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C_n microspheres as surrogate membranes in glycosidase-catalysed hydrolysis of glycolipids

José A. R. Martins, David H. G. Crout* and Peter Critchley
Department of Chemistry, University of Warwick, Coventry, UK CV4 7AL.
E-mail: d.h.g.crout@warwick.ac.uk

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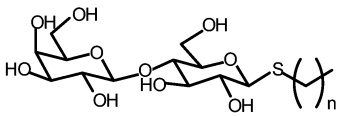
Glycosidase catalysed hydrolysis of glycolipids non-covalently attached to C_n microspheres proceeds to completion for appropriate glycolipid-microsphere combinations in contrast with hydrolysis of covalently immobilised analogues which in all cases studied stops significantly short of complete hydrolysis.

Glycolipids play important roles in many biological systems,[1] particularly in relation to adhesion phenomena. In an attempt to shed light on the behaviour of glycolipids in natural systems, we have studied aspects of their behaviour in an artificial system that acts as a surrogate for biological membranes, namely C_n microspheres. Although these cannot be claimed to serve as close models of biological membranes, they have certain properties in common which makes the study of the behaviour of glycolipids attached non-covalently to them relevant to the behaviour of membrane-associated glycolipids in nature.

The four lactose thioglycolipids **1–4** (Fig. 1)) were synthesised by standard methods.[2] These were deposited on either C_8 or C_{18} microspheres (silica gel 100 (Fluka), 0.015–0.035 particle size,



	n
1	7
2	9
3	11
4	17

Fig. 1

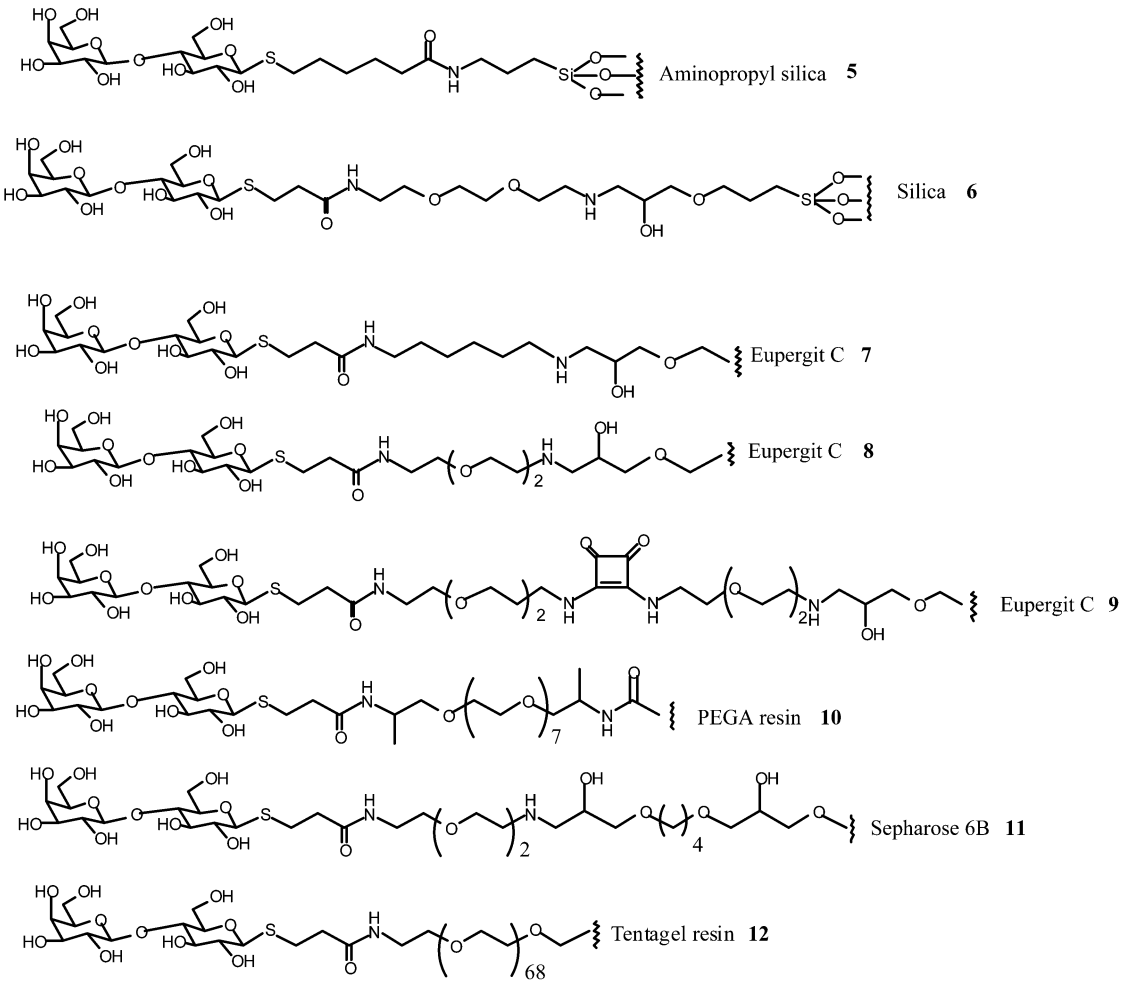


Fig. 2

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Table 2 Hydrolysis of glycolipids **1–4** non-covalently attached to Cn microspheres by the β-galactosidase from *Bacillus circulans*

