Supramolecular architectures based on lipopeptides and liposaccharides*

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Lipopeptides and liposaccharide macromonomers with acrylamide, methacrylamide, acrylate and methacrylate polymerizable groups have been synthesized and polymerized. Depending upon the molecular characteristics of the macromonomers, lamellar (smectic), cylindrical and nematic architectures have been obtained and characterized by X-ray diffraction. The utilization of these systems as models of biological systems and their biomedical applications are discussed.

(Keywords: lipopeptides; liposaccharides; comb polymers; liquid crystals; biomimetics)

INTRODUCTION

Biomembranes are very complicated systems[1,2], therefore the understanding of physicochemical processes that govern the stability and the behaviour of biomembranes entails the use of models.

The biomembrane can be divided into three layers[2,3]: glycocalix, protein–lipid bilayer and cytoskeleton (*Figure 1*). The middle layer — the lipid bilayer containing proteins — is coated on the outside by a carbohydrate-rich layer, the glycocalix. It consists mainly of oligosaccharide head groups of glycolipids and of oligosaccharide chains of glycoproteins. The carbohydrate chains at the cell surface act as antennae and govern the relations between neighbouring cells and between cells and the external medium. The carbohydrate chains of glycoproteins are receptor sites for viruses, proteins, enzymes and hormones. They are antigenic determinants in reactions of the immune system. They play an important part in intercellular adhesion and recognition and in cell contact inhibition[2,4]. In animal cells, stabilization of the cell membrane is mainly achieved by the cytoskeleton which is linked to the inner side of the central lipid bilayer[2,3].

The functions and properties of biomembranes and of their main constituents are based on a combination of molecular mobility and high ordering. Such combinations of properties are typical of liquid crystalline behaviour, so mesophases are good models for the study of biomembranes and their main constituents.

As the basis of our liquid crystalline systems, we have chosen amphiphilic molecules of biological interest; that is to say polymerizable lipopeptides and liposaccharides of general formulas:

$$H_2C{=}CH{-}Y{-}(CH_2)_n{-}Z{-}W$$

or

$$H_2C{=}C(CH_3){-}Y{-}(CH_2)_n{-}Z{-}W$$

where W = amino acid, peptide or saccharide, Y = NH–CO, CO–NH or COO and Z = NH, CO or $N(CH_3)$.

These molecules consist of a polymerizable group, $H_2C{=}CH{-}CO$ or $H_2C{=}C(CH_3){-}CO$, a hydrophobic lipid chain, $(CH_2)_n$, and a hydrophilic moiety, W, formed by an amino acid (glycine, alanine, serine, thyrosine, aspartic acid or glutamic acid), a peptide (polysarcosine) or a saccharide (*N*-methylglucamine, glucose, aminoglucose or aminogalactose).

In this paper we will first recall the principle of the synthesis and polymerization of the polymerizable lipopeptides and liposaccharides and then describe their properties.

EXPERIMENTAL

Synthesis of macromonomers

Polymerizable lipo-amino acids, lipopeptides and liposaccharides were synthesized as described elsewhere[5–10].

Structural determination

The structure of comb-like polymers was studied by low angle X-ray scattering in a Guinier-type camera operating under vacuum. Strictly monochromatic X-rays were used (CuKα1), isolated by a bent quartz monochromator and focused as a sharp line on the film. The camera was equipped with a device for recording the diffraction patterns from samples held at various temperatures between 25 and 200°C with an accuracy of 1°C.

Samples for X-ray studies were prepared as described elsewhere[5–10].

RESULTS

Synthesis

Synthesis of macromonomers. To synthesize polymerizable lipopeptides or liposaccharides, the starting materials have to be α,ω-bifunctional lipids with a functional group to link the polymerizable group and a functional group to link the peptidic or saccharidic chain.

