His-tagged tobacco etch virus (TEV) protease purification protocol

The TEV protease gene was cloned into the pET-24d(+) vector (a gift from EMBL, Hamburg). BL21 (DE3) *Escherichia coli* cells were transformed with the recombined plasmid and plated on a Luria broth (LB) agar plate containing 50 μg ml^{-1} kanamycin. Colonies from this plate were used to start a 200 ml LB overnight pre-culture supplemented with 50 μg ml^{-1} kanamycin. 25 ml of this overnight pre-culture was transferred into a 2 l LB culture flask supplemented with 50 μg ml^{-1} kanamycin. A total of six flasks (12 l) were prepared; the culture was grown to an optical density of 0.8 at 600 nm at 310 K and expression was induced by the addition of 1 m*M* isopropyl β-D-1-thiogalactopyranoside. After induction the cells were grown overnight at 293 K. The following day the cells were centrifuged at 12 000*g* for 15 min and resuspended in 100 ml buffer *A* (50 m*M* Tris–HCl pH 8, 400 m*M* NaCl, 20 m*M* imidazole, 5 m*M* β-mercaptoethanol and 1 m*M* benzamidine). The cells were disrupted by sonication and centrifuged at 30 000*g* for 45 min. The crude extract was incubated with 20 ml NTA agarose beads (Qiagen; pre-equilibrated with buffer *A*) for 1 h. The beads were washed with buffer *B* (50 m*M* Tris–HCl pH 8, 1 *M* NaCl, 50 m*M* imidazole, 5 m*M* β-mercaptoethanol and 1 m*M* benzamidine) and the protein was eluted with buffer *E* (50 m*M* Tris–HCl pH 8, 400 m*M* NaCl, 500 m*M* imidazole and 5 m*M* β-mercaptoethanol). The eluted protein was dialyzed in dialysis bags (cutoff 12-15 kDa) overnight at 277 K against 2 l anion-exchange buffer (50 m*M* Tris-HCl pH 8 and 5 m*M* β-mercaptoethanol). The day after, the proteins were spun down at 30 000*g* for 10 min to remove protein aggregates. The supernatant was loaded onto a 2 × 5 ml Hi-Trap Q-FF anion-exchange column (GE Healthcare Life Sciences) equilibrated with anion-exchange buffer. The TEV protease was collected in the flowthrough (while the rest of the contaminants bound to the column). The TEV protease was then concentrated to 1 mg ml^{-1} and dialyzed against storage buffer (50 m*M* Tris–HCl pH 7.6, 5 m*M* β-mercaptoethanol and 50% glycerol). The protein was finally stored at 193 K. Following this protocol, 200 mg of pure TEV protease was obtained.