

Serine proteases are involved in the excystation and metacystic development of *Entamoeba invadens*

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SUMMARY

The functions of cysteine proteases involved in the pathogenicity and differentiation of *Entamoeba histolytica* have been demonstrated, but little is understood about the functions of serine proteases (SP). This study examined the involvement of SP in amoebic excystation and metacystic development, using *E. invadens* as a model of *E. histolytica*. Four SP inhibitors (PMSF, AEBSF, TPCK, and DCI), given at different concentrations, decreased the number of metacystic amoebae in a dose-dependent manner, but did not affect the survival of cysts. PMSF inhibited not only the increase, but also the development, of metacystic amoebae. PMSF was effective in inhibiting SP activity in cystic lysates. The protease band on gelatin SDS-PAGE was weaker than controls when treated with PMSF. These data demonstrate involvement of SP in amoebic excystation and development. Searches of the genome databases of *E. histolytica* and *E. invadens* found that *E. invadens* has three types of enzymes in SP family S28, two types of S9 and one type of S24. Real-time RT-PCR revealed the expression levels of these SP mRNAs five hours after induction of excystation were higher than those observed prior to induction; an increase in expression of one type of S9 enzyme was most significant. Also, this S9 enzyme mRNA level was higher in trophozoites than in cysts. These results indicate that SP mediates the excystation and metacystic development of *Entamoeba*, and that SP mRNA levels in amoeba cysts increase after induction of excystation, especially for one type of S9.