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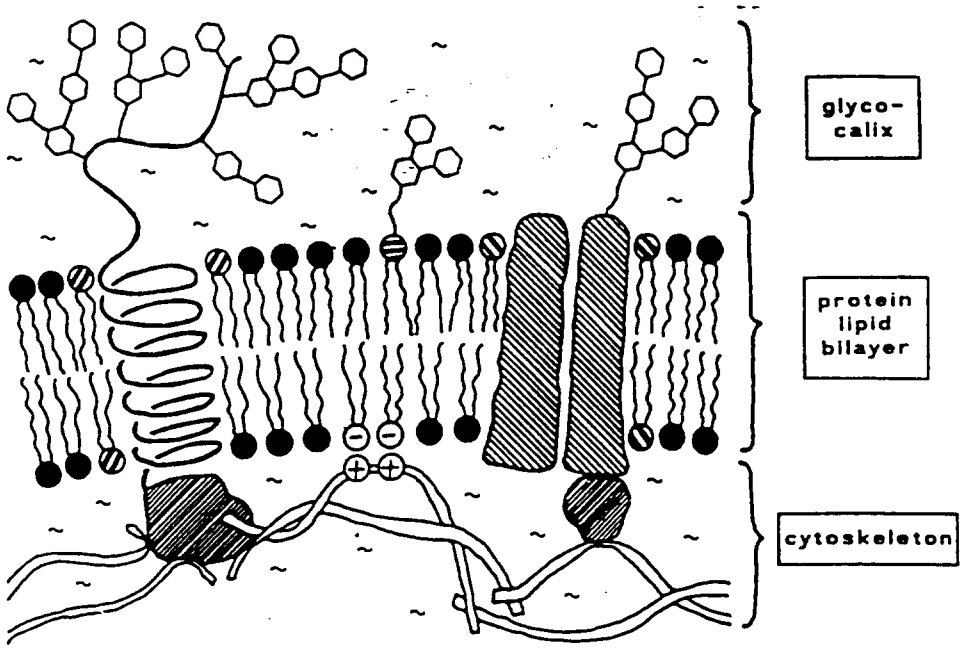

Figure 1 Schematic representation of a biomembrane (adapted from references 2 and 3)

We used two types of bifunctional lipids: α,ω-diamine and α,ω-amino acids.

The α,ω-fatty diamines allow the fixation of the polymerizable group at their α-amino end, by action of a vinyl acid chloride. At their ω-amino end, initiation of polymerization of the *N*-carboxyanhydride of an amino acid or the fixation of a sugar lactone[5–7,9,10] takes place. The α,ω-fatty amino acids allow the fixation of the polymerizable group at their α-amino end, by action of a vinyl acid chloride, and the fixation of an α-amino acid or an amino sugar at their ω-carboxylic acid end[8,9].

Polymerization of the macromonomers. Macromonomers were transformed into comb-like polymers by radical polymerization in solution in different solvents (the nature of the solvent is determined by the solubility of the macromonomer). The polymerization was carried out at 60°C, using azobis(isobutyronitrile) as initiator[5–10].

In the case of lipo-amino acid macromonomers, at the end of the polymerization the carboxyl groups of the amino acids were transformed into sodium salts by the addition of sodium hydroxide.

Gel permeation chromatography performed in aqueous solution, using polyoxyethylene standards for calibration, gave polymerization degrees between 70 and 100 and a polydispersity index of less than 1.5.

Liquid crystalline properties

Comb-like polymers with lipopolysarcosine, lipo-amino acid or liposaccharide side chains exhibit mesophases in the dry state and in water solution for water concentrations less than about 50%.

We determined the phase diagram temperature/water concentration of comb-like polymers by differential scanning calorimetry (d.s.c.) and X-ray diffraction. D.s.c. allowed determination of the transitions between phases and thus determination of the domain of existence of the mesophases; X-ray diffraction allowed determination of the structure of the mesophases[5].

Phase diagram. Figure 2 gives an example of phase diagram temperature/water concentration obtained for polymers with polyacrylamide main chains and lipopolysarcosine side chains. It illustrates the fact that our polymers are both thermotropic and lyotropic. The smectic *A* phase (S_A) observed for the dry polymer at room temperature transforms into a nematic (*N*) phase at temperatures higher than 90°C and into an isotropic phase at temperatures higher than about 150°C, illustrating the thermotropic behaviour. The addition of increasing amounts of water to the smectic *A* structure of the dry polymer at first swells the smectic structure and then transforms it into a nematic one, illustrating the lyotropic behaviour.

Structural determination. X-ray diagrams obtained with comb-like polymers can be classified into three families corresponding to three types of liquid crystalline structures.

