Substrate selection and processing in endocytosis

JOHN B. LLOYD, MARGARET K. PRATTEN, RUTH DUNCAN, TEAKE KOOISTRA and SUSAN A. CARTLIDGE
Biochemistry Research Laboratory, Department of Biological Sciences, University of Keele, Keele, Staffs. ST5 5BG, U.K.

In an earlier contribution to these Transactions (Lloyd & Williams, 1984) the characteristics of non-specific adsorptive pinocytosis were discussed. Evidence was presented that many macromolecules captured by adsorptive pinocytosis display either cationic or hydrophobic determinants, and that many types of cell possess surface features that interact positively with such ligands. Internalization of these macromolecules is a consequence of the constant formation and inflow of pinocytic vesicles. Thus constitutive pinocytosis has a high degree of built-in substrate specificity and there is no need, in most instances of adsorptive uptake, to invoke a specific substrate-triggered pinocytic event. The earlier article also discussed critically the techniques available for quantifying substrate uptake by pinocytosis.

In this second contribution we review our recent work on the substrate specificity of pinocytosis and the subsequent processing of internalized macromolecules within the lysosomes. A common theme of most of this work is the use of synthetic polymers as custom-designed probes for these cellular phenomena.

Substrate selection in endocytosis

Rates of pinocytosis of different proteins have been reported by investigators using a range of techniques in vitro or in vivo. Unfortunately it is impossible to ascribe differences in rate to any particular molecular parameter, since proteins differ from one another in molecular size and shape, hydrophobicity, charge distribution, and other characteristics. With synthetic polymers it is possible to vary one parameter at a time.

Molecular size. We have reported data on the pinocytosis of 125I-labelled polyvinylpyrrolidone of different molecular weight ranges by an epithelial cell type (rat yolk sac) and by rat peritoneal macrophages (Duncan et al., 1981a). It was clear that very large macromolecules were discriminated against by the yolk sac but somewhat preferred by macrophages. A subsequent study, using a 125I-labelled methacrylamide-based polymer fractionated to yield samples of different mean molecular weight and low polydispersity, confirmed that yolk sac discriminates against large polymer molecules (Cartledge et al., 1982).

Phagocytosis is the substrate-induced endocytosis of particulate matter, and is a phenomenon seen in macrophages and a few other cell types. But particles may also be captured in pinocytosis, provided they are small enough. As reported elsewhere in these Transactions (Pratten et al., 1984a), endocytosis of Percoll, a 30nm diameter polyvinylpyrrolidone-coated silica particle, is by pinocytosis, not phagocytosis, in the rat yolk sac. In the rat peritoneal macrophage, a cell capable of both pinocytosis and phagocytosis, a study using 125I-iodinated latex beads demonstrated a shift from pinocytosis to phagocytosis as the mean diameter of the substrate was increased in the range 100-1000nm (Pratten & Lloyd, 1983a).

Surface features. Only those characteristics of a macromolecule that are displayed at its surface will be perceived by a cell's plasma membrane. Thus, as explained previously (Lloyd & Williams, 1984), the rate of uptake of bovine serum albumin varies with the state of its tertiary structure and the degree to which hydrophobic residues are unmasked. We have recently studied the uptake by macrophages of a block copolymer comprising polyethyleneoxide linked to a poly-L-lysine highly substituted with (hydrophobic) palmitoyl groups (Pratten et al., 1984b). Its rate of pinocytic uptake did not differ from that of a simple polyethyleneoxide of the same mean molecular weight, and we were able to show that the block copolymer formed a (unimolecular) micelle in which the hydrophobic domains are buried in a central core and so were not a determinant for pinocytosis.

We find that few macromolecules are wholly without affinity for the plasma membranes of pinocytic cells. Polyvinylpyrrolidone and polyhydroxypropylmethacrylamide are two such (Williams et al., 1975; Duncan et al., 1981b), which consequently enter cells wholly by fluid-phase pinocytosis. They are therefore molecules that can be used to study the effect of known amounts of particular substrates on the mode of pinocytic uptake. Using this approach we have shown that the inclusion of relatively few positively charged species dramatically increases the rate of uptake into macrophages of a polymer composed principally of vinylpyrrolidone units (Pratten et al., 1982). In another study it has been found that the uptake of polyhydroxypropylmethacrylamide by yolk sac increases if side-chains terminating in tyrososamine are present (Duncan et al., 1984a). We assume that this increase is due to increased hydrophobicity, although at present we cannot exclude the possibility that the phenolic residues are effective for another reason. In these experiments, and in a parallel study with a polyspartamide substituted with tyramine residues (Duncan et al., 1982a, 1984b), the phenolic residues had relatively little effect until their density on the molecule reached approx. 10mol%. Above this value rate of uptake increased with increasing degree of substitution.

Synthetic polymers may even be used to probe the recognition moieties in much more specific membrane-ligand interactions. The bisazo dye Trypan Blue and the trypanocide suramin for glycoproteins that contain galactose-terminating oligosaccharides was found to recognize a polyhydroxypropylmethacrylamide with Gly-Gly-Gal-NH₂ side-chains (Duncan et al., 1983a). This polymer was captured by the liver more rapidly than glucosamine- and mannosamine-containing analogues, demonstrating that the hepatocyte receptor recognizes not only galactose but galactosamine, recognizes the sugar not only when glycosidically linked but also when conjugated through the 2-position, and recognizes the ligand even when attached to a wholly unnatural macromolecule.

