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SELECTIVITY IN ENDOCYTOSIS OF SERUM AND CYTOSOL PROTEINS BY MACROPHAGES IN CULTURE

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Summary

It was found that cultured mouse macrophages preferentially endocytose and degrade short half-life serum and cytosol proteins. With increasing duration of endocytosis, the components of longer half-life are increasingly endocytosed and degraded. The relevance of these observations to the selectivity of protein turnover is discussed.

The mechanisms by which proteins are degraded at characteristic and widely varied rates remain unclear (1,2). One possibility is that proteins undergoing degradation in lysosomes (2,3) enter at different rates. It is known that extracellular materials can enter cells at different proportional rates (4,5) by virtue of different degrees of interaction with the plasma membrane involved in endocytosis. Bohley (6) suggested that such a mechanism might occur at the surface of the lysosome involved in autophagy, and contribute selectivity to turnover of intracellular proteins.

Recent work has shown that selective binding of cytosol and serum proteins can occur on hydrophobic supports (7,8) and membranes (9), and that the short half-life proteins tend to bind more than those of long half-life. This paper shows that such a selective binding at the cell surface can lead to selective internalisation and degradation of proteins. Previous preliminary observations on uptake and degradation by a rat liver cell line are consistent with this idea (10). These observations render more likely the hypothesis that selective entry and degradation might occur on intracellular membranes performing autophagy.

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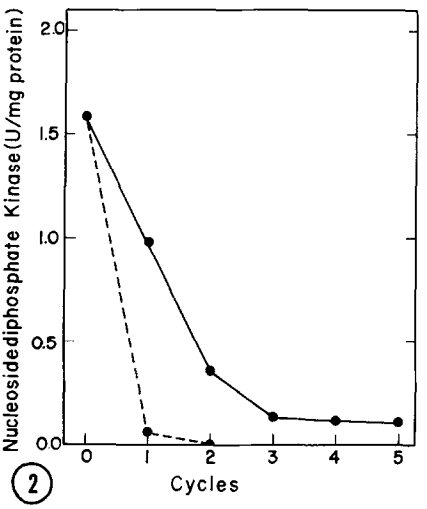
Materials and Methods

Mouse peritoneal macrophages were collected from normal TO mice as described previously (11) and 2 - 4 x 10^6 cells were cultured on 5 cm plastic dishes in 5 ml Medium 199 containing 10% heat inactivated swine serum. Serum and liver cytosol proteins double labelled with [^{3}H]- and [^{14}C]-leucine (Radiochemical Centre, Amersham), in which $^3H/^{14}C$ ratios are an index of *in vivo* half-life (high ratios corresponding to short half-life), were obtained from rats as described earlier (12). Three separate preparations of each component were used, each derived from three rats. They were dialysed to remove TCA-soluble radioactivity and sterilised by millipore filtration. Usually about 50,000 d.p.m. of [^{3}H]-leucine were supplied to each culture. Triplicate cultures were then incubated for various times before harvesting. At harvesting, the medium was removed and the cells lysed with 0.1% Triton X-100 in phosphate-buffered saline, and scraped off with rubber bungs. 0.5 ml aliquots of medium and cell suspensions (to which 50 µl of 10% bovine serum albumin was added as carrier) were then precipitated with 0.5 ml of 10% trichloracetic acid (TCA)-containing 10 mM-leucine. After centrifugation, aliquots of the supernatant were counted in a Triton-toluene scintillant. The pellets were washed twice with 5% TCA-10 mM leucine, redissolved in formic acid and counted similarly.

Results and Discussion

The uptake of cytosol proteins by macrophages is shown in Fig. 1a. The macromolecular (TCA precipitable) radioactivity associated with the cells reaches a roughly constant level by about 48 h. The rate of uptake of radioactivity stays roughly constant throughout the 96 h experiments. The endocytic indices (5) for the various cytosol protein preparations ranged from 0.9 to 2.1 µl/h/10^6 cells (measured over 48 h, with reference to the tritium label). The uptake of serum proteins was of similar form, with endocytic indices from 0.2 to 1.1 µl/h/10^6 cells. These relatively high indices (c.f. 5) are indicative of entry by adsorptive pinocytosis. At a maximum about 10% of the cytosol and 4% of the serum proteins were endocytosed in 96 h.

Such experiments were done at least twice with each batch of protein, and they were also evaluated in terms of selectivity of uptake. The $^3H/^{14}C$ ratio of the macromolecules associated with the cells and of the degradation products, were assessed at various times. Fig. 1b shows the selectivity of uptake and degradation of the cytosol proteins in one particular experiment. Very little protein radioactivity was associated with the cells after five minutes of incubation, presumably because adsorption was largely suppressed by the competing medium proteins. But the internalised and degraded materials had elevated ratios,



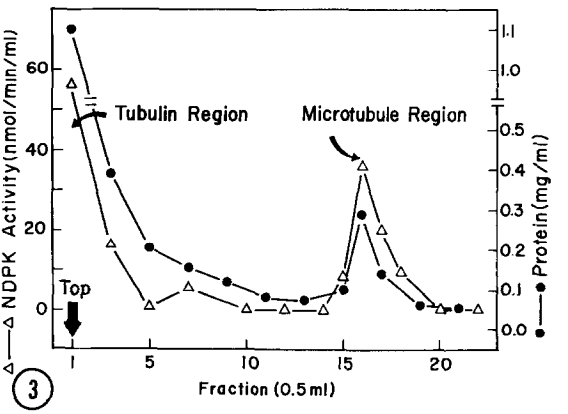


Figure 2. Bovine brain tubulin was purified through 5 assembly-disassembly cycles. The upper curve (solid line) shows the nucleosidediphosphate kinase specific activity after each cycle. The lower curve (broken line) shows the highest estimate of enzyme specific activity expected by simple dilution in each cycle.

