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# STUDY BY X-RAY DIFFRACTION OF THE GEOMETRICAL SHAPE OF GLYCOPROTEIN SUGAR CHAINS IN TWO MODEL GLYCOCONJUGATES, A LIPOSACCHARIDE AND A PHOSPHOLIPOSACCHARIDE, HAVING THE SAME SUGAR CHAIN*

BERNARD GALLOT, XAVIER SANTARELLI, AND ANDRÉ DOUY
*Centre de Biophysique Moléculaire, C.N.R.S. 1A, Avenue de la Recherche Scientifique, F-45071 Orléans (France)*

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ABSTRACT

Two amphipatic, model glycoconjugates having the same sugar chain but differing in their hydrophobic component were studied by X-ray diffraction in concentrated water solution and in the dry state. The liposaccharide **2**, obtained by linking the $NH_2$-4 group of the asparagine residue of the glycoaminoacid obtained from hen ovotransferrin with the activated carboxylic acid group of palmitic acid exhibited a cubic structure in which the sugar chain adopted a slightly deformed, "T-shaped conformation". The phospholiposaccharide **3**, obtained by linking the $NH_2$-4 group of the asparagine residue of the same glycoamino acid with the primary amine group of dipalmitoylphosphatidylethanolamine through a suberyl bridge exhibited a lamellar structure in which the sugar chain adopted a "Y-shaped conformation". Thus, it was possible to induce a conformational change of the hen ovotransferrin sugar chain by changing the "hydrophobic residue" to which it is linked.

## INTRODUCTION

Glycoprotein sugar chains are involved in such biological processes as cellular recognition, cellular adhesion, and contact inhibition[1]. Unfortunately, glycoproteins are complex molecules and the oligosaccharides corresponding to their glycan chains do not crystallize. Consequently, model glycoconjugates in which a sugar chain from an *N*-glycoprotein is covalently bound to a hydrophobic peptidic[2] or lipidic chain[3,4] were synthesized. The amphipatic character of the model glycoconjugates led to the formation of aqueous mesophases which exhibited a periodic structure allowing their study by X-ray diffraction.

In the case of model glycoconjugates formed by the asialo, but galactose-con-

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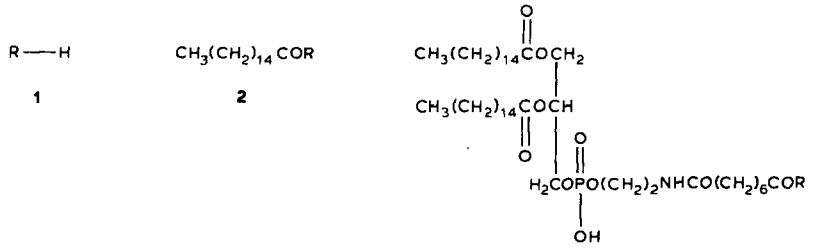
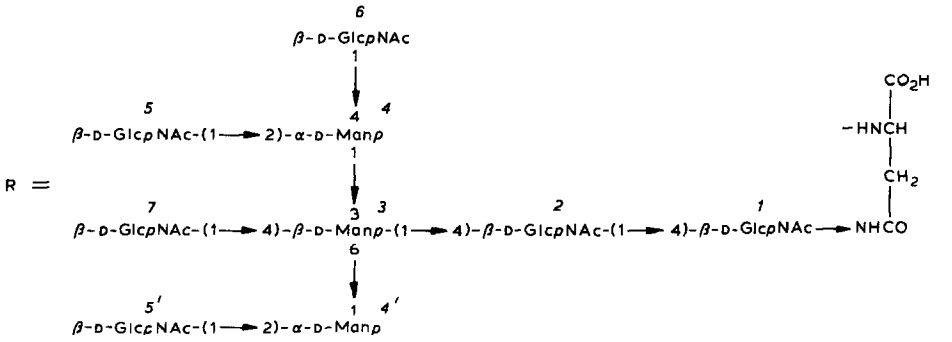
taining, sugar chain from hen ovomucoid linked to a poly(5-benzyl L-glutamate) chain in an $\alpha$-helix conformation, it was possible to resolve a well organized lamellar structure and to show that, in such a structure, the sugar chains adopts a "T-shaped conformation" or a "Y-shaped conformation" depending upon the molecular weight and the organization of the polypeptide chains[5]. Furthermore, the existence of a change of the sugar chains from T into Y conformation as a function of the phospholipid content[6] of the systems was demonstrated for ternary liposaccharide–phopsholipid–water systems.