The first family of X-ray diagrams is characterized by the presence in the low-angle region of two or three sharp reflections with reciprocal spacings in the ratio 1, 2, 3, and by the presence in the wide-angle region of a diffuse band. They are characteristic of a lamellar smectic *A* structure[5].

The second family of X-ray diagrams is also characterized by the presence in the wide-angle region of a diffuse band and in the low-angle region of a set of sharp reflections, but their reciprocal spacings are in the ratio 1, $\sqrt{3}$, $\sqrt{4}$, $\sqrt{7}$, $\sqrt{9}$, characteristic of a hexagonal structure[8].

The third family of X-ray diagrams is characterized by the presence in the low-angle region of a diffuse band and by the presence in the wide-angle region of a diffuse band, characteristic of a nematic mesophase[5].

Description of the structures. The repeat unit of the comb-like polymers can be divided into two parts: the hydrophilic part $A=Z-W$, and the hydrophobic part $B=CH_2-CH-Y-(CH_2)_n$ or $CH_2-C(CH_3)-Y-(CH_2)_n$.

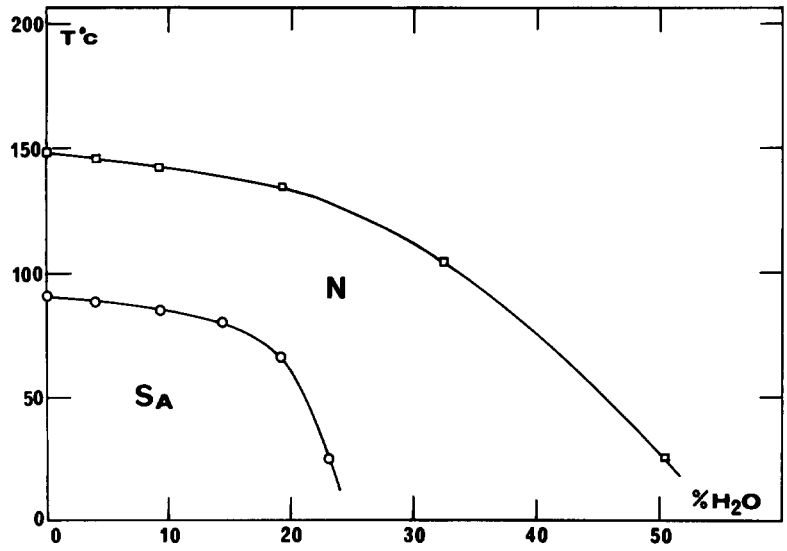

Figure 2 Phase diagram. Temperature/water concentration of a comb like polymer with polyacrylamide main chain and lipopolysarcosine side chains

These two parts are incompatible, tend to segregate in space and give rise to a phase separation at the molecular level. So we can describe the lamellar and hexagonal structures as follows.

The lamellar structure consists of plane, parallel, equidistant sheets. Each sheet, of thickness d, results from the superposition of two layers: one, of thickness d_A, contains the peptidic or saccharidic hydrophilic chains and the water, while the other, of thickness d_B, contains the polymer main chain and the lipidic side chains (*Figure 3*).

The hexagonal structure consists of long, parallel cylinders of radius R, assembled in a hexagonal array of parameter D. The cylinders are filled with the polymer main chain and the lipidic side chains, while the space between the cylinders is occupied by the peptidic or saccharidic hydrophilic chains and the water (*Figure 4*).

The lattice parameters d for the lamellar structure and D for the hexagonal structure were obtained directly from the X-ray patterns. The other parameters — d_A, d_B, R and S_L and S_H (the average surface occupied by a chain at the interface for the lamellar and hexagonal structures, respectively) — were obtained using the following formulae based on simple geometric considerations:

$$d_B = d\left[1 + \frac{CX_A V_A + (1-C)V_S}{C(1-X_A)V_B}\right]^{-1} \quad (1)$$

$$d_A = d - d_B \quad (2)$$

$$S_L = 2M_B V_B / N d_B \quad (3)$$

$$R^2 = \frac{D^2\sqrt{3}}{2\pi}\left[\frac{1 + CX_A V_A + (1-C)V_S}{C(1-X_A)V_B}\right]^{-1} \quad (4)$$

$$S_H = 2M_B V_B / NR \quad (5)$$

where C is the weight content of comb-like polymer in solution, X_A is the weight fraction of hydrophilic peptidic or saccharidic chains in the polymer, V_A is the specific volume of the hydrophilic moiety A, V_B is the specific volume of the hydrophobic moiety B, V_S is the specific volume of the water, M_B is the molecular weight of the hydrophobic moiety and N is the Avogadro number.

