Short communication

Enzymatic Digestion of Liposome-Bound Polysaccharides: Evidence of Bridging Mechanism

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Abstract. Laminarin, a water-soluble polysaccharide is known to induce aggregation and fusion of liposomes. Using laminarin specific lysing enzymes for multilamellar liposomes their aggregation was found to be fully reversible, in contrast to small unilamellar liposomes for which irreversible fusion is the main process. Moreover, our results indicate that these processes are probably mediated by the formation of polysaccharide cross-bridges between adjacent liposomes.

Key words: Polysaccharides — Aggregation — Liposomes — Lysing enzymes digestion — Bridging mechanism

The majority of cell surface receptors involved in antigen recognition by T cells and in the orchestration of the subsequent cell signalling events are glycoproteins. The length of a typical N-linked sugar is comparable with that of an immunoglobulin domain (3 nm). Thus, by virtue of their size alone, oligosaccharides may be expected to play a significant role in the functions and properties of cell surface proteins to which they are attached. Unfortunately, the biological role of polysaccharides on cell surfaces is not fully understood (Sverremark 1998; Rudd et al. 1999).

Interactions between lipid vesicles (liposomes) continue to be studied as simplified models for cellular and subcellular adhesion, aggregation, and fusion processes (Kang et al. 1994; Meyuhas et al. 1996; Babincová and Machová 1997, 1999; Hianik et al. 1999; Hincha et al. 2000). Liposomes are not only a suitable model for the understanding of these fundamental biological processes but polysaccharide-liposome complexes are also very promising for the treatment of cancer (Vodovozova et al. 1998; Miao et al. 1999; Yamamoto et al. 2000).

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Another system used to study the influence of polysaccharides are red blood cells (RBC) which, in the presence of high molecular weight polymers, aggregate to form rouleaux and rouleaux networks (Chien and Jan 1973). As a mechanism of aggregation the formation of bridges between the cells mediated by the polymer molecules (the bridging mechanism) has been proposed (Chien et al. 1983; Barshtein et al. 1998).

Our study is focused on laminarin induced aggregation and fusion of multilamellar (MLL) and small unilamellar (ULL) liposomes and on the influence of lysing enzymes on polysaccharide-liposome complexes.

To prepare MLL, soybean phosphatidylcholine (Sigma, USA) was dissolved in a mixture of chloroform and metanol (2:1 v/v) and this lipid solution was evaporated in a rotational evaporator. After evaporation of the solvent, 50 mmol/l sodium citrate buffer, pH 5.3 (Radelkis, Hungary), was added into the retort with the lipid film (final lipid concentration 5mg/ml) and the solution was intensively shaken mechanically for 10 min. Small unilamellar liposomes were prepared by one hour sonication of MLL suspension in ultrasound bath.

The relative extent of aggregation and/or fusion of liposome suspension, was quantified by measuring the increase of decadic turbidity given as $-(1/L)\log(I/I_\mathrm{o})$, where L is the optical path length, I_o and I are intensities of light transmitted through pure liposomes and liposomes after the addition of laminarin, respectively, measured at 600 nm on a Specol 210 spectrophotometer (Carl Zeiss Jena, Germany). The turbidity was recorded 2 min after changing laminarin (molecular weight 6,000) (Sigma, USA) concentration.

Addition of the polysaccharide laminarin to MLL or ULL suspension leads immediately to an increase of turbidity (Figure 1). There are two possible processes responsible for this effect, namely aggregation and fusion of liposomes. To distinguish between them lysing enzymes from *Trichoderma harzianum* (Sigma, USA), having hydrolytic specificity to polysaccharides, were added to MLL- and ULL-laminarin complexes. *Trichoderma harzianum* is a soil-borne filamentous fungus capable of parasitizing several plant pathogenic fungi. Secretion of lysing enzymes, mainly glucanases and chitinases, able to hydrolyse the glycosidic linkage in polysaccharides, is considered as the crucial step of the mycoparasitic process. The pH optimum for these enzymes is 5.3 and maximum activity is ~ 3 U/ml (Noronha and Ulhoa 2000).

Two different scenarios were observed. For MLL, the enzymatic digestion of the polysaccharide resulted in a change from initial decadic turbidity of 1.64 cm^{-1} to almost the same turbidity of MLL suspension after 143 min as before the addition of laminarin (0.34 cm^{-1}); laminarin thus promoted completely reversible aggregation of MLL. For ULL, addition of lysing enzymes lead to just a slight decrease of turbidity (from 0.72 cm^{-1} to 0.48 cm^{-1}), suggesting that in this case irreversible fusion of ULL prevailed.

