Uptake of rat and human α_2-macroglobulin-trypsin complexes into rat and human cells

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Uptake of rat and human α_2-macroglobulin-trypsin complexes was measured in rat hepatocytes, rat and human adipocytes and human fibroblasts. Uptake and degradation of ^{125}I-labelled rat complex were about one-third of that of the human complex in the various isolated cell types. In rat hepatocytes, the apparent K_m for cell association of the rat complex was about 16 nM as compared to about 6 nM for the human complex. The V_{max} values were similar, about 1×10^4 molecules · cell^{-1} · min^{-1}. Thus, rat α_2-macroglobulin (an acute-phase protein) complexed with trypsin follows the same pathways of uptake as the human homologue, although with a somewhat lower affinity for the uptake system.

α_2-Macroglobulin *Adipocyte* *Hepatocyte* *Species difference*

1. INTRODUCTION

Recent studies have shown that human α_2-macroglobulin-trypsin complex is specifically associated to and degraded by isolated rat hepatocytes [1]. Most of the cell-associated material is internalised at 37°C [1] and we therefore refer to cell association as uptake. Other studies showed that the rapid removal of human α_2-macroglobulin-trypsin from the circulation in rats [2] and mice [3] is mainly accounted for by its uptake into hepatocytes. However, rat plasma contains 2 macroglobulins which are homologous to human α_2-macroglobulin. One of them, rat α_1-macroglobulin, is structurally quite different from human α_2-macroglobulin [4]. The concentration of this homologue in rat plasma is high (2–4 mg/ml) and relatively constant, and in this sense it resembles human α_2-macroglobulin. Rat α_2-macroglobulin is structurally and serologically similar to human α_2-macroglobulin. However, its concentration in plasma of adult rats is normally low (15–30 μg/ml) and may increase to 2–4 mg/ml during acute inflammation [5,6]. In this sense it is different from human α_2-macroglobulin which is not an acute-phase protein. We decided to compare cell association and degradation of rat and human α_2-macroglobulin in different cell types from rats and humans since the physiology of the macroglobulins seems quite different in the 2 species and since the rat is a commonly used laboratory animal.

2. MATERIALS AND METHODS

Trypsin (60% active) was from Boehringer; soybean trypsin inhibitor and bovine serum albumin (fraction V) were from Sigma. Collagenase (type 1) was from Worthington and $^{125}I^-$ (about 2 Ci/μmol) from Amersham International, England. Male rats (about 250 g) received subcutaneous injections of 0.5 ml croton oil in the subscapular area and blood was collected after 48 h. α_2-Macroglobulin was prepared from rat or human plasma by Zn^{2+} chelate chromatography and Sephacryl S300 gel chromatography according to Nelles and Schnebli [7] as described [8]. Alternatively, rat α_2-macroglobulin was prepared in the following way. EDTA plasma (100 ml) from rats with adjuvant-induced arthritis was mixed with

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5 mM benzamidine and 2 mM phenylmethanesulfonyl fluoride. Globulins were fractionally precipitated at 0°C with 6–14% polyethylene glycol 4000 and redissolved in 0.02 M Tris-HCl, pH 7.4. α_2-Macroglobulin was then purified by sequential chromatography on the following columns: (1) Fractogel TSK DEAE-650M (Merck, Darmstadt) (3.2 × 27 cm) using 0.02 M Tris-chloride, pH 7.4, and a 600 ml gradient of 0–0.5 M NaCl; (2) Fractogel TSK HW-55F (Merck) (3.2 × 80 cm) using Tris-chloride, pH 7.8, and 0.15 M NaCl; (3) zinc chelated to immobilized iminodiacetic acid on Fractogel TSK (Pierce, Rockford, IL) (1.5 × 15 cm) loading in Tris-chloride, pH 7.8, and eluting with 0.05 M sodium phosphate, pH 6.0, in 0.15 M NaCl [8]; (4) Mono Q (Pharmacia, Uppsala) with 0.05 M Tris, pH 7.8, and 0.1–0.4 M NaCl. This preparation was obtained through the courtesy of Drs K. Lonberg-Holm and D.L. Reed, DuPont, Central Research Station, Wilmington, DE, and the method will be described in detail elsewhere.