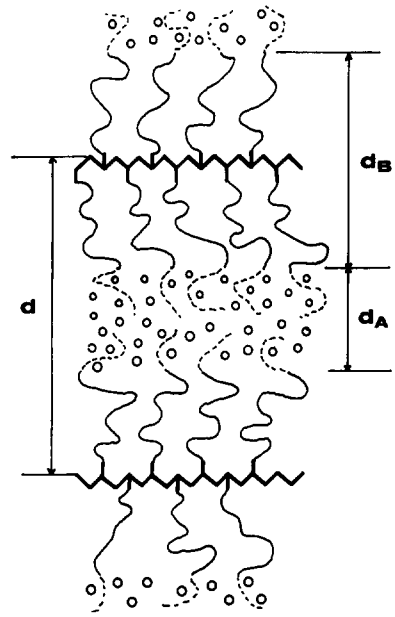

Figure 3 Schematic representation of the lamellar structure: ⋀⋀, polyvinyl main chain; —, paraffinic chain; - - - -, hydrophilic moiety (amino acid, peptide or saccharide); OOO, water

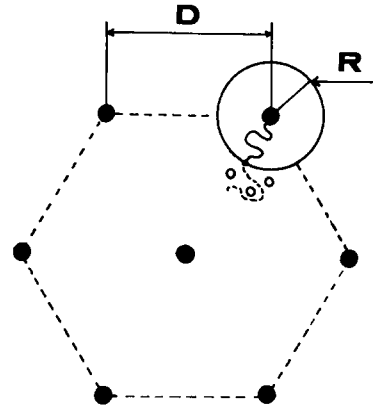

Figure 4 Schematic representation of the hexagonal structure. Notation as in *Figure 3*

Influence of the water concentration

The water concentration has an influence on the nature of the mesophases and on the values of their structural parameters.

Influence on the nature of the mesophase. As in the case of block copolymers[11], the type of structure is determined by the ratio of the volumes of the hydrophilic and hydrophobic domains. The addition of water to comb-like polymers increases the relative volume of the hydrophilic domains and is therefore able to transform a lamellar structure into a cylindrical hexagonal one. This transformation has been observed for the following amino acids: aspartic acid, glutamic acid and tyrosine[8,12].

Influence on the structural parameters. To illustrate the influence of the water concentration on the structural parameters of the mesophases, we have plotted in *Figure 5* the variation of the parameters of the lamellar and hexagonal structure of comb-like polymers with polyacrylamide main chain and lipo(glutamic acid sodium salt) side chains.

One can see that the increase of the water concentration involves a discontinuity in the variation of the structural parameters at the transition between the lamellar and the hexagonal structure, but within the domain of stability of each mesophase the structural parameters vary in a continuous manner with the water concentration.

For the lamellar structure, when the water concentration increases, the total thickness d of a sheet, the thickness d_A of the hydrophilic layer and the average surface S_L per chain at the interface all increase, whereas the thickness d_B of the hydrophobic layer decreases as the hydrophobic chains have to maintain a constant density.

For the hexagonal structure, when the water concentration increases, the distance D between the cylinders and the average surface area S_H both increase, whereas the diameter $2R$ of the hydrophobic cylinders decreases.

CONCLUSION

We have shown that comb-like polymers with polyvinyl main chains the lipo-amino acid, lipopeptide or lipo-saccharide side chains exhibit liquid crystalline properties.

