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Trypsin purification using magnetic azocasein composite

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This contribution presents an inexpensive, simple and fast procedure to purify trypsin from fish viscera based on affinity binding onto magnetic particles of azocasein composite (MAzo). This casein derivative, nonspecific protease substrate, was magnetized by co-precipitation of Fe+2 and Fe+3 ions. Intestines of fish Nile tilapia (Oreochromis niloticus) were homogenized in 0.01 M Tris-HCl buffer, pH 8.0 (one gram of tissue/ml). Afterwards, the homogenate was partially purified with ammonium sulfate (20-40% of saturation), dialyzed and then incubated with MAzo. The adsorbed proteins were firstly extensively washed with 0.01 M Tris-HCl buffer, pH 8.0, and subsequently with 3 M NaCl by collecting the magnetic azocasein composite under a magnetic field. The fractions collected by these washings were used for protein and enzyme activity determinations as well as electrophoresis. The specific activity of the protein collected with 3 M NaCl (41.82 unit/mg) showed to be 220 times higher than that found for the crude extract (0.19 unit/mg). The SDS-PAGE showed that the size of the purified protein was approximately 24 kDa, agreeing with the previously reported for the Nile tilapia trypsin molecular weight. This procedure presents the advantages of magnetic azocasein compostite reuse and can be applied for trypsin purification from other sources.