Molecular recognition on giant vesicles: coating of phytyl phosphate vesicles with a polysaccharide bearing phytyl chains†

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The molecular recognition between phytyl phosphate giant vesicles and a polysaccharide (pullulan) bearing phytyl or cholesteryl groups and a fluorescent tag was investigated; the pullulan bearing phytyl chains did coat the surface of the vesicles, in contrast with the pullulan bearing cholesteryl groups.

We have postulated that polyprenyl phosphates possibly formed the most primitive vesicles.[1] We have also suggested plausible processes for 'primitive' vesicles becoming more complex 'proto-cells'.[2,3] We have now studied whether giant vesicles (5 μm or more)[4] made from single-chain polyprenyl phosphates[5] could be coated by a polysaccharide (pullulan, MW ~55000 Da) bearing hydrophobic polyprenyl chains (Fig. 1). This would lead to an assembly somewhat reminiscent of the cell wall of microorganisms. To the polysaccharide was also covalently attached a fluorescent tag, to make it possible to clearly observe coated vesicles with optical fluorescence microscopy. We have recently reported that POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) vesicles can indeed be coated with pullulan, to which had been linked cholesteryl groups (FITC-CHP, 1.17

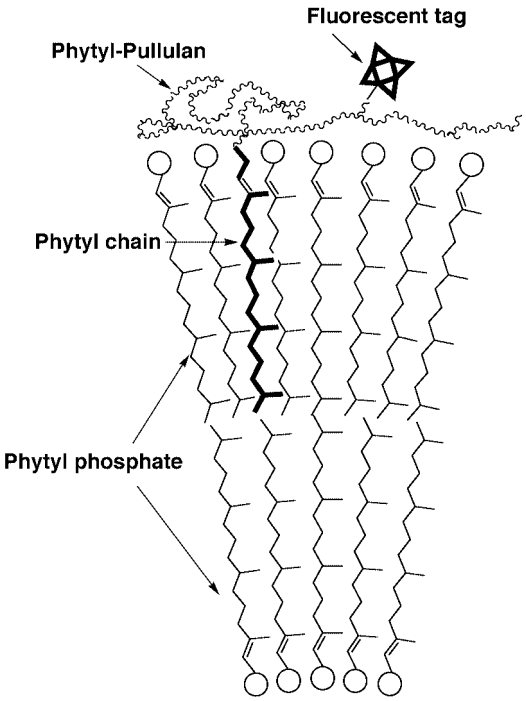
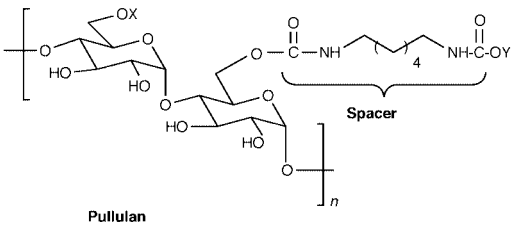
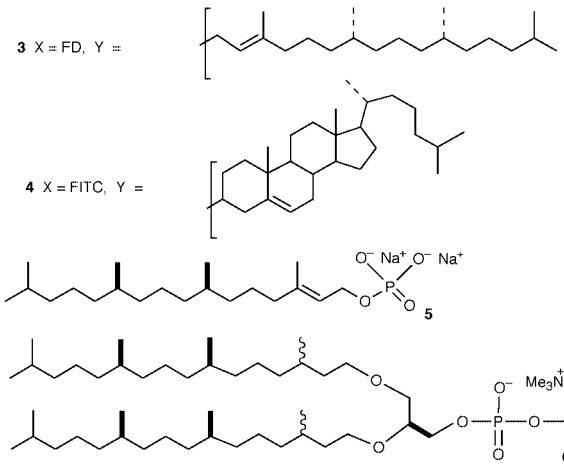


Fig. 1 Schematic representation of the coating of phytyl phosphate giant vesicles by phytyl-pullulan carrying fluorescent tags.

† Synthetic and spectroscopic data are available as supplementary data from the RSC web site, see http://www.rsc.org/suppdata/cc/a9/a908827j/



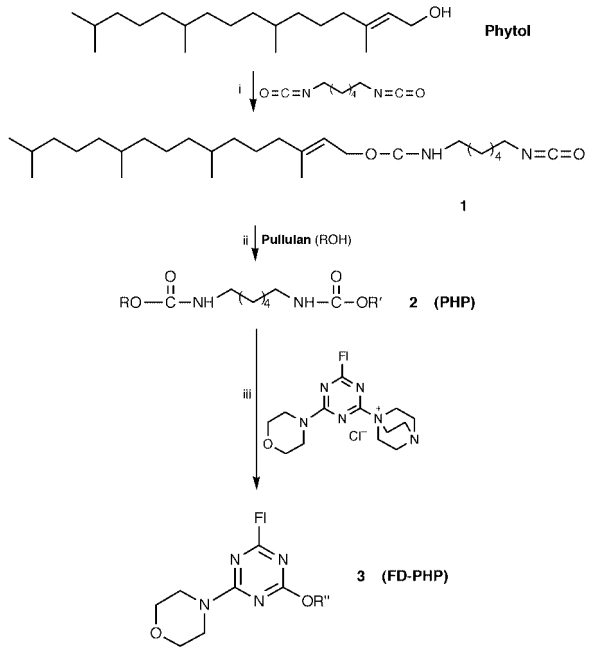
Pullulan



cholesteryl groups per 100 monosaccharide units) **4** (Scheme 1).[6] The cholesteryl groups of this 'cholesteryl-pullulan' insert themselves spontaneously into the membrane, in agreement with the fact that cholesterol itself is a widespread reinforcer of eucaryotic membranes, thereby spreading and anchoring the polysaccharide on the outer surface of the vesicles.