More recently, the induction of similar conformational changes of the glycoprotein glycan chains was attempted by changing the nature of the hydrophobic nonglycan component of the model glycoconjugates but using only hydrophobic chains that are monodisperse and can be easily incorporated in model membranes such as liposomes. For that purpose the synthesis of two model glycoconjugates was undertaken, liposaccharide **2** in which the asparagine residue of a glycoaminoacid derived from ovotransferrin (**1**) is linked through a peptide bond to a fatty acid residue[7], and phospholiposaccharide **3** in which the asparagine residue of the same glycoaminoacid **1** is linked to the polar head of a phospholipid through a spacer arm[8]. The liquid–crystalline type structures of **2** and **3** were determined by X-ray diffraction (in concentrated water solution and in the dry state), showing the influence of the nature of the "hydrophobic residue" of the molecule on the conformation of the sugar chain.

## EXPERIMENTAL

*Materials.* — Hen ovotransferrin was extracted and purified by a combination of the methods of Williams[9], and Azari and Baugh[10] adapted to the processing of large amounts of egg white[7]. Glycoaminoacid **1** was obtained by enzymic degradation of ovotransferrin, purified and characterized as previously described[7,11,12]. Liposaccharide **2** was synthesized by coupling[7] the glycoaminoacid **1** with activated palmitic acid[13]. Phospholiposaccharide **3** was synthesized by a three-step method as described elsewhere[8].

The liposaccharide or phospholiposaccharide was dissolved in a small excess of water and, when perfect homogeneity had been reached, the desired concentration was obtained by evaporation at room temperature at a very slow rate. The samples were, then, kept at room temperature in tight cells for one week to be certain that equilibrium had been reached. After each X-ray experiment, the concentration of the sample was controlled by evaporating to dryness *in vacuo*.

*Methods.* — X-Ray diffraction studies were performed *in vacuo* with a Guinier-type focussing camera, equipped with a bent quartz monochromator giving a linear collimation and a device for recording the diffraction patterns from samples held at various temperatures with an accuracy of ±1°.

## RESULTS AND DISCUSSION

The liquid crystalline-type structures exhibited in concentrated water solution (<45% water) and in the dry state by liposaccharide **2** and by phospholiposaccharide **3** were studied by X-ray diffraction. Two regions could be distinguished in the X-ray patterns: the central region (corresponding to low angles) that gave information about the long range order (lamellar, hexagonal, or cubic structure) adopted by the system, and the external region (wide angles) that gave information about the organization of the paraffinic chains[14].

*Liquid–crystalline structure of* **2**. — The X-ray patterns obtained for **2** exhibited in the central region a set of reflections with Bragg spacings in the ratio 1, $\sqrt{2}$, $\sqrt{3}$, $\sqrt{4}$..., characteristic of a cubic structure, and in the wide-angle region a diffuse band typical of liquid paraffin chains[14]. Therefore, the structure of **2** is cubic and consists of spheres of radius $R_B$, filled with the hydrophobic paraffin chains and assembled on a cubic array of side $a$; the sugar chains and the water occupy the space between the spheres (Fig. 1).

The characteristic parameters of the cubic structure of **2** are: (a) the side $a$ of the cubic lattice directly obtained from the Bragg spacings of the X-ray patterns with an accuracy of ±1%, (b) the radius $R_B$ of the spheres calculated by Eq. (*1*) based on simple geometrical considerations, and (c) the area $S_B$ available for a molecule at the interface and calculated by Eq. (*2*), where $c$ is the concentration (mass ratio) of **2**, $G_B$ the weight fraction of the paraffin chains B ($G_B$ 0.097), $v_A$ the specific volume of the hydrophilic moiety A of the model glycoconjugate ($v_A$ 0.624

