGF) [1]. Precursor expression and the target protein purification were performed as described in [1]. The only modification was the composition of the elution buffer (0.5 mM NaCl, 1 mMEDTA and 50 mM sodium phosphate, pH 6.0). Homogeneous aFGF isolation validates the use of the Cys+1/Val mutant, which builds up precursor in vivo, for protein purification.

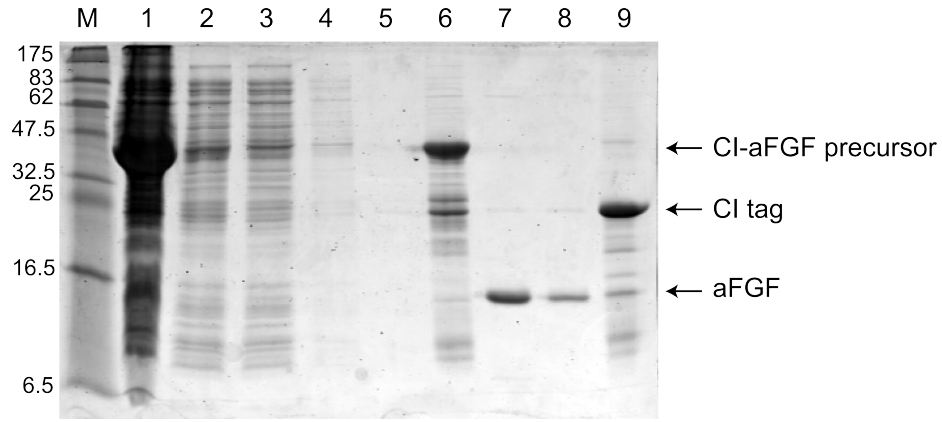

FIG. 3. On-column purification using the Cys+1/Val mutant. On-column purification of aFGF was performed using CBD as the affinity tag and the Cys+1/Val mutant. Lanes: M, molecular weight marker (kDa); 1, cell lysate, 2, diluted, clarified cell lysate; 3, column flow-through; 4-5 column washes; 6, resin sample before intein cleavage, containing precursor bound to the chitin resin and some prematurely cleaved CBD-I tag; 7-8, purified protein fractions; and 9, resin sample after product elution, containing cleaved CBD-I tag.

[1] W. Y. Wu, A. R. Gillies, J. F. Hsii, L. Contreras, S. Oak, M. B. Perl, and D. W. Wood, Biotechnology Progress **26**, 1205 (2010).