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MECHANISM OF CELL DISSOCIATION WITH TRYPSIN AND EDTA

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Abstract. The mechanism of cell dissociation with trypsin and EDTA was examined. Cell dissociation was possible when trypsin and EDTA were given simultaneously, when trypsin was given after EDTA treatment, but not when trypsin was given before EDTA treatment.

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When cultured cells are not easily dissociated with trypsin or EDTA in subculturing or cloning, a combination of trypsin and EDTA is generally used (1). However, the mode of cell dissociation with trypsin and EDTA is poorly understood. We investigated this aspect using cultured hepatoma cells, which show little dissociation into a single cell suspension even with high doses of EDTA or trypsin.

MATERIALS AND METHODS

dRLa-74 cells (1) derived from liver tissue (cancerous portion) of a Donryu rat fed with 4-dimethylaminoazobenzene for 191 days were used as model hepatoma cells. This cell line, which showed an epithelial-like morphology, was maintained in stationary culture. It gave rise to tumors with few metastases in rats by the back-transplantation test. The medium employed was Eagle's minimal essential medium (Chiba Pref. Serum Inst.) with 20% bovine serum inactivated at 56°C for 30 min. Trypsin (Difco, Detroit, U. S. A.), ethylenediaminetetraacetic acid (EDTA, Sigma, Saint Louis, Missouri, U. S. A.) or glycolethylenediaminetetraacetic acid (GEDTA, Sigma) was dissolved in Ca^{2+} and Mg^{2+}-free phosphate buffered saline (PBS). Cell dissociation was carried out as follows: A suspension of the cells was prepared by trypsin and EDTA treatment. One point five ml of the suspension containing 1×10^5 cells was distributed into short test tubes (15 mm diameter with a flattened bottom). The tube was stationarily incubated at 37°C to make cells attach to the glass surface. Two days later, the medium was decanted, replaced with an equal volume of the dissociation solution and incubated at 37°C. After an appropriate time, the number of viable cells floating in the medium was counted using a hemocytometer and the erythrosine exclusion method. The relative proportion of cells in each of the stages of dissociation was estimated.

RESULTS AND DISCUSSION

dRLa-74 cells in a monolayer detached from the glass surface with trypsin alone; however, they did not dissociate. On the other hand, cells subjected to EDTA (GEDTA) treatment or vigorous pipetting rarely detached or dissociated. Cell dissociation occurred when cultures were treated with a combination of trypsin and EDTA (GEDTA) (Table 1). Whether such cell dissociation was only possible when trypsin and EDTA were given simultaneously was examined by pretreatment with trypsin and EDTA separately (Table 2). Namely, after

TABLE 1. DISSOCIATION OF dRLa-74 CELLS WITH TRYPSIN AND EDTA. I, SIMULTANEOUS TREATMENT WITH TRYPSIN AND EDTA

Treatment	Stage of dissociation (%)[a]				
	I	II	III	IV	V
0.2% trypsin 10 min	0	0	0	0	100.0
0.1% EDTA 10 min	0	0	0	0	0
0.1% GEDTA 10 min	0	0	0	0	0
0.2% trypsin + 0.1% EDTA 10 min	39.7	16.9	6.9	8.2	28.2
0.2% trypsin + 0.1% GEDTA 10 min	33.8	19.4	8.3	7.9	30.6

a Mean of three experiments; I, Single cells; II, Cells in groups of two; III, Cells in groups of three; IV, Cells in groups of four; V, Cell clumps containing more than five cells each.

TABLE 2. DISSOCIATION OF dRLa-74 CELLS WITH TRYPSIN AND EDTA. II, PRETREATMENT WITH TRYPSIN OR EDTA

Experimental design :

1, 0.2% trypsin 5 min·····W[a]·····0.1% EDTA 10 min
2, 0.2% trypsin 10 min ·····W[a]·····0.1% EDTA 10 min
3, 0.1% EDTA 5 min ·····W[a]·····0.2% trypsin 10 min
4, 0.1% EDTA 10 min ·····W[a]·····0.2% trypsin 10 min
5, 0.2% trypsin+0.1%EDTA 5 min

	Stage of dissociation (%)[b]				
	I	II	III	IV	V
1,	0	0	0	0	100.0
2,	0	0	0	0	100.0
3,	23.5	10.5	7.4	3.1	55.6
4,	35.8	8.0	8.0	6.2	42.0
5,	10.6	16.1	4.6	4.6	64.2

a Washing; *b* Mean of three experiments; I, Single cells; II, Cells in groups of two; III, Cells in groups of three; IV, Cells in groups of four; IV, Cell clumps containing more than five cells each.

treatment with, for example, trypsin for 5–10 min at 37°C, cells were washed twice with PBS and then treated with EDTA for 10 min at 37°C. The order was then reversed with EDTA being used initially, followed by washing in the same buffer as that used before trypsin treatment. Cell dissociation occurred not only when trypsin and EDTA were given simultaneously, but also when trypsin was given after EDTA treatment. However, it did not occur when trypsin was given before EDTA treatment. These results suggested that cell dissociation with trypsin and EDTA occurred without any interaction of trypsin with EDTA. However, cell dissociation resulting from EDTA pretreatment followed by trypsin may have been due to trace amounts of EDTA in intercellular spaces, owing to insufficient washing after EDTA treatment. The effect of washing after EDTA treatment was therefore examined. As shown in Table 3, the effect of

TABLE 3. DISSOCIATION OF dRLa-74 CELLS WITH TRYPSIN AND EDTA. III, NUMBER OF WASHING AFTER EDTA PRETREATMENT

Experimental design:

1, 0.1% EDTA, 10 min·····W[a] (1)·····0.2% trypsin 10 min
2, 0.1% EDTA, 10 min·····W[a] (2)·····0.2% trypsin 10 min
3, 0.1% EDTA, 10 min·····W[a] (3)·····0.2% trypsin 10 min
4, No treatment ·····W[a] (1)·····0.2% trypsin+0.1% EDTA 10 min

	Stage of dissociation (%)[b]				
	I	II	III	IV	V
1,	37.1	18.8	6.4	4.0	33.7
2,	27.0	15.9	11.1	4.8	41.3
3,	42.7	18.3	7.9	11.0	20.1
4,	19.7	14.1	9.3	7.4	49.5

a Washing. Number of washings is shown in parentheses.

b Mean of three experiments, I: Single cells II: Cells in groups of two III: Cells in groups of three IV: Cells in groups of four V: Cell clumps containing more than five cells each.

EDTA pretreatment was profound with an increased number of washings. This finding indicates that interaction of trypsin with EDTA remaining in the intercellular spaces was probably not of consequence. The effect of EDTA pretreatment on trypsin-induced cell dissociation was thus established. It is difficult to explain the molecular mechanism of this cell dissociation from such limited data. However, it is likely that unkown factors or conditions interfering with cell dissociation by trypsin are present in intercellular spaces. EDTA treatment may affect these adhesive factors or otherwise modify surface components involved in intercellular adhesion (3–5), thus facilitating cell dissociation by trypsin. In general, preparation of single cell suspensions involves cell dissociation and detachment of cells from solid substrate. Whether cell suspensions obtained after

EDTA pretreatment resulted from cell separation from cell clumps detached from the glass surface with trypsin or from detachment from the glass surface after cell separation with trypsin is not clear. However, cell separation in this experiment system seemed to be due to cell dissociation, since cells were not detached by EDTA from the glass surface. Whether this method of cell dissociation with trypsin and EDTA using hepatoma cells is generally applicable needs to be investigated further.

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