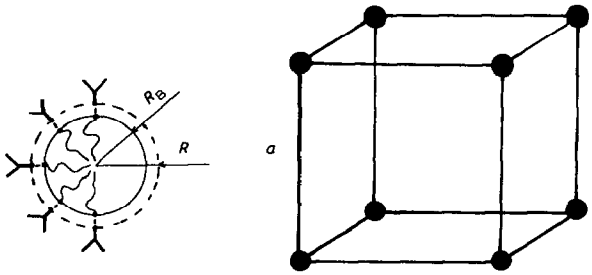



Fig. 1. Schematic representation of the cubic structure of liposaccharide **2**. Y, sugar chains.

$cm^3 \cdot g^{-1}$), $v_B$ the specific volume of the hydrophobic paraffin chains ($v_B$ 1.235 $cm^3 \cdot g^{-1}$), $v_S$ the specific volume of the solvent, $M_B$ the mol. wt. of the paraffin chains ($M_B$ 211), and $N$ the Avogadro number.

$$R_B^3 = \frac{3a}{4\pi}\left[1 + \frac{c(1 - G_B)\,v_A + (1 - c)\,v_S}{c\,G_B\,v_B}\right]^{-1} \qquad (1)$$

$$S_B = \frac{2M_B\,v_B}{NR_B} \qquad (2)$$

*Liquid–crystalline structure of* **3**. — The X-ray patterns obtained for **3** exhibited in their central region a set of reflections with Bragg spacings in the ratio 1,2,3..., characteristic of a lamellar structure, and in the wide-angle region a sharp reflection characteristic of extended paraffin chains, hexagonally packed and tilted as in the case of the $L'_\beta$ phase of synthetic phospholipids[15,16]. Therefore, the structure of **3** is lamellar and consists of plane, parallel, and equidistant sheets; each elementary sheet or thickness $d$ results from the superposition of two layers: a layer of thickness $d_A$ formed by the hydrophilic part of the phospholiposaccharide (the polar head group of the lipid residue, the suberyl bridge, and the glycoamino acid **1**), and a layer of thickness $d_B$ formed by the hydrophobic paraffin chains of the lipid residue, assembled on an hexagonal array of lattice parameter $D$ and tilted (Fig. 2).

The characteristic parameters of the lamellar structure of **3** are: (a) The total thickness $d$ of a sheet directly obtained from the Bragg spacings of the low-angle region of X-ray patterns with an accuracy of ±1%, (b) the parameter $D$ of the hexagonal lattice formed by the paraffinic chains and directly deduced from the Bragg spacings of the wide angle region of X-ray patterns, (c) the thickness $d_B$ of the hydrophobic layer calculated by Eq. (*3*) based on simple geometrical considerations, (d) the thickness $d_A = d - d_B$ of the hydrophilic layer, (e) the surface $S_L$ available for a molecule at the interface and calculated by Eq. (*4*), (f) the surface $\Sigma$ available for paraffin chain ($D^2\sqrt{3}/2$), and (g) the angle of tilt $\phi$ of the paraffin chains to the normal at the interface calculated by Eq. (*5*) [for the dry phospholiposaccharide **3**, the value of the angle of tilt ($\phi$ 27°) is similar to that found for the

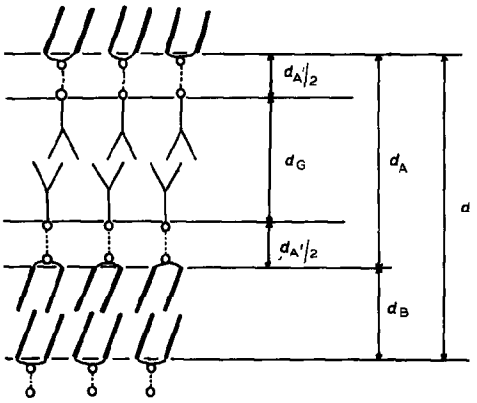



Fig. 2. Schematic representation of the lamellar structure of the phospholiposaccharide **3**. Y, sugar chains.