The α_2-macroglobulins were iodinated and complexed with trypsin as described [1] except that 0.5 mol iodine was used per mol protein. The concentrations were determined using $E_{280}^{1\%} = 8.9$. All treatments of human and rat α_2-macroglobulin were carried out in parallel. After complexing with trypsin the 2 preparations of rat α_2-macroglobulin were compared in several experiments and no difference was found in their ability to compete with uptake of ^{125}I-labelled human complex (cf. fig.1).

^{125}I-labelled rat α_2-macroglobulin-trypsin was injected intravenously into anesthetized 200 g rats and the cellular localization of the radioactivity was determined using light microscopic autoradiography as in [2]. Rat hepatocytes were prepared by in vitro perfusion of the liver from animals weighing 190–230 g with collagenase [1,9]. Aliquots of cell suspension (0.5 ml with 2 × 10^5 cells/ml unless otherwise stated) were incubated in buffer (37°C, pH 7.6) containing Krebs' salts, 10 mM Hepes and 5% (w/v) albumin. By the end of the incubation, 400 μl cell suspension was transferred to 550 μl microfuge tubes containing 75 μl dibutylphthalate and centrifuged for 1 min at 10000 × g. Finally, the tube was cut through the oil layer and the cell pellet counted [1,10]. Rat [1,11] and human [12] adipocytes were prepared and incubated (5 × 10^5 cells/ml) following standard methods. Human fetal fibroblasts were maintained in Dulbecco's modification of Eagle's medium fortified with 2 mM L-glutamine, 10% fetal calf serum, 50 mg/l neomycin, and 2.5 × 10^4 U/l nystatin and buffered with 20 mM Hepes. Two days before the experiment the cells were trypsinized, washed, filtered through a 56 μm nylon mesh and seeded in plates with a culture area of 4.5 cm^2 [13]. On the day of the experiment the cells were washed and incubated in situ using the same buffer as for the other cell types. The incubations were stopped by washing the cells 3 times with 1 ml ice-cold 0.9% NaCl. Finally, the cells were dissolved by treatment with 0.5 M NaOH at 80°C for 6 h and the cell-associated radioactivity determined.

3. RESULTS

The half time of disappearance of rat α_2-macroglobulin-trypsin complex from the blood in anesthetized rats was about 4 min, which is twice that of the human complex [2] and uptake into the liver accounted for 80–90% of the clearance (not shown). Table 1 shows that the uptake was mainly into hepatocytes in agreement with previous data for human α_2-macroglobulin-trypsin [2].

Fig.1A shows the effect of unlabelled rat and human α_2-macroglobulin-trypsin on the uptake of 20 pM labelled human complex into isolated rat hepatocytes. Half-maximal inhibition was ob-

Table 1

Distribution of grains over liver tissue as analyzed by light microscopic autoradiography of tissue fixed 10 min after injection of about 50 μCi ^{125}I-labelled rat α_2-macroglobulin-trypsin

	Hepatocytes	Non-hepatocytes	Space of Disse and lumen of sinusoides
Grains	1318	28	10

Four sections from different parts of the liver were analyzed and 1356 grains were counted over 3.8 × 10^4 μm^2 using a magnification of × 2048. The autoradiographic background was negligible, i.e. less than 50 grains/10^5 μm^2