Figure 3. Sucrose gradient centrifugation of twice polymerized bovine brain tubulin. Samples were centrifuged in 30-60% sucrose in 10 mM buffer, pH 6.5, and 1 mM $MgCl_2$ at 38,000 rpm in a Beckman SW41 rotor for 1 hr at 30°C. Each fraction was assayed for nucleosidediphosphate kinase and protein as described in the Material and Methods section.

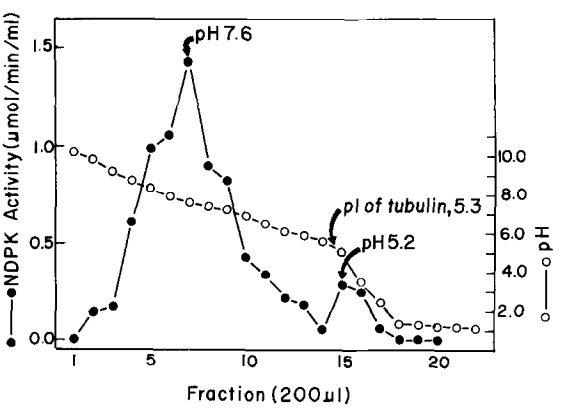


Figure 4. Isoelectric focusing of twice polymerized tubulin at constant voltage (150 V) for 18 hr. Fractions were assayed for nucleosidediphosphate kinase and pH.

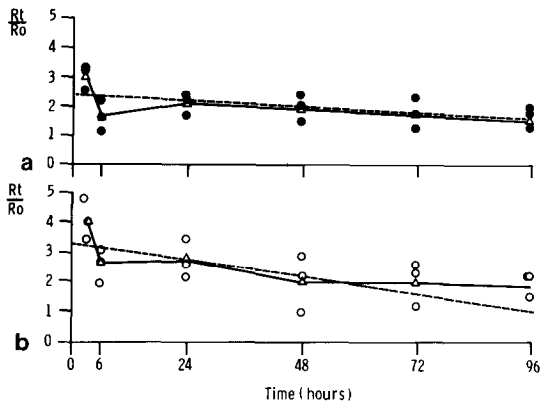


Figure 2

Selectivity in uptake and degradation of cytosol (a) and serum (b) proteins by macrophages. In (a) the dependence of $^{R}t/R_{o}$ of cell associated TCA-precipitable radioactivity on time is shown for three groups of three cultures (each point (•) represents the average of three cultures). The solid line joints the means (△) of all nine cultures at each point. The dotted line shows a best fit linear regression ($r = -0.48$, gradient = -0.008, intercept on the $^{R}t/R_{o}$ axis = 2.36). The correlation coefficient is significant at the 5% level. In (b) the same data are displayed for the total TCA-soluble radioactivity in cultures exposed to labelled serum proteins (○). The solid line again joins the averages (Δ) and the dotted line is the linear regression (correlation coefficient -0.60, gradient -0.017, intercept on the $^{R}t/R_{o}$ axis 3.27). The correlation coefficient is significant at the 1% level.

correlation is confirmed by linear regression analysis. The data of Fig. 2 fit linear regressions given in the legends with correlation coefficients that are statistically significant. Other regression functions could probably be chosen to give larger correlation coefficients.

Thus macrophages selectively endocytose and degrade short half-life proteins of serum and cytosol, confirming earlier preliminary observations. These short half-life proteins are progressively (but irregularly) succeeded by others of greater half-life. The hypothesis that selective internalisation of proteins into lysosomes may be a mechanism responsible for differential turnover, therefore receives some experimental support.

It remains to be established whether a similar selectivity obtains at the forming surface of autophagosomes. Evidence for selective binding to the surface of lysosomes in conditions of physiological ionic strength (9) but not at lower

ionic strength (9,14) is available. And preliminary evidence from studies of autophagy of microinjected proteins (15) and proteins loaded into the cytosol by means of liposomes (10,16) is indicative of such selective autophagy.

The selectivity of turnover of serum and liver cytosol proteins in diabetes and starvation is drastically changed (17). For instance the normal correlations between molecular size, isoelectric point and half-life (1) are abolished. It will be of interest to see whether this reflects changes in substrate molecular characteristics or selectivity of the degradative mechanisms. In the case of serum proteins, while the selectivity of uptake by a particular cell type might be altered, there is the additional possibility that the quantitative contribution of different cell types (each with distinct selectivities) to the uptake, might be changed.

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