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Purification of untagged ubiquitin from bacterial cells

Wildtype and mutant ubiquitins were expressed in Rosetta(DE3) pLysS cells and purified to apparent homogeneity by a variation of the procedure of Baboshina and Haas (J. Biol. Chem. 271: 2823-2831, 1996). The bacterial cell pellet was collected by centrifugation (RC5C Sorvall centrifuge; 5,000 rpm in SLA3000 rotor), resuspended in an equal volume of "no-salt" Buffer A (25mM ammonium acetate, 10mM 2-mercaptoethanol, 10% glycerol, and proteases inhibitor) at pH 7.0, and lysed by sonication. Cell debris was then removed by centrifugation (RC5C Sorvall centrifuge; 12,000 rpm in SLA600 rotor) and the supernatant adjusted to pH 4.5-5.0 with concentrated acetic acid. Acid-precipitable proteins were removed by centrifugation (RC5C Sorvall centrifuge; 12,000 rpm in SLA600 rotor), and the supernatant was adjusted to pH 5.1 with 6N NaOH. This supernatant, containing the ubiquitin monomers, was passed through a 0.45 mm PES filter (Nalgene catalog # 194-2545) and loaded onto a 4 ml SP Sepharose (Amersham Biosciences) column equilibrated with "low-salt" (25mM NaCl) Buffer A at pH 5.1. The column was then washed with five column volumes of the same buffer, and the ubiquitin monomers were eluted with "high-salt" (100 mM NaCl) Buffer A at pH 5.1. After pooling the peak protein fractions, the eluted ubiquitin monomers were desalted and neutralized on a PD10 column (Amersham Biosciences) equilibrated with Ni Wash Buffer (25mM Tris-HCl pH 7.9, 50mM NaCl, 10mM 2-mercaptoethanol, 10% glycerol, protease inhibitors), and stored in aliquots at –80°C. Ubiquitin preparations were analyzed by SDS-PAGE, and the yield of each was determined by comparing coomassie staining with that of known quantities of BSA and ubiquitin (Affinity Research).