	C_{18} silica			C_8 silica		
Glycolipid	Loading/ mmol g^{-1}	Hydrolysis time/h	% Hydrolysis	Loading/ mmol g^{-1}	Hydrolysis time/h	% Hydrolysis
1	0.14	20	100	0.042	21	100
2	0.20	20	37	0.084	21	100
2	0.20	74	88	—	—	—
3	0.14	20	8	0.16	21	100
3	0.14	74	33	—	—	—
4	0.05	74	20	0.039	90	31

Table 1 Hydrolysis of immobilised lactose derivativers **5–12** by the β-galactosidase from *Bacillus circulans*

Preparation	Loading/ mmol g^{-1}	Hydrolysis time/h	% Hydrolysis
5	0.28	117	< 5
6	0.26	117	5–10
7	0.50	91	56
8	0.46	91	37
9	0.25	91	35
10	0.33	48	50
11	0.20	50	51
12	0.20	140	< 5

surface coverage 17–18% C (C_{18}) or 10–12% C (C_8), fully end-cappped) by precipitation from methanolic solution with water. To probe the properties of the resulting systems it was proposed to study the hydrolysis of the thioglycolipids using a β-galactosidase. To provide a set of references that would permit comparison with related covalently-immobilised systems, the set of eight solid supported β-thiolactosides **5–12** (Fig. 2) were prepared. The presence of sulfur in these compounds served two purposes. From the C,H,N and S elemental analyses of derivatised and underivatised support the loading of β-thiolactoside could be calculated. Also, following enzymatic hydrolysis, cleavage of the carbohydrate component with mercuric trifluoroacetate released a mixture of lactose and glucose, from the ratio of which the extent of hydrolysis could be determined. In some cases, loadings were estimated from the amount of carbohydrate released by mercuric trifluoroacetate treatment. The lactose:glucose ratio was determined by HPLC using a light scattering (Sedex) detector.

The results of enzymatic hydrolysis of the covalently-linked materials are given in Table 1. The silica-based system **5** showed negligible hydrolysis after 117 hours. Since this result might have been attributed to the relative shortness of the linker with consequent steric inhibition of hydrolysis, the related system **6** with an extended linker was studied. However, this again showed very little hydrolysis after 117 hours. We next turned to Eupergit C (Rohm Pharma), a polyacrylamide-based material with pendant glycidyl groups. This material was derivatised so as to give linkers of increasing length and polarity (preparations **7–9**). With these, a significant degree of hydrolysis (56, 37 and 37% respectively) was observed after 91 hours. From the work of Norberg[3] who observed significantly increased activity in fucosylation with increased linker length in a solid-supported system, we anticipated that the degree of hydrolysis in our system might show a similar effect. However, linker length was clearly not related to degree of hydrolysis (Table 1). Indeed, if anything, the results show a small inverse correlation with linker length. PEGA resin and Sepharose were derivatised with extended hydrophilic linkers to give the materials **10** and **11**. In both cases, the results were comparable with those obtained with the Eupergit-based systems. Finally, Tentagel resin was derivatised with a long polyethyleneglycol (PEG) linker (**12**). However, the corresponding β-thiolactoside was resistant to hydrolysis. In all cases, the data reported in Table 1 represents the limiting extents of hydrolysis.

With the microsphere system (Table 2) strikingly different results were obtained. Two clear trends were obvious. The rate and degree of hydrolysis increased as the microsphere coating changed from C_{18} to C_8 and also increased with decreasing length of the lipid component. In both microsphere systems, complete hydrolysis could be achieved by using the appropriate combination of lipid chain and microsphere coating.

The results are particularly striking in relation to the behaviour of the silica-based covalently bound systems **5** and **6**. It is clear that with these systems, a significant proportion of the immobilised lactose (~ 50%) was not accessible to the enzyme. The complete reaction obtained with four out of eight microsphere systems (Table 2) suggests that even if some material is bound in inaccessible regions of the silica, diffusion over the surface eventually brings much or all of the material to locations where hydrolysis can take place. A possibility considered was that hydrolysis, at least in some cases, was taking place on substrate that had diffused away from the microsphere surface. When the microspheres were washed with aqueous buffer before exposure to the enzyme, no leaching of any material from the C_{18} microspheres was observed and only traces from the C_8 microspheres. To test the possibility that hydrolysis might be taking place on material that had diffused into solution, experiments were carried out in which the microspheres were contained in a dialysis bag and the enzyme was introduced into the outer compartment. Under these conditions no hydrolysis was observed. This indicates that diffusion away from the microsphere surface does not play a part in the hydrolysis process.

The reduction in hydrolysis as the hydrophobicity (chain length) of both microsphere coating and glycolipid increases probably reflects the kinetic consequence of the increased rates of diffusion on the more hydrophobic surface and the increase in the hydrophobicity of the glycolipids. Parallel results have been reported with respect to the diffusion of lipopeptides in biological membranes, where the rates of diffusion correlated with the hydrophobicity of the lipid components of the peptides.[4] To this extent, the microsphere system appears to exhibit behaviour similar to that of biological membranes.

The glycolipids used in this study could be recovered completely from the microspheres by washing with methanol. The system therefore has clear potential for synthetic applications. It can be predicted that lipidated peptides and oligonucleotides, for example, would exhibit similar behaviour. The system clearly also has potential for various types of affinity chromatography.

Notes and references

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