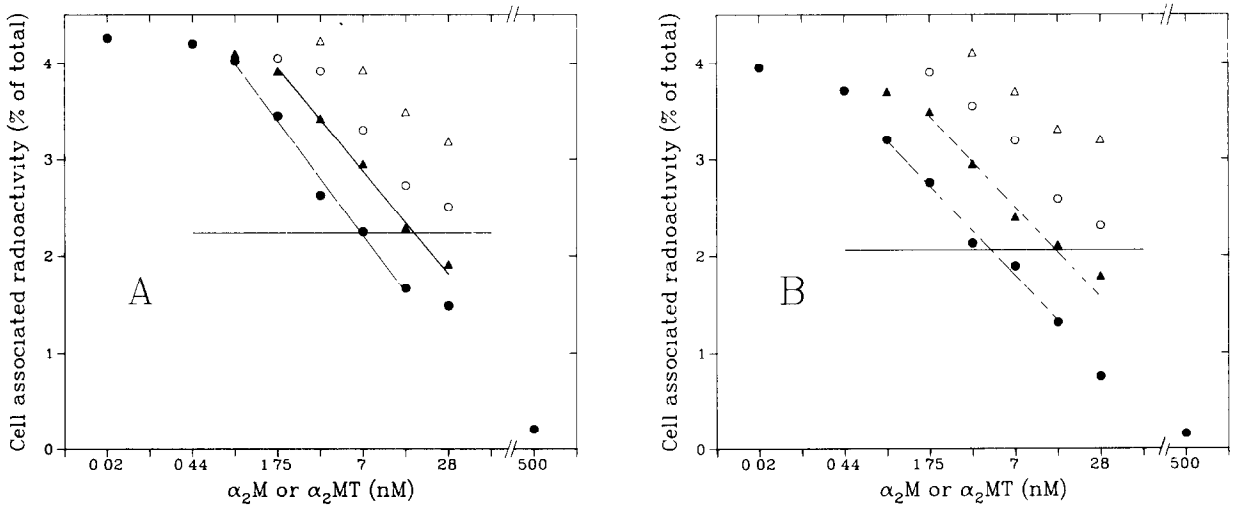

Fig.1. Inhibition of the uptake of ^{125}I-labelled human α_2-macroglobulin-trypsin by unlabelled complexes in rat hepatocytes (A) and rat adipocytes (B). The cells were incubated for 3 h with 20 pM human complex in the absence or presence of unlabelled human (●—●) or rat (▲—▲) complexes. The open symbols represent the equivalent data for the uncomplexed macroglobulin. The points represent the means of 3 replicates. The coefficient of variation was about 6%. The lines are calculated by linear regression on the points representing the straight part of the competition-inhibition curves. The radioactivity associated with the cells in the presence of 500 nM unlabelled human α_2-macroglobulin-trypsin was regarded as non-specific and the horizontal lines indicate half of the maximal specific cell association.

tained with 13.9 nM of the rat complex and 5.1 nM of the human complex, yielding a relative potency of the rat complex of 37%. The mean relative potency of the rat complex was calculated from this and similar experiments as 32 ± 5% (SD, n = 4). Fig.1A also shows the low potencies of the uncomplexed macroglobulins. Fig.1B shows the equivalent data using rat adipocytes. The mean relative potency of rat α_2-macroglobulin-trypsin was 38 ± 4% (SD, n = 4) in this system.

Table 2 shows that the uptake of 20 pM ($\ll K_m$) ^{125}I-labelled rat α_2-macroglobulin-trypsin is 30–50% of that of the labelled human complex in the 4 cell types tested. Other experiments (not

Table 2

Specific uptake of 20 pM rat or human ^{125}I-labelled α_2-macroglobulin-trypsin in rat and human cells

	Rat hepatocytes	Rat adipocytes	Human adipocytes	Human fibroblasts
Human α_2-macroglobulin-trypsin	4.5 ± 0.3	4.0 ± 0.3	0.9 ± 0.1	5.6 ± 0.2
Rat α_2-macroglobulin-trypsin	2.0 ± 0.2	1.7 ± 0.2	0.4 ± 0.1	3.4 ± 0.1

Incubations were carried out for 3 h at 37°C and the percent of cell-associated radioactivity was determined. The results with the rat cells are the mean values of 4 independent experiments ± 1 SD. The results with the human cells are given as the mean of at least 4 replicate values ± 1 SD, and this experiment was repeated with essentially identical results

shown) demonstrated that the degradation (formation of products soluble in 12% trichloroacetic acid) of ^{125}I-labelled rat α_2-macroglobulin-trypsin in suspensions of rat hepatocytes was about half of that of the human homologue.

Fig.2 shows that the apparent affinity for uptake of the rat complex was about one-third of that for the human complex, whereas the maximal velocities were not significantly different. The K_m values of 6 nM for the human complex and 16 nM for the rat complex are in agreement with the competition experiment shown in fig.1A. The V_{max} value of 16 pM · min^{-1} corresponds to a rate of uptake of 6×10^3 molecules · cell^{-1} · min^{-1}. Three additional experiments gave similar K_m values whereas V_{max} varied from 14 to 26 pM · min^{-1}.