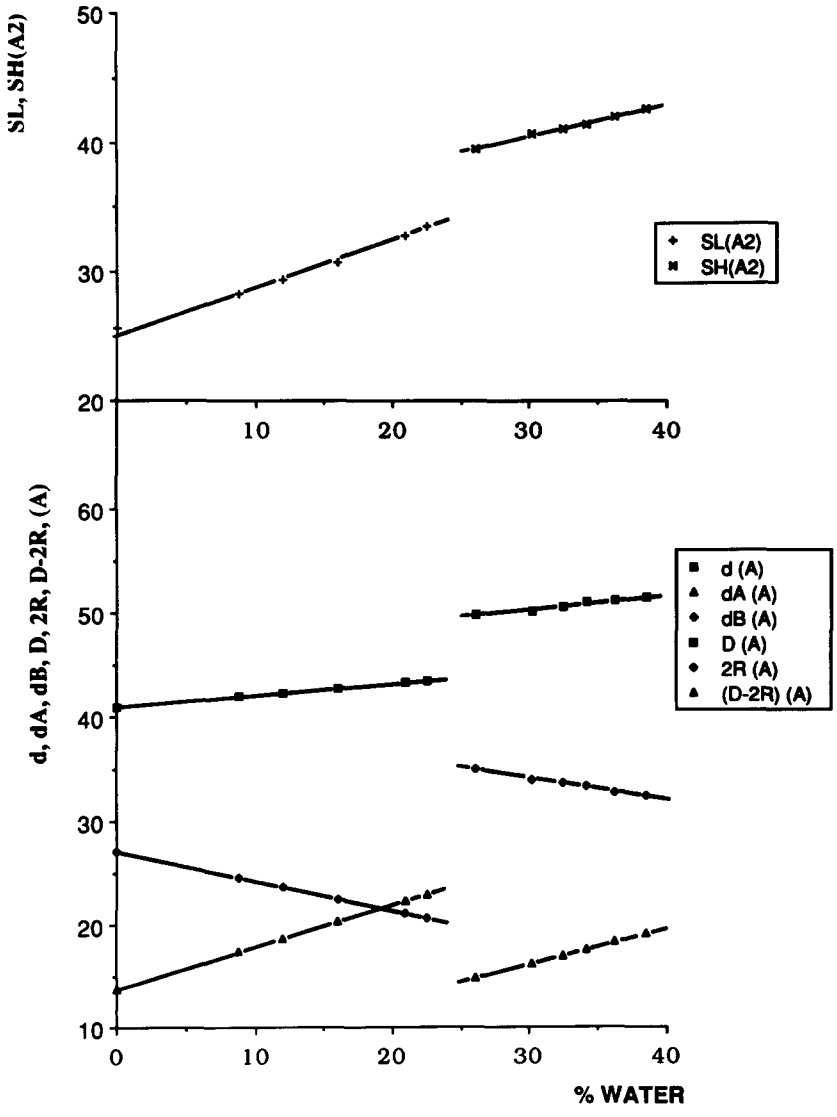

Figure 5 Variation with water concentration of the structural parameters of the lamellar and hexagonal structures exhibited by the comb-like polymer with polyacrylamide main chain and lipoglutamic acid sodium salt side chains

The nature of the mesophases (lamellar, cylindrical hexagonal or nematic) and the number of mesophases (one or two) are mainly determined by the nature of the hydrophilic part of the side chains. For example in the case of comb-like polymers with polyacrylamide main chain one observes: (i) only a lamellar structure when the amino acid is glycine, alanine or serine and when the sugar is *N*-methylglucamine, glucose or aminoglucose[8,9]; (ii) a lamellar and a cylindrical hexagonal structure when the amino acid is tyrosine, aspartic acid or glutamic acid[8,12]; (iii) lamellar and nematic structures when the hydrophilic chains are formed of polysarcosine[5,6]; (iv) a nematic structure when the sugar is aminogalactose[9].

How can we go further in biomimetics with these systems? Some years ago we synthesized models of glycoproteins formed by carbohydrate chains of glycoproteins covalently linked to a hydrophobic peptidic[13], lipidic[14–17] or phospholipidic[17,18] moiety. The carbohydrate chains of hen egg white ovomucoid[19,20], hen egg ovotransferrin[21] and human serrotransferrin[22] were obtained by enzymatic degradation of glycoproteins[23] followed by separation and purification by column chromatography. Finally they were linked to polymerizable or non-polymerizable fatty acid chains or to phosphatidyl-ethanolamine with fatty acid chains[14–18]. The carbohydrate chains were formed of a rigid core of three saccharide residues (one mannose and two *N*-acetylglucosamine)[4,24] and antennae containing neuraminic acid, galactose, *N*-acetylglucosamine and mannose for serrotransferrin[25], galactose, *N*-acetylglucosamine and mannose for ovomucoid and asialo-serrotransferrin[4,24,25] and *N*-acetylglucosamine and mannose for ovotransferrin[26], the antennae being bound to the rigid core by mannose–mannose linkages[4,24].

