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Interaction between Chondroitin-6-Sulfate and *Entamoeba histolytica* as Revealed by Force Spectroscopy

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Entamoeba histolytica is a protozoan parasite that causes amebic dysentery in humans. Infection with this parasite may result in invasion of intestine. During this process, *Entamoeba* is able to kill and ingest epithelial cells, cells from the immune system and red blood cells. The cytoadhesion process exerted by the pathogen is regulated by chemical surface molecules. Essential components for the successful ingestion of cells are surface receptors of *Entamoeba* that mediate rapid recognition and uptake. *Entamoeba histolytica* has a 10 nm thick plasma membrane, with an external and continuous coat mainly composed by carbohydrate residues of glycoprotein. Only few surface molecules of *Entamoeba* have been described, as the 270 kDa galactose/*N*-acetyl galactosamine (Gal/GalNAc) and 220 kDa *N*-acetyl glucosamine-binding lectins, and the 112 kDa surface adhesin[1]. The Gal/GalNAc lectin has been shown to mediate adherence of the parasite to exposed terminal galactose/N-acetyl-galactosamine residues of target cell glycoproteins or colonic mucins [2]. Trophozoites poorly adhere to target cells defective in production of N- and O-linked Gal-terminal oligosaccharides, thus confirming this carbohydrate specificity [3]. The Gal/GalNAc lectin is a heterodimer molecule composed of a heavy (170 kDa) transmembrane subunit and a light (31/35 kDa) glycosylphosphatidylinositol-anchored subunit linked by disulfide bonds [4].
Some interaction properties of Gal/GalNAc lectin and its ligands have been characterized using isolated *E. histolytica* membranes. For instance, Adler *et al.*, (1995) showed that the interaction of GalNAc with membrane fractions is stronger when carried out in solutions with high ionic concentrations (above 50 mM NaCl). It was also observed that complete inhibition of *E. histolytica* adherence to target cells or colonic mucins is not observed, even when the lectin is blocked with high concentrations of galactose (100 to 500 mM) or GalNAc monomers [5], suggesting that other molecules than lectin could participate in the adhesion event. We have observed by Atomic Force Microscopy (AFM) the ameba surface and probed the interaction force between *E. histolytica* and chondroitin-6-sulphate (C6S). We have used several substrates to adhere trophozoites. The best reproducibility in sample preparation was obtained with fibronectin-coated coverslips and when the cells were fixed with paraformaldehyde. The images obtained with the AFM showed that the trophozoite exhibits an irregular surface. Pseudopods and waving adhesion plaques could be observed (fig.1). Force spectroscopy analysis showed that the trophozoite surface strongly interacts with C6S-functionalized tips (fig.2). During cantilever retraction, attractive force peaks were observed at distances up to 1.3 μm above the trophozoite surface. Statistical analysis of the force distributions collected for five samples showed a reproducible 2.2 nN mean adhesion force. We observed a reduction of the adhesion force and of the interaction distance after addition of galactose to the buffer solution suggesting that the observed interaction is also Gal/GalNAc-lectin-mediated (fig.3).
Supports: CNPq-PRONEX, Faperj, CAPES

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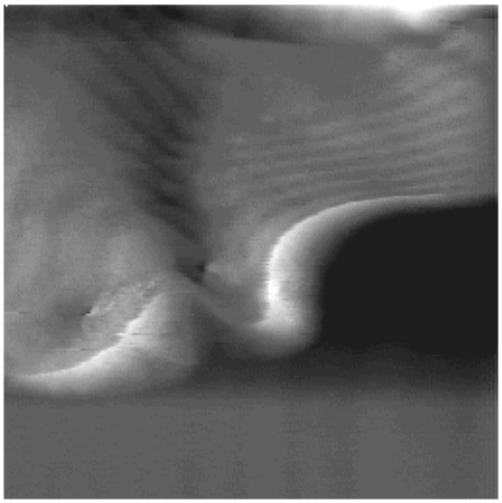

FIG 1

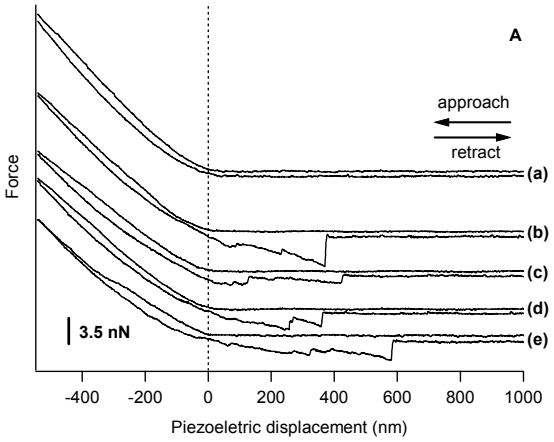

FIG 2

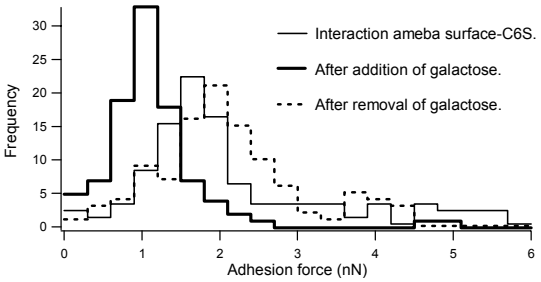

FIG 3

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