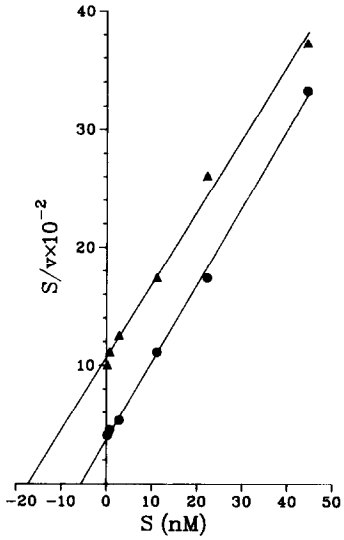

Fig.2. Concentration dependence of uptake of rat (▲—▲) and human (●—●) α_2-macroglobulin-trypsin complex in rat hepatocytes. The hepatocyte concentration was 1.2×10^6/ml and the incubation time was 40 min. The data are plotted according to Hanes' modification of the Michaelis-Menten equation:

$$S/v = 1/V_{max} \cdot S + K_m/V_{max}$$

using linear regression. The K_m values were calculated from the negative intercepts with the abscissa as 6.0 nM for the human complex and 16.4 nM for the rat complex. The V_{max} values were calculated from the reciprocal slopes as 15.3 pM · min^{-1} for the human complex and 15.9 pM · min^{-1} for the rat complex.

4. DISCUSSION

Previous results have shown that human, rat and mouse α_2-macroglobulin-trypsin complexes are removed from the blood by the same saturable pathway in mice [14]. This was thought to involve reticuloendothelial cells and mainly Kupffer cells [15,16]. The present results show that rat α_2-macroglobulin-trypsin, like the human complex [2], is mainly taken up by hepatocytes in the rat. However, the apparent affinity for uptake of the rat complex in these cells is only about one third of that of the human complex. The same applies to uptake in human fibroblasts and in adipocytes from both rats and humans. This moderate difference in affinity may well be due to structural differences between the rat and human macroglobulins (cf. [17]). In any case, it seems appropriate, in view of the present results, to use human α_2-macroglobulin complexes in studies employing the rat as the experimental animal.

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REFERENCES

[1] Gliemann, J., Larsen, T.R. and Sottrup-Jensen, L. (1983) Biochim. Biophys. Acta 756, 230–237.
[2] Davidsen, O., Christensen, E.I. and Gliemann, J. (1985) Biochim. Biophys. Acta, in press.
[3] Feldman, S.R., Rosenberg, M.R., Ney, K.A., Michalopoulos, G. and Pizzo, S. (1985) Biochem. Biophys. Res. Commun. 128, 795–802.
[4] Schaaufele, J.T. and Koo, P.H. (1982) Biochem. Biophys. Res. Commun. 108, 1–7.
[5] Gordon, A.H. (1976) Biochem. J. 159, 643–650.
[6] Okubo, H., Miyanaga, O., Nagano, M., Ishibashi, H., Kudo, J., Ikuta, T. and Shibata, K. (1981) Biochim. Biophys. Acta 668, 257–267.
[7] Nelles, L.P. and Schnebli, H.P. (1982) Hoppe-Seyler's Z. Physiol. Chem. 363, 677–682.
[8] Sottrup-Jensen, L., Stepanik, T.M., Wierzbicki, D.M., Jones, C.M., Lønblad, P.B., Kristensen, T., Mortensen, S.B., Petersen, T.E. and Magnusson, S. (1984) Ann. NY Acad. Sci. 421, 41–60.

[9] Linde, S., Sonne, O., Hansen, B. and Gliemann, J. (1981) Hoppe-Seyler's Z. Physiol. Chem. 362, 573–579.
[10] Andreasen, P., Schaumburg, B., Østerlind, K., Vinten, J., Gammeltoft, S. and Gliemann, J. (1974) Anal. Biochem. 59, 110–116.
[11] Foley, J.E., Foley, R. and Gliemann, J. (1980) Biochim. Biophys. Acta 599, 689–698.
[12] Pedersen, O., Hjøllund, E., Beck-Nielsen, H., Lindskov, H.O., Sonne, O. and Gliemann, J. (1981) Diabetologia 20, 636–641.
[13] Sonne, O. (1985) Mol. Cell. Endocrinol. 39, 39–48.
[14] Gonias, S.L., Balber, A.E., Hubbard, W.J. and Pizzo, S.V. (1983) Biochem. J. 209, 99–105.
[15] Ohlsson, K. (1971) Acta Physiol. Scand. 81, 269–272.
[16] Fuchs, H.E., Shifman, M.A. and Pizzo, S.V. (1982) Biochim. Biophys. Acta 716, 151–157.
[17] Sottrup-Jensen, L., Stepanik, T.M., Kristensen, T., Wierzbicki, D.M., Jones, C.M., Lønblad, P.B., Magnusson, S. and Petersen, T. (1984) J. Biol. Chem. 259, 8318–8327.