



Fig. 2. SDS-PAGE (a) and Western blot (b) of proteins from *L. vannamei* midgut gland. MWM, molecular weight marker; I, Coomassie stained; II, TAP antibody labeled; III, IP antibody labeled. Arrows indicate the different bands.

Granules, containing aggregated molecules (Fig. 5), were darker than the cytoplasm background. Similar cells in different sections of the midgut gland were labeled with both treatments.

DISCUSSION

Results showed that the *L. vannamei* midgut gland synthesizes and stores trypsinogen to keep trypsin inactive while stored and ready to be secreted and activated. Evidence of trypsinogen storage in the penaeid midgut gland is provided by Western blot analysis and immunohistology. As shown in Fig. 1, anti-TAP antibodies specifically detected trypsinogen only, but anti-IP detected both trypsin and trypsinogen. The criterion to identify a true trypsinogen molecule was that antibodies had no cross-reaction, and that the antibody against the trypsinogen activation peptide detected the same molecule as the internal peptide antibody elsewhere in the Western blot (molecular weight) or the immunohistology (cells stained). Antibodies generated by the two peptides were

specific and were suitable for distinguishing two different sequences in the trypsin molecule, one of them specific to the inactive form of trypsin. Trypsinogen purification was not possible (Klein *et al.*, 1996), but eliciting antibodies from synthetic peptide was an option.

Detection of *L. vannamei* trypsinogen is difficult because it is activated quickly, possibly by itself (Van Wormhoudt *et al.*, 1995). To prevent possible activation during homogenization, protein from the midgut gland was precipitated with acetone, and EDTA, reducing agents, and heat were used. These treatments eliminated enzyme activity by chelating calcium ions needed for trypsin stabilization (Rypniewski *et al.*, 1994), breaking disulfide bonds, and denaturing protein.

The trypsin activation peptide sequence is different among species. In vertebrates, the peptide is small, around six amino acid residues long, and highly acidic because of the Asp content (Light and Janska, 1989). The enzyme enteropeptidase is responsible for cleavage and activation of trypsinogen because of its high and precise affinity for the peptide bond linking the trypsinogen activation peptide and the enzyme forming the trypsinogen (Light and Janska, 1989). In *L. vannamei*, the activation peptide is larger, with 14 amino acid residues, and the composition suggests that the activation enzyme is trypsin itself, in contrast to vertebrate trypsinogen that protects itself from activation (Light and Janska, 1989; Van Wormhoudt *et al.*, 1995; Klein *et al.*, 1996).

By comparing the pattern of the total protein stained with Coomassie with that of proteins labeled with antibodies by Western blot, it was observed that trypsin sequences obtained by Klein *et al.* (1996) were transcribed in the midgut gland of *L. vannamei*. Substrate gel electrophoresis analysis and inhibition (Lemos *et al.*, 2000) showed that at least three different trypsins were present, as demonstrated by Le Moullac *et al.* (1996).

The mass of denatured trypsins detected by Western blot analysis was similar to that estimated for the trypsin pool (30 kDa) by Klein *et al.* (1996). Three protein bands were detected with anti-TAP, and two protein bands of sizes close to those of the two heavier proteins detected with anti-TAP were detected with anti-IP antibodies. Differences in band number could be related to differences in trypsin isoforms among IP sequences (Klein *et al.*, 1996). The TAP sequence was conserved in the