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Research Note

Localization of Toxin-Like Activity in Lysosome-Rich Fractions of *Entamoeba histolytica*

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Since Lushbaugh *et al.* (1979) reported the presence of a toxin-like activity in cell-free extracts of trophozoites of *Entamoeba histolytica,* which caused detachment and rounding of tissue-cultured cells, its properties have been investigated in several laboratories. Mattern *et al.* (1980) attributed this toxin-like activity to a lectin-like substance, because the toxic activity was reversed by fetuin, N-acetylgalactosamine or serum. Koblier and Mirelman (1980), however, reported that the toxin-like activity was present only in the supernatant fluid of the extract of amoebae isolated by centrifugation at 100,000 g for 60 min, although most of the lectin-like activity was found in the pellet.

While investigating cytopathogenecity of *E. histolytica,* we found that membrane fractions isolated from homogenate of amoebae caused detachment and rounding of NIH:3T3 cells. In the present communication, we report that the toxin-like activity of *E. histolytica* is at least partially associated with its lysosome-rich fractions.

Trophozoites of *E. histolytica* (strain HM-1:IMSS) were axenically grown in BI-S-33 medium (Diamond *et al.*, 1978) as described previously (Takeuchi *et al.*, 1977). Amoebae were finally suspended in 50 mM Tris-HCl buffer, pH 7.4 containing 0.25 M sucrose so that the protein concentration was 20–25 mg/ml.

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Immediately after harvesting, amoebae were disrupted, and subcellular fractions were isolated by differential centrifugation as described previously (Takeuchi *et al.*, 1981). The 15,000 g×20 min pellet isolated by the differential centrifugation was further fractionated on a discontinuous sucrose density gradient centrifugation as described (Takeuchi *et al.*, 1981).

To measure the toxin-like activity, the isolated pellets were washed twice in Hanks balanced salt solution (BSS) containing 5 μM paramethyl sulfonyl fluoride and 5 mM 2-mercaptoethanol, and finally suspended in the BSS to make 0.2–0.6 mg protein/ml. NIH:3T3 cells were cultivated using a plastic dish of 3.5 cm diameter in Eagle's MEM supplemented with 10% new-born calf serum (Flow Laboratory, Rockville, Maryland) at 37 C. Confluent NIH:3T3 cells were rinsed and overlaid with 0.5 ml of the BSS. Approximately one half milliliter of these fractions was added to the plastic dish, and the dish was incubated for 60 min at 37 C. When 110,000 g ×120 min supernatant fluid was examined, 5 μM paramethyl sulfonyl fluoride and 5 mM 2-mercaptoethanol were supplemented before the fraction was added. Detachment and rounding of NIH:3T3 cells were observed with a light microscope.

Detachment and rounding of NIH:3T3 cells were readily observed when either 15,000 g pellet or 110,000 g supernatant fluid was added. Detached cells were found to be agglutinated. The toxin-like activities of 700 g×5 min and 110,000 g×120 min pellets were much lower than those of these two fractions.

The sucrose density gradient centrifugation of 15,000 g pellet resulted in isolation of four bands (Takeuchi *et al.*, 1981), which were designated B-1 to B-4 from the top of the gradient. Among these four bands, the toxin-like activity was found only in B-2 and B-3 (Figs. 1, 2), whereas B-1 and B-4 had little toxic activity (Figs. 3, 4). It was also demonstrated that detached NIH:3T3 cells agglutinated (Figs. 1, 2).

We have previously demonstrated that marker enzymes for lysosome of amoeba such as acid phosphatase were particularly concentrated in B-2 and B-3 (Takeuchi *et al.*, 1981). These data, therefore, led us to envision that the toxin-like activity was associated with lysosome of amoeba at least in part. Because marker enzymes for plasma membrane of amoeba were also detected in B-2 and B-3 (Takeuchi *et al.*, 1981), it seems possible that the toxin-like activity is also on the plasma membrane.

These data appear incompatible with Koblier and Mirelman (1980) who reported that the toxin-like activity, which caused detachment and rounding of tissue-cultured cells, was present only in 100,000 g×60 min supernatant fluid of the amoebal extract prepared by freeze-thawing. This difference may result from the procedures of disruption of amoebae. We disrupted amoebae by homogenization for 2 min at 4 C.

Although our finding that detached NIH:3T3 cells agglutinated may suggest that the lectin-like activity is also present in B-2 and B-3, it is not known whether a single substance has both toxin- and lectin-like properties. As Koblier and Mirelman (1980) suggested, these activities may be catalyzed by different substances. It is also not known whether there is any difference between the toxin-like activity of the lysosome -rich fractions and that of 110,000 g×120 min supernatant fluid. Further characterization of the toxin-like activities of these two fractions is still in progress in our laboratory and will be presented elsewhere.

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短　報

赤痢アメーバのトキシン様活性の局在

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赤痢アメーバ栄養型におけるトキシン様活性の局在を分別遠沈，及びショ糖密度勾配遠沈を用いて得た分画を材料とし，NIH：3T3 細胞を標的として検索した．活性は 15,000 g×20 分沈渣，及び 110,000 g×20 分上清に見いだされ，更に 15,000 g 沈渣においては主としてライソゾーム，及び細胞膜を多く含む分画に活性が存在することが判明した．

Legends to Figures

Fig. 1　Detachment and rounding of NIH:3T3 cells caused by B-2. Agglutination of detached cells was also observed. (×200)
Fig. 2　The same changes as in Fig. 1 of NIH:3T3 cells caused by B-3. (×200)
Fig. 3　NIH:3T3 cells incubated with B-1. Note that the cells appear undamaged. (×200)
Fig. 4　NIH:3T3 cells incubated with B-4. The cells also appear undamaged. (×200)