We first attempt to coat giant vesicles of phytyl phosphate **5** or of 2,3-diphytanyl-*sn*-glycero-1-phosphocholine (DPhPC) **6**, an archæal membrane constituent,[7] with 'cholesteryl-pullulan' **4**. This was unsuccessful, and cholesterol did *not* insert into either the monopolyprenyl phosphate or the dipolyprenyl phosphatidylcholine membranes. This was somewhat surprising, as cholesterol and phytol have similar amphiphilicity and overall shape. The non-incorporation of cholesteryl moiety does not depend on the nature of the head-group and must depend on the texture of the lipid part of the membrane. Indeed, Yamauchi *et al.*[8,9] have shown, by leakage studies, that diphytanyl phosphatidylcholine **6** membranes are relatively more rigid than those made of *n*-acyl lipids, which they explained by the lateral interdigitation of the phytanyl chains, which could also exclude the cholesteryl groups.

We then synthesized a novel pullulan derivative, replacing the cholesteryl moiety of (FITC-CHP) **4** by a phytyl chain (FD-



Scheme 1 *Reagents and conditions*: i, toluene–pyridine, 60 °C, 4 days, 100%; ii, DMSO–pyridine, 75 °C, 3 days, 55%; iii, rt, 24 h, 45%. ROH = pullulan, R′ = phytyl, R″ = hydrophobized pullulan (PHP), Fl = fluorescamine unit.

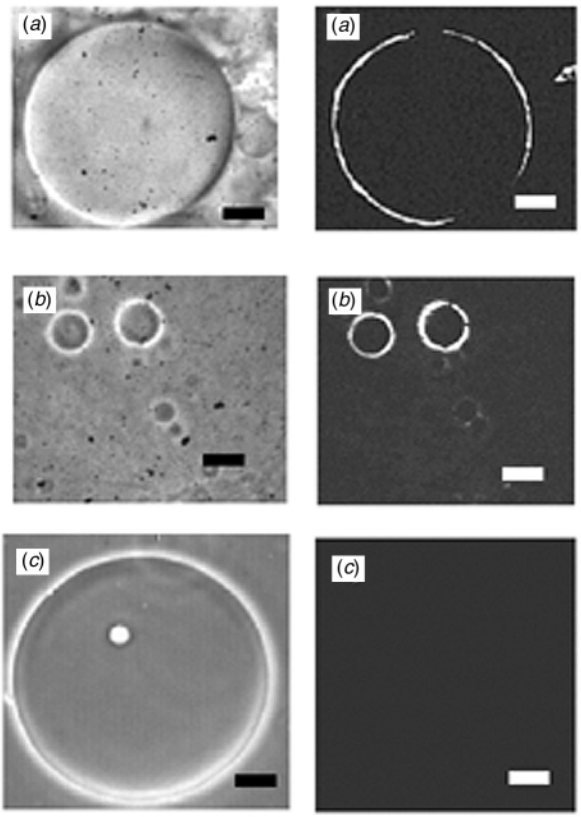


Fig. 2 Phase contrast image (left) and the corresponding fluorescent microscopic image (right) of coating (or not) of the surface of the preformed giant vesicles: (*a*) coating of phytyl phosphate vesicles by phytyl-pullulan (FD-PHP) **3**; (*b*) coating of diphytanyl phosphatidylcholine vesicles by phytyl-pullulan (FD-PHP) **3**; (*c*) absence of coating of phytyl phosphate vesicles by cholesteryl-pullulan (FITC-CHP) **4**. Bar: 10 μm.

PHP) **3**; this should of course be compatible with bilayers of phytyl phosphate **5** and permit the coating process. The synthetic route to obtain the fluorescent 'phytyl-pullulan' (FD-PHP) **3** is shown in Scheme 1. The phytyl moiety was linked to pullulan through an α,ω-dicarbamoylhexyl spacer, affording quantitatively a half-carbamate **1**, which was condensed with pullulan to produce the 'phytyl-pullulan' (PHP) **2** (55%). Finally, PHP was coupled to a morpholinyltriazine labelled with fluorescamine, followed by purification on a Sepharose gel, to yield a pure 'phytyl-pullulan' labelled with fluorescamine **3** FD-PHP (45%); content of phytyl groups: 2.9 per 100 glucose units; fluorescamine: 0.8 per 1 PHP molecule).‡

In contrast to the experiments with 'cholesteryl-pullulan', the entire outer surface of giant vesicles composed of phytyl phosphate **5** or DPhPC **6** could easily be coated with 'phytyl-pullulan' **3** (Fig. 2). Control experiments carried out with 'FITC-pullulan' (pullulan bearing the fluorescent tag but no hydrophobic moiety) confirmed that the coating of vesicles required the hydrophobic chains. Confocal microscopy showed that FD-PHP **3** spreads only on the surface of vesicles (not shown).

These results indicate that the criteria for efficient insertion of a lipophilic anchor into a bilayer are more stringent than we had initially expected; a near identical structure of the bilayer phospholipids and of the anchoring chains is required, or else the fit must be as closely adapted as is the case for cholesterol and *n*-acyl lipids.[10]

The recognition of a 'phytyl-pullulan' by a membrane of phytyl phosphate **5** or of diphytanyl phosphatidylcholine **6** is an interesting example of solubility guided by close structural similarity.

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Notes and references

‡ Optical, fluorescence and confocal microscopies were carried out according to the procedures described in refs. 11–14. To coat the surface, it is sufficient to use oligosaccharide carrying 3% only of the stoichiometric amount of lipophilic chains.

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