Two conclusions can be drawn from this study: Firstly, laminarin is able to promote fusion of ULL but is able only to induce MLL aggregation. Secondly, the aggregation of MLL proceeds probably via polysaccharide bridges between the indi-

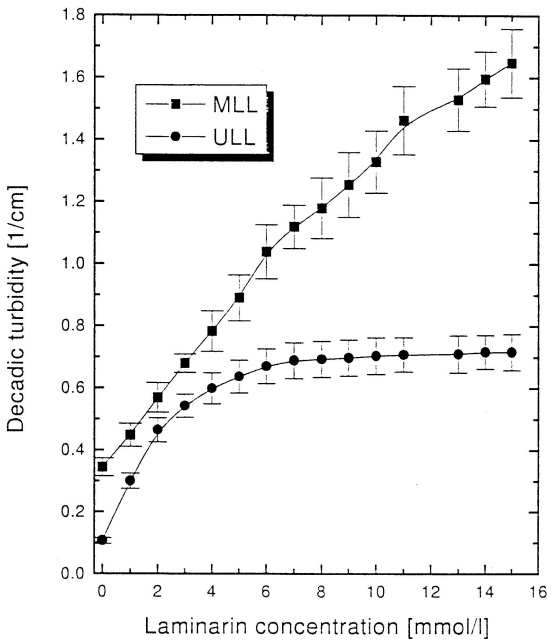

Figure 1. Decadic turbidity of multilamellar (MLL) and small unilamellar (ULL) phosphatidylcholine liposomes as a function of the added laminarin concentration. Each data represents the average from three independent experiments ± calculated standard deviations.

vidual MLL, where polysaccharides are present in the interliposome space and the terminal of them cross-bridge adjacent liposome surfaces, in analogy to the formation of RBC rouleaux networks, and after polysaccharide digestion, non-aggregated liposomes' initial state is recovered. Our results are in agreement with previous related studies of polysaccharide-liposome interactions (Minetti et al. 1979; Sunamoto et al. 1980; Iwamoto and Sunamoto 1982; Babincová and Hianik 1994). In our preliminary studies using the phase contrast microscopy technique we also observed disintegration of the laminarin induced RBC rouleaux upon addition of lysing enzymes (work in progress). Thus, the results presented herein are supportive of the bridging mechanism for the aggregation of both red blood cells and liposomes.

Possibly, the attractive interactions between polysaccharides and liposomes are based on hydrogen bonds, although other types of interaction may also be important. For example, it has been found recently that monosaccharides can expose a significant area of their hydrophobic surface in aqueous solution (Sigurskjold and Bundle 1992). For glucose, the ratio of the difference between the heat capacity of the solid state and the partial molar heat capacity of an infinitely diluted aqueous solution (ΔC_p^o), to the exposed non-polar surface area (ΔA_{np}), is -1.12 J mol^{-1} K^{-1}Å^{-2}. This figure is of the same sign and approximately the same value as that for the transfer of hydrocarbons in water at infinite dilution to the liquid state ($\Delta C_p^o/\Delta A_{np} = -1.05 \pm 0.13$ J mol^{-1} K^{-1}Å^{-2}), processes which are dominated by hydrophobic interactions.

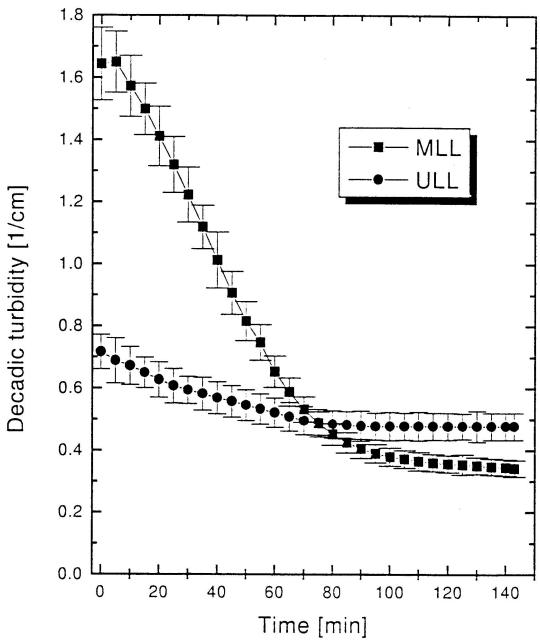

Figure 2. Time dependence of the decadic turbidity of polysaccharide-liposome complex upon the addition of lytic enzymes. Each data represents the average from three independent experiments ± calculated standard deviations.

The results obtained may be of potential importance also for the electric pulse mediated cell fusion, where polysaccharides, e.g. dextrans, are used as mediators of cell adhesion as a prerequisite of fusion, without the understanding of their role in this process (Jiang and Berg 1995).

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