1,2-diarachinoylphosphatidylethanolamine[15] ($\phi$ 29°)], where $c$ is the concentration (mass ratio) of **2**, $G_B$ the weight fraction of the paraffin chains B ($G_B$ 0.153), $v_A$ the specific volume of the hydrophilic moiety A of **3** ($v_A$ 0.62 $cm^3 \cdot g^{-1}$), $v_B$ the specific volume of the hydrophobic paraffin chains ($v_B$ 1.235 $cm^3 \cdot g^{-1}$), $v_S$ the specific volume of the solvent, and $M_B$ the mol. wt. of the paraffin chains ($M_B$ 422).

$$d_B = d\left[1 + \frac{c(1 - G_B)\,v_A + (1 - c)\,v_S}{c\,G_B\,v_B}\right]^{-1} \tag{3}$$

$$S_L = \frac{2M_B\,v_B}{Nd_B} \tag{4}$$

$$\cos\phi = \frac{2\Sigma}{S_L} \tag{5}$$

The values of the specific volumes $v_i$ used for the calculation of some geometrical parameters of the cubic structure and of the lamellar structure exhibited, respectively, by **2** and **3** were calculated by the formula $M.v = M_i.v_i$, starting from the specific volumes of **1** (ref. 7), dipalmitoylphostidylethanolamine[15], and the $CH_3$, $CH_2$, and CO groups[17].

*Influence of the water concentration.* — In order to facilitate the comparison between the behavior of **2** and **3**, the variation of the geometrical parameters *vs.* the water content $c_1$ of the hydrophilic domains was plotted according to Eq. (*6*). (See Figs. 3 and 4).

$$c_1 = \frac{1 - c}{(1 - c) + c\,(1 - G_B)} \tag{6}$$

When $c_1$ increases, (a) the lattice parameters (side $a$ of the cubic cell for the cubic structure of **2** and total thickness $d$ of a sheet for the lamellar structure of **3** increase, (b) the characteristic parameters of the hydrophobic domains occupied by

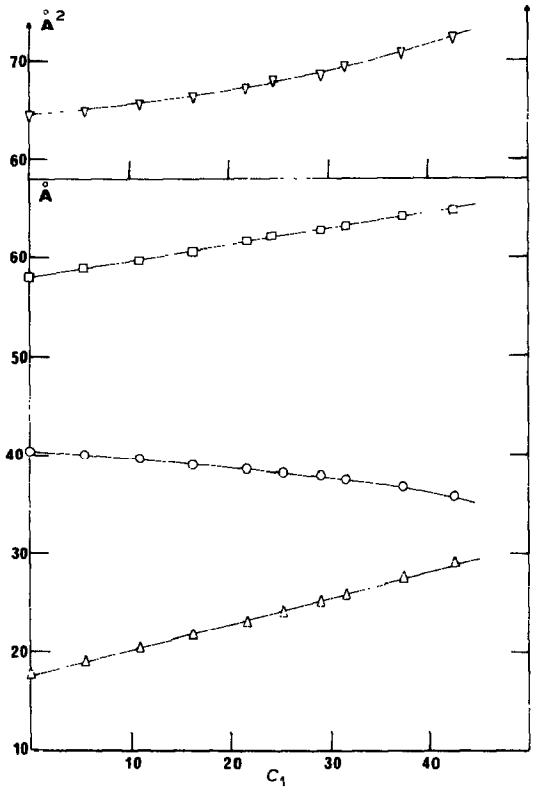



Fig. 3. Variation of the geometrical parameters of the cubic structure exhibited by **2** *vs.* the water content $c_1$ of the hydrophilic domains: (—□—□—) Side $a$ of the cubic cell, measured with an accuracy of ±1%; (—○—○—) diameter $2R_B$ of the spheres filled with the paraffinic chains; (—△—△—) $a - 2R_B$; and (—▽—▽—) average surface area $S$ available for a molecule at the surface of the spheres.

the paraffin chains (diameter $2R_B$ of the spheres for the cubic structure and thickness $d_B$ of the hydrophobic layer for the lamellar structure) decrease, (c) the characteristic parameters of the hydrophilic domains ($a - 2R_B$ for the cubic structure and $d_A$ for the lamellar structure) increase, and (d) the surfaces