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THE RELATIVE PERMEABILITY OF LYSOSOMES FROM *TETRAHYMENA PYRIFORMIS* TO SOME AMINO ACIDS AND DIPEPTIDES

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SUMMARY

It has been shown that lysosomes in homogenates of *Tetrahymena pyriformis* are permeable to the amino acids glycine, L-alanine, D-alanine and L-valine (probably by means of a simple diffusion process). These lysosomes are impermeable to the dipeptides glycylglycine, glycyl-L-alanine, L-alanylglycine, L-valyl-L-leucine and L-leucylglycine although they are permeable to glycyl-L-leucine. The reason for the behaviour of glycylleucine is not understood.

INTRODUCTION

The permeability of the lysosomal membrane to salts, low-molecular-weight carbohydrates and other organic compounds has been studied using lysosomes both from rat liver[1–4] and from the ciliate protozoan *T. pyriformis*[5]. However, in spite of considerable work on lysosomal cathepsins and the digestive activity of lysosomes[3,6–10], the only study of the permeability of the lysosomal membrane to amino acids and peptides has been done with intact macrophages[11].

The results reported in this paper demonstrate that the lysosomes of *T. pyriformis* are permeable to all the amino acids studied, although they are impermeable to all of the dipeptides, with the exception of glycyl-L-leucine.

MATERIALS AND METHODS

Sucrose and glycine (Analar) were supplied by Hopkin and Williams Ltd., Chadwell Heath, Essex. L-Alanine, D-alanine, L-valine, glycylglycine and some samples of glycyl-L-leucine were from British Drug Houses Ltd., Poole, Dorset. β-Glycerophosphate (Grade 1), Triton X-100 and all other amino acids and peptides were obtained from Sigma (London) Ltd., London, S. W. 6.

Growth and harvesting of cells

T. pyriformis, strain S, was grown in the dark, harvested in late log phase and disrupted by brief exposure to pH 11 at 0° in the manner previously described[5]. After preparation, homogenates were placed in ice and an aliquot was diluted with 4 vol. of an ice-cold 0.25 M solution of the amino acid or peptide which had been

adjusted to a pH between 6.3 and 7.3. At timed intervals thereafter, samples of the diluted homogenates were assayed for acid phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2) activity at 0° and pH 4.75. Assays were done in in triplicate in assay mixtures which contained 0.25 M sucrose in addition to the sucrose present in the homogenate[5]. In the determination of total activity, the assay mixture contained 0.05 % Triton X-100 and latency was defined as the percentage of the total activity unmasked by incorporation of Triton X-100 into the assay mixture. Permeability was assessed from the inability to prevent the release of acid phosphatase from previously intact lysosomes.

Chromatography of amino acids and peptides in homogenates

To determine whether there had been any degradation of the amino acids and peptides during the course of these experiments samples of the diluted homogenates were applied to sheets of Whatman No. 1 chromatography paper and developed using the solvent system *n*-butanol–acetic acid–water (65:10:25, by vol.).

Chromatographic behaviour and amino acid composition of glycyl-L-leucine

Paper chromatography with the solvent systems *n*-butanol–acetic acid–water (65:10:25, by vol.) and 80 % (w/v) phenol–0.3 % ammonia was used. Hydrolyses were in 5.7 M HCl at 105° for 18 h.

RESULTS

Studies were carried out at least twice with each of the compounds, and paper chromatography of the homogenate at the end of each experiment showed that there had been no substantial degradation of the amino acids or peptides.

Fig. 1 shows a comparison of the behaviour of the latency of acid phosphatase

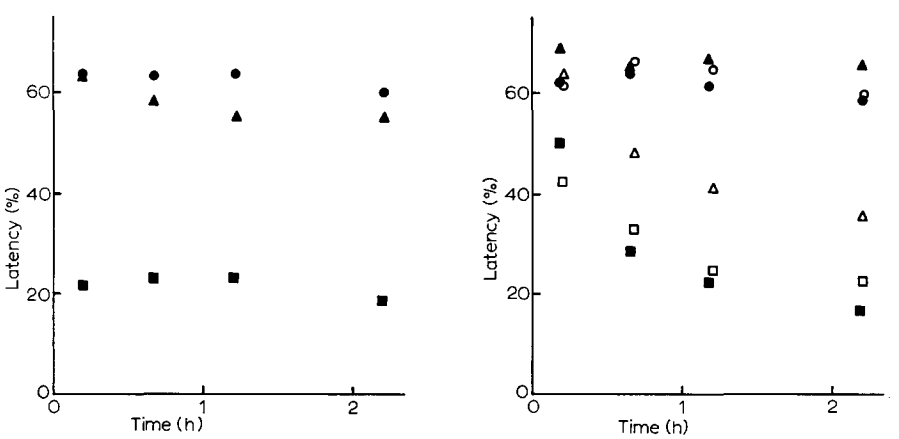

Fig. 1. Latency of acid phosphatase in an homogenate prepared with 0.25 M sucrose and diluted with sucrose (●), with glycine (■) and with glycylglycine (▲). The concentration of the diluting solutions was 0.25 M.

Fig. 2. Latency of acid phosphatase in two homogenates prepared with 0.25 M sucrose. 1. After dilution with sucrose (●), with L-alanine (■) and with L-valyl-L-leucine (▲). 2. After dilution with sucrose (○), with D-alanine (□) and with glycyl-L-leucine (△). The concentration of the diluting solutions was 0.25 M.

in a control sample of homogenate diluted with sucrose and two other samples diluted either with glycine or glycylglycine. It can be seen that the latency in the samples diluted with sucrose and glycylglycine behaved similarly and remained at a value of about 60 % throughout the subsequent 2-h period while that in the sample diluted with glycine had already reached a value of about 20 % within 15 min of dilution and it remained essentially unchanged over the next 2 h.