With these models we establish the existence of two conformations (a 'T' and a 'Y' conformation) for the carbohydrate chains of glycoproteins (*Figure 6*) and different ways to induce conformational changes by rotation of the antennae around the mannose–mannose linkages[13,15–18].

We also introduce these liposaccharides into phospholipid multilayers (*Figure 7*) and showed the existence of two conformations (a 'T' and a 'Y' conformation) for their glycoprotein carbohydrate chains[27].

In the near future we will prepare liposomes of mixtures of glycoprotein-derived liposaccharides and polymerizable lipo-amino acids with two hydrophobic chains.

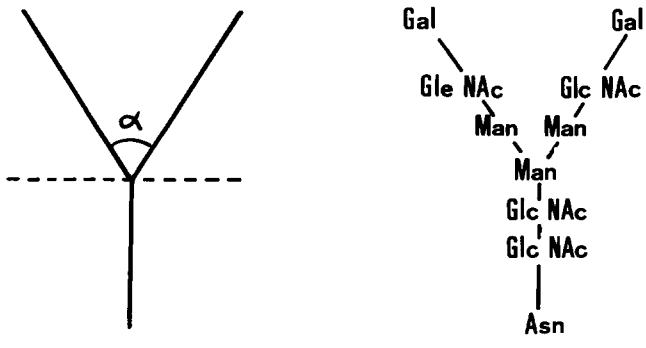

Figure 6 Schematic representation of the carbohydrate chain of asialo-serrotransferrin in the 'Y' conformation: right and left (full line); for the 'T' conformation: left, $\alpha = 180°$ (dotted line)

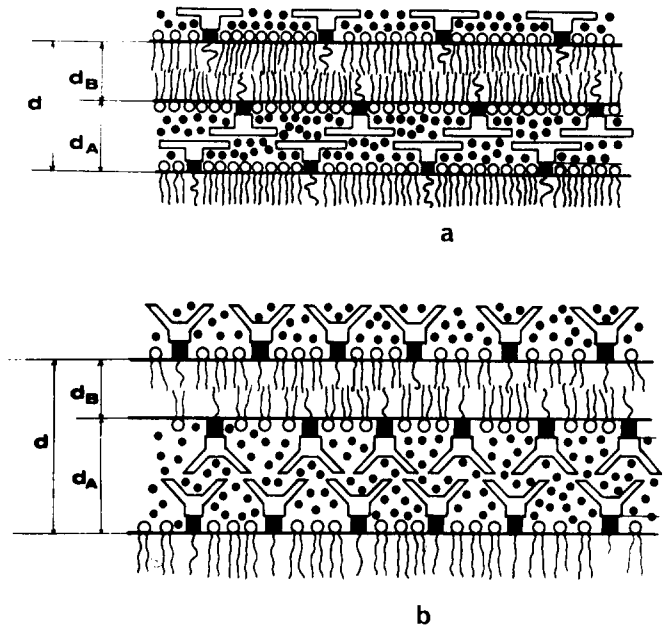

Figure 7 Schematic representation of (a) the 'T' and (b) the 'Y' lamellar structures of ternary systems liposaccharides–phospholipids–water. d_B = thickness of the hydrophobic layer containing the aliphatic chains of the liposaccharides and the phospholipids; d_A = thickness of the hydrophilic layer containing the carbohydrate chains of the liposaccharides, the polar heads of the phospholipids and the water (adapted from reference 26)

Such liposomes will be used to study interactions of glycoprotein carbohydrate chains with lectins[3] and to transport drugs, the carbohydrate chains playing the part of antennae and carrying the liposomes towards the target cells.

We will copolymerize liposaccharides containing glycoprotein carbohydrate chains with lipo-amino acids. The terminal functional groups of amino acids will allow the linkage of antitumour substances and we hope that glycoprotein carbohydrate chains work as transport systems and carry the polymer to the target cells. Furthermore it is known that the endocytosis power of tumour cells is higher than the endocytosis power of normal cells, so we hope that the macromolecular nature of our systems will increase their specificity.

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