

Identification and purification of actin in the subpellicular network of *Toxoplasma gondii* tachyzoites

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Abstract

Toxoplasma gondii infects cells through actin-dependent dynamic events. Although presence of cortical actin has been widely suggested, visualization of actin filaments and their location have not been possible yet. The subpellicular cytoskeleton network is a recently described structure in where dynamic events would take place. By non ionic detergent extractions, the cortical cytoskeleton network was enriched and used for the isolation and identification of actin. Actin was detected by Western blot in extracts of cytoskeleton networks, and it was localized by immuno-gold staining at the network, the apical end and the posterior polar ring. Actin was isolated from subpellicular cytoskeleton extracts by binding to DNase I, and it polymerized *in vitro* as filaments that were gold-decorated by a monoclonal anti-actin antibody. Filaments bound the sub-fragment 1 of heavy meromyosin, although with atypical arrangements in comparison with the arrowheads observed in muscle actin filaments. Treatment with cytochalasine D and colchicine altered the structural organization of the subpellicular network indicating the participation of actin filaments and microtubules in the maintaining of this structure. Actin filaments and microtubules, in the subpellicular network, participate reciprocally in the maintaining of the parasite's shape and the gliding motility.