The decrease in latency of acid phosphatase after dilution with L-alanine is shown in Fig. 2 and it differs from that obtained with glycine in that the decay in latency occurred more slowly. Fig. 2 also shows that the response to dilution with D-alanine was similar to that found with L-alanine and, although the latency remained as high after dilution with L-valyl-L-leucine as with sucrose, dilution with glycyl-L-leucine (Sigma) resulted in progressively decreasing latency.

Fig. 3 demonstrates that dilution of an homogenate with L-valine resulted in decreasing latency similar to that found on dilution with alanine although the latency was well maintained after dilution with glycyl-L-alanine, L-alanylglycine or L-leucylglycine.

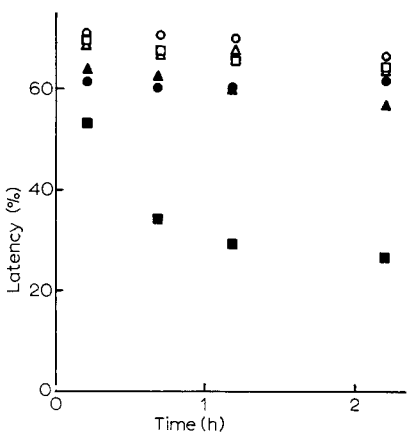

Fig. 3. Latency of acid phosphatase in two homogenates prepared with 0.25 M sucrose. 1. After dilution with sucrose (●), with L-valine (■) and with glycyl-L-alanine (▲). 2. After dilution with sucrose (○), with L-leucylglycine (□) and with L-alanylglycine (△). The concentration of the diluting solutions was 0.25 M.

DISCUSSION

Previous work on the digestive activity of lysosomes has usually contained the tacit assumption that macromolecules are degraded into smaller units which pass freely through the lysosomal membrane and, while it has long been known that small molecules can readily pass through the membrane of rat liver lysosomes[1–3], it has only recently become clear that the "cut-off" point for the passage of carbohydrates through the membrane of rat liver, macrophage and *T. pyriformis* lysosomes lies between monosaccharides, which are freely permeable, and disaccharides which are not readily permeable[4,5,12]. In view of the work of EHRENREICH AND COHN[11] it seemed likely that proteins in the lysosomes of *T. pyriformis* would have to undergo

extensive degradation in order that the products could pass through the lysosomal membrane, but it was not at all certain that the passage of dipolar amino acids and peptides would be similar to that of uncharged monosaccharides[5].

Fig. 1 shows that glycine passes through the membrane of *T. pyriformis* lysosomes just as rapidly as uncharged glycerol and dimethylsulphoxide molecules[5] although Figs. 2 and 3 indicate that the presence of a side-chain reduces the speed of passage of amino acids to a rate similar to that found with hexoses[5]. It is also shown in Fig. 2 that D-alanine passes through the lysosomal membrane just as readily as the stereoisomeric L-alanine and it appears unlikely that amino acids pass through the membrane by means of a specific transport system. Most probably they pass through by simple diffusion.

The results shown above indicate that the latency of acid phosphatase is as well maintained by glycylglycine, glycyl-L-alanine, L-alanylglycine, L-leucylglycine and L-valyl-L-leucine as by sucrose, and it would appear that the lysosomal membrane, although permeable to amino acids, is impermeable to dipeptides. However, Fig. 2 demonstrates that glycyl-L-leucine is unable to maintain the latency of acid phosphatase in spite of the fact that it is maintained by L-leucylglycine. This behaviour has been found consistently with glycylleucine from two commercial suppliers and neither the amino acid composition nor the chromatographic behaviour has given reason to doubt the identity of the products. It is possible that glycylleucine itself or some minor contaminant of the commercial preparations has a direct lytic action on the lysosomal membrane but, for the moment, it must be assumed that this peptide has the ability to traverse the lysosomal membrane.

The proteolytic system of *T. pyriformis* has received considerable attention from DICKIE AND LIENER[13,14] and they have identified one intracellular and two extracellular proteases from strain W. However, the fact that the two extracellular enzymes have lower molecular weights than the intracellular proteinase, and that all three have a common N-terminal histidine residue makes it appear that they are structurally related and it is possible that the extracellular proteinases are autolytic fragments produced from the intracellular proteinase. The substrate specificity of all three enzymes has been studied[14] and it has been found that they have a limited ability to digest the A and B chains of oxidised insulin as compared, for example, to the procine pepsins[15,16]. This fact, together with the observation that they were unable to digest a series of peptide substrates including glycylglycine and L-leucylglycine, makes it unlikely that the proteolytic system in strain W can digest proteins down to the level of amino acids. Hence, if the permeability of the lysosomal membrane in strain W is similar to that found for strain S, and if it is not radically altered by increased temperature, then it seems reasonable to predict the existence of intralysosomal peptidase(s) similar to that described in the lysosomes from rat liver[17]. However, it should be pointed out that the instability of the lysosomes of *T. pyriformis* in sucrose solutions at temperatures greater than about 10° (refs. 18, 19) could be due to the lysosomal membrane becoming more permeable to sucrose.

The amino acids and peptides used in this study have either hydrophobic side chains or none at all and the question arises as to the results which would ensue if the amino acids possessed ionised side-chains. Previous studies[5] have indicated that the presence of ionised groups slowed down the passage of ionised carbohydrate derivatives as compared to similar molecules which were uncharged and it seems

probable that a similar hindrance would be observed with amino acids and peptides possessing a net electrical charge.

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