'Piggy-back' pinocytosis

This term refers to the enhanced uptake of one substrate as a consequence of its binding to another substrate that is concurrently being captured by adsorptive pinocytosis. The second substrate must of course be a bivalent (at least) ligand in order to bind both to the first substrate and also to the plasma membrane.

The bisazo dye Trypan Blue and the trypanocide suramin are both polysulphonated aromatic compounds. They both enhance the pinocytic uptake of 125I-labelled polyvinylpyrrolidone, a macromolecule that enters cells by fluid-phase pinocytosis. This enhancement is attributed to 'piggy-back' pinocytosis, since Trypan Blue and suramin have no effect on the uptake of several other substrates (Roberts et al., 1980; Pratten & Lloyd, 1983b). The polycations poly-L-lysine and poly-L-ornithine provide further examples: these substances enhance the uptake of colloidal 119Au-gold, but not of 125I-labelled polyvinylpyrrolidone (Pratten et al., 1978; Duncan et al., 1979).

These examples of 'piggy-back' pinocytosis were discovered through investigating claims in the literature that...
certain substances (polyanions, polycations) stimulate pinocytosis. Our experience leads us to believe that the stimulation of pinocytosis, in the sense of an increase in the rate of formation of pinocytic vesicles, is an uncommon phenomenon and one that should only be considered as substantiated if "piggy-back" pinocytosis has been excluded as an explanation.

Substrate processing within the lysosomes

At least in the case of proteins, there is strong evidence that the degradative capacity of the lysosomal enzymes is more than adequate to process the macromolecules entering the lysosome compartment after pinocytosis. In experiments using rat yolk sac, in which proteins were pinocytosed at very high rates because of their high affinity for plasma membrane, there was no evidence of an accumulation of substrate awaiting digestion in the lysosomes (Moore et al., 1977; Ibbotson & Williams, 1979). In other words pinocytic uptake is the rate-limiting step in the uptake and degradation of proteins.

In certain circumstances, however, digestion can become the rate-limiting step. Pinocytosis of several proteins was studied in normal human fibroblasts and in cells from patients with the recessive disease cystinosis (Kooistra & Lloyd, 1983). In cystinosis there is a massive accumulation of cystine within the lysosomes, which decreases their proteolytic activity. The processing of most proteins in the cystinotic cells was unimpaired. However, one protein, formaldehyde-denatured bovine serum albumin, which is a poor substrate for lysosomal proteases, was pinocytosed faster than it could be digested, both in normal and (particularly) in cystinotic cells. When the cells were treated with leupeptin, the cysteine-proteinase inhibitor, degradation became rate-limiting with some other protein substrates.

We have used soluble synthetic polymers to study post-pinocytic digestion. The carbon backbones of polymers such as polyvinylpyrrolidone are totally resistant to lysosomal enzymes, and so these macromolecules accumulate within the lysosomes at rates that simply reflect the rates of their pinocytic uptake (see above). Polyhydroxypropylmethacrylamide is such a polymer, but to it may be conjugated to oligopeptide side-chains which are potentially degradable in the lysosomes. Polymers with side-chains of graded susceptibility to peptidase attack have been prepared and side-chain degradation studied with isolated lysosomal enzymes (Tritosomes) (Duncan et al., 1982a, 1983b). Side-chains vary in both their innate degradability and the susceptibility of their degradation to inhibition by leupeptin. Within cells after pinocytosis (Duncan et al., 1981b) these polymeric substrates show a continuum of response from accumulation without degradation to degradation without accumulation.

Where the side-chain degradation is leupeptin-sensitive, this inhibitor converts a non-accumulated polymer into one that is retained within the lysosomes.

Much of the work discussed has been done as a collaborative project with polymer chemists in continental Europe. Professor H. Ringsdorf (Mainz) and Dr. J. Kopeček (Prague) have been responsible for synthesizing the polymers used, and we acknowledge gratefully their major contribution to the work. The work has been generously supported by grants from the Cancer Research Campaign, the Science and Engineering Research Council and the National Kidney Research Fund. We also thank the British Council and the Royal Society for several travel grants.

Characterization of clathrin and clathrin-associated proteins

ERNST UNGEWICKELL
Max-Planck-Institute for Biophysical Chemistry, D-3400 Goettingen, Federal Republic of Germany

The limiting membranes of organelles in eukaryotic cells differ significantly from each other with respect to their protein composition, and to a large extent in their lipid composition. During endocytosis and secretion membranes from different compartments fuse with each other and their membrane components are likely to get mixed. To prevent the randomization of their membranes as a consequence of intracellular vesicle traffic eukaryotes must have developed means that ensure the specificity of budding vesicles. In principal this could be achieved by direct or indirect fixation of membrane proteins to elements of the cytoskeleton, when they are not destined for transport. Alternatively the budding vesicle may be by itself capable of selecting those membrane proteins that are at a given time due to be moved to different cell compartments. There is strong evidence which indicates that coated pits and coated vesicles have the ability to select membrane proteins specifically and to facilitate their transport to a different