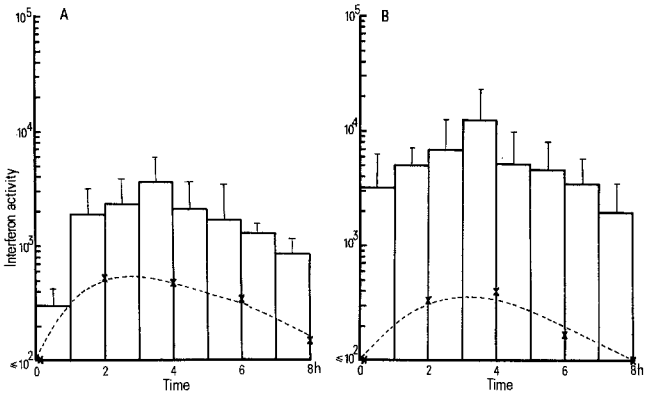
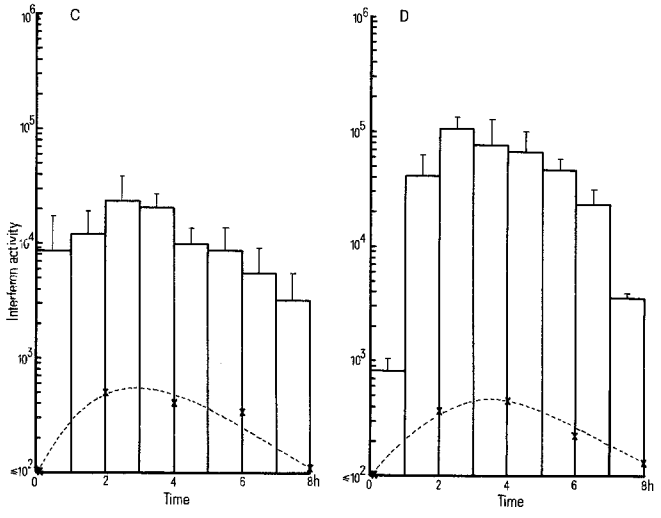


fore, for optimizing IFN absorption via lymphatics, the albumin concentration needs to be increased. The consistency of the edema induced at the site of injection is clearly an important driving force facilitating IFN absorption through the lymphatics.

A striking finding is the very low recovery of IFN in lymph; although IFN entering into the plasma pool is very rapidly eliminated[6–8], we were expecting a somewhat higher IFN recovery in lymph. At present we can only speculate that the low yield is due not so much to dilution in the lymph pool as to extensive cell-binding of IFN. In fact the concentration of lymphoid cells is normally from 15- to 1000-fold higher in lymph and nodes, respectively, than in plasma[9] so that little IFN emerges free into the thoracic lymph. Further studies with radioactively labeled IFN are necessary for quantitating this phenomenon, but the possibility of increasing the IFN concentration in the lymph and lymph nodes, where most of the effector cells with important anti-tumor activities are concentrated, is well worth studying.



Mean plasma levels, IU/ml (x—x) and recoveries in thoracic lymph (mean ± SE, total units/h) after s.c. injection of rec. IFN-α_2 in saline: *A* one site, and *B* five sites; *C* in 4% ALB, and *D* with HYAL.

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Cellular and secreted tumor plasminogen activator: the effects of NaCl

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Summary. Plasminogen activator, secreted by metastatic tumor cells, was strongly inhibited in buffer or tissue culture medium containing physiological concentrations of NaCl. Intact cells, however, expressed strong activity under similar conditions. Thus, if plasminogen activator is involved in invasion and metastasis, the cellular activity, acting as an ectoenzyme, may be more important than secreted enzyme under physiological conditions.
Key words. Plasminogen activator; NaCl; metastasis; MAT 13762 cells.

The serine proteinase plasminogen activator (PA), by virtue of its generation of the broad specificity proteinase plasmin from the circulating zymogen plasminogen, may be involved in tumor metastasis and host tissue destruction[1-3]. Some studies have shown a correlation between secreted PA activity, in vitro, and the metastatic ability of tumor cell lines[4,5], whereas others have not[6].

PA, but not plasmin, activity in both the fibrin plate[7-9] and colorimetric[10-12] assays is strongly inhibited by NaCl. Indeed, at physiological concentrations (0.15 M) inhibition can be over 95%, although PA from different sources vary greatly in their susceptibility to inhibition[8,10,11].

There is recent evidence for the involvement of PA in the metastatis of the MAT 13762 rat mammary tumor[13]. In view of the reported inhibitory effects of NaCl on PA activity we have therefore measured the effect of NaCl on MAT 13762 cell-secreted soluble PA, and the direct plasminogen-dependent fibrinolytic activity of these cells in culture media containing physiological concentrations of NaCl.

Although soluble PA was strongly inhibited by NaCl, when assayed in Tris-HCl buffer, viable cells expressed high levels of activity in the presence of NaCl which was comparable to that of Triton X-100 cell lysates, assayed in Tris-HCl.

Materials and methods. Bovine fibrinogen (type 1-S) from the Sigma Chemical Co., dog serum from Gibco, Long Island, N.Y., and human urokinase from Calbiochem, La Jolla, CA. MAT 13762 cells were originally from the Mason Research Laboratories, Worcester, MA and were routinely cultured in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum. These cells were poorly adherent and grew mainly in suspension.

Plasminogen activator was assayed, at 37°C, as described by Barrett et al.[14], using [^{3}H]-fibrin coated 24-well Linbro plates (2.0 cm^2 wells, Flow Laboratories, VA). Each well contained 100 µg of fibrin and 100,000 cpm. Assays were carried out in 1 ml of 50 mM Tris-HCl buffer, pH 7.5, containing 1% (v/v) acid-treated dog serum as a source of plasminogen, over 2 h. Background release was < 5% and results were expressed as the percentage release of cpm released by 50 ploug U of human urokinase (total cpm). No activity was measured in the absence of added dog serum.

Serum-free MAT 13762 cell culture supernatants were used as a source of soluble PA. Cells (10^6) were incubated for 18 h in 2 ml of RPMI 1640 medium; after removal of cells by centrifugation supernatants were stored in aliquots at −20°C. Release of cpm was linear with increasing amounts of enzyme up to 60–70% release of total cpm. Cell pellets were extracted with 0.2% (v/v) Triton X-100 in saline for 30 min at room temperature, followed by centrifugation at 100,000 × g 60 min. The supernatants were used as a source of Triton X-100 solubilized cellular PA.

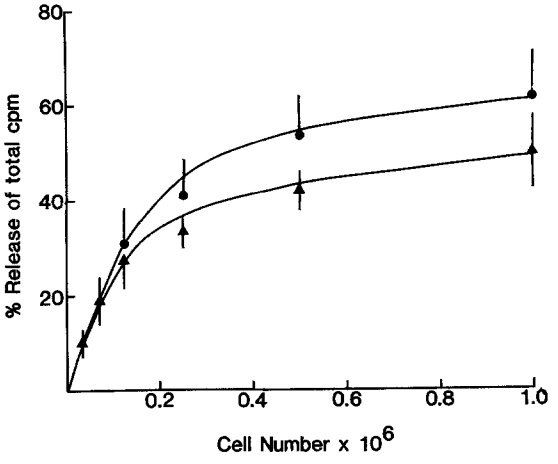

Figure 2. Direct plasminogen-dependent fibrinolytic activity of intact MAT 13762 cells. Cells were added to assay wells containing 1 ml of RPMI 1640 medium, ▲, or 1 ml of 0.125 M NaCl buffered with 50 mM Tris-HCl, pH 7.5, ●, both supplemented with 1% (v/v) acid-treated dog serum as a source of plasminogen. Release of cpm was measured in aliquots of supernatant (500 µl) after 2 h. Results are mean ± SEM for 3 separate cell preparations. The release of cpm increased linearly with the log of the cell numer (r = 0.996).

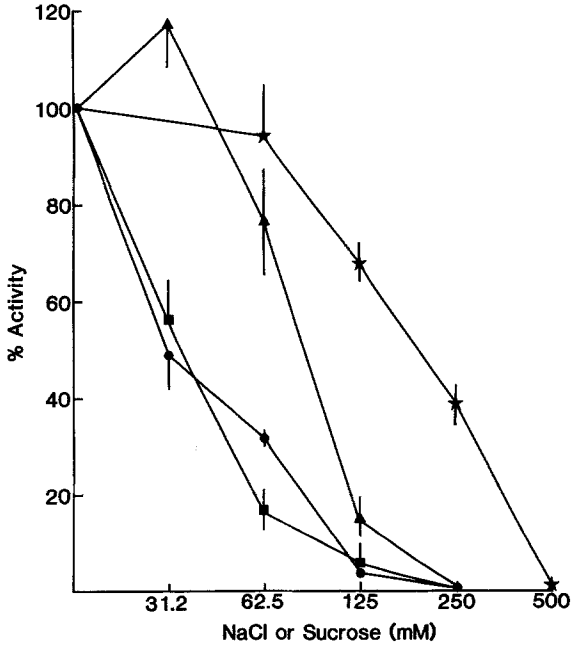

Figure 1. The effect of NaCl on soluble secreted PA, ■, Triton X-100 solubilized cellular PA, ●, and human urokinase, ▲. The effect of sucrose, ★, on soluble secreted PA is also shown. Assays were carried out in 50 mM Tris-HCl, pH 7.5, containing increasing amounts of NaCl or sucrose. Results are mean ± SEM for 3 separate experiments.

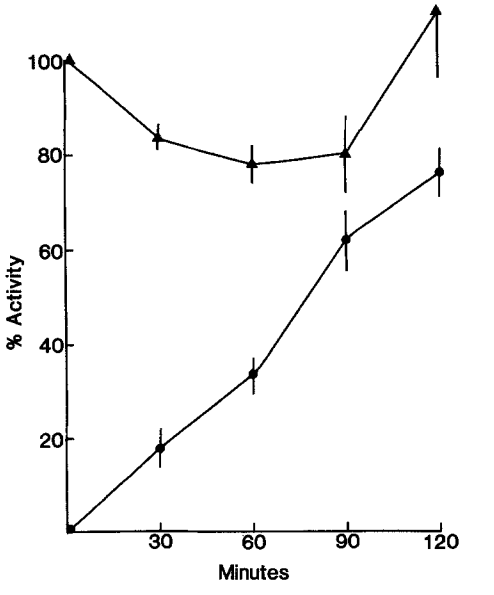

Figure 3. Time course of cell-associated, ▲, and secreted, ●, PA activity during incubation of 2 × 10^6 cells/ml in serum-free RPMI 1640 medium at 37°C. Cells were lysed with 0.2% (v/v) Triton X-100 and the activity of 100 µl of lysate standardized as 100% activity and represented 34.7±8.8% release of total cpm. Results are mean ± SEM of 3 separate cell preparations.

Results. As shown in figure 1, addition of NaCl to the assay buffer (50 mM Tris-HCl, pH 7.5) resulted in a strong inhibition of both soluble secreted PA and of cellular PA extracted with 0.2% Triton X-100. With 0.125 M NaCl present inhibition was approximately 95%; human urokinase was also inhibited although to a lesser degree. Inhibition was not specific for NaCl and was also found with sucrose, although this was less pronounced. Increasing the molarity of the Tris-HCl buffer from 50 mM to 100 mM to 150 mM was also inhibitory and reduced activity from 100% to 80.4 ± 4.4 to 45.2 ± 12.5 (n = 5) respectively. The inhibition of MAT 13762 – PA by NaCl was very similar to that reported for rabbit fibroblast PA, assayed under the same conditions[8].

When intact cells were assayed directly in the fibrin wells, in 1 ml of RPMI 1640 medium, activity was measured which increased linearly with the log of the cell number (fig. 2). Similar results were obtained with cells suspended in the assay wells in 0.125 M NaCl buffered with 50 mM Tris-HCl, pH 7.5. The different dose response curves of soluble PA and activity expressed by intact cells (linear-linear and log-linear respectively) makes comparisons between these two activites difficult. However, at low cell numbers where relationship of cellular PA activity to increasing cell number approximated to linearity, PA activities of the intact cells were similar to those of Triton X-100 lysates of the same cell preparations (10.8 ± 4.7% release of total cpm compared to 15.0 ± 4.0%, n = 3).

The cellular (Triton X-100 lysate) and soluble secreted PA activities, over a 2-h incubation period, of cells in serum-free medium are shown in figure 3. There was an approximately linear release of soluble PA, and a net increase in the total activity of incubation mixture. This soluble secreted activity was, however, reduced by approximately 95% when assayed in RPMI 1640 medium compared with 50 mM Tris-HCl, 3.2 ± 0.7% release of total cpm compared to 59.2 ± 0.4% (n = 3). One interpretation of this result is that during the assay of PA activity expressed by intact cells in RPMI 1640 medium, although soluble activity was released it was largely inactive, and only a cellular ectoenzyme was being measured.

Discussion. Plasminogen activators are found in many cell types both as cell-associated and soluble secreted forms[11, 15–17]. Cell-associated PA activities are generally membrane-bound and can only be solubilized with detergents or chaotropic membrane-disrupting ions[11,16]. Additionally, they act as ectoenzymes in some cells, such as macrophages[11,17].

In the present study, viable MAT 13762 cells expressed plasminogen-dependent fibrinolytic activity under conditions where soluble PA activity was suppressed by 95%. Thus, although cells in RPMI 1640 medium release considerable soluble activity over 2 h (fig. 3), the presence of NaCl in the medium would largely inhibit this. The fact that viable cells and Triton X-100 lysates showed comparable activities, at low cell numbers, suggests that most of the cellular activity acts as an ectoenzyme, as cells would not be permeable to plasminogen. Furthermore, this represents high potential fibrin-degrading activity in the presence of NaCl; 62.5×10^3 cells digested 20 µg of fibrin over 2 h, with only 10 µl (1% in 1 ml assay volume of acid-treated dog serum as the source of plasminogen. Because of the release of large amounts of soluble PA, demonstration of the ectoenzyme nature of the cell-associated activity must necessarily be indirect. Previous fractionation of MAT 13762-PA has shown the main type to be of approximately 50,000 mol. wt, with minor higher mol. wt species[18]; the mol. wt distribution of Triton lysates was similar (unpublished). Thus, there would not seem to be a differential effect of NaCl on secreted and cellular PA due to these being different PA types. This is of importance in view of the differential inhibitory effect of NaCl on MAT 13762-PA and urokinase (fig. 1).

Cellular, unlike soluble, PA activity increased linearly with the log of the amount of enzyme (cell numbers). Other workers have found a similar cell density-dependent expression of PA activity where tumor cells, at different densities, were incubated in phosphate buffered saline containing dog serum for 30 min. Aliquots of supernatant were then removed and assayed for generated plasmin by active site titration[19]. This and the present study emphasize the need to measure activities of viable cells at low cell numbers (or densities) for an accurate comparison with soluble activities. The observed decrease in activity per cell with increasing cell density may be due to the geometry of the assay system where cells settle as a monolayer on top of the insoluble fibrin. Thus, unlike soluble PA, the cellular enzyme is not evenly mixed with the substrate, plasminogen, in the assay wells.

Most studies of the possible involvement of PA in metastasis have concentrated on the secreted activity as this is quantitatively higher when assayed in NaCl free buffers. Indeed, a good correlation between secreted PA activity and metastatic potential has been shown for clonal cell lines of the MAT 13762 tumor[13]. However, in some cell types at least (macrophages) plasminogen-dependent fibrin degradation can occur in the absence of detectable soluble PA activity[17]. The data presented here suggests that cell-associated PA may be an important activity expressed by MAT 13762 cells, in the presence of physiological concentrations of NaCl.

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