

Involvement of cysteine proteases in the excystation and metacystic development of *Entamoeba invadens*

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SUMMARY

We examined the effects of six cysteine protease inhibitors (Z-Phe-Ala-DMK, E-64d, Z-Phe-Phe-DMK, E-64, ALLM and cathepsin inhibitor III) on the excystation and metacystic development of *Entamoeba invadens*. Excystation was assessed by counting the number of metacystic amoebae after the induction of excystation. We found that excystation was inhibited by the cysteine protease inhibitors Z-Phe-Ala-DMK and E-64d, and that inhibition was concentration-dependent during incubation. Metacystic development was assessed by counting the number of nuclei in amoeba, and was also found to be inhibited by the same protease inhibitors. The other four cysteine protease inhibitors (Z-Phe-Phe-DMK, E-64, ALLM and cathepsin inhibitor III) had a weak or little effect on the excystation, but they inhibited cysteine protease activity in the lysates of *E. invadens* cysts. During gelatin substrate gel electrophoresis, we detected broad bands with gelatinase activity of metacystic amoebae, as well as cysts and trophozoites that were inhibited by Z-Phe-Ala-DMK. There was a difference in the protease composition of cysts and trophozoites, and the protease composition of metacystic amoebae changed from cyst-type to trophozoite-type during development. These results strongly suggest that cysteine proteases contribute to the excystation and metacystic development of *Entamoeba invadens*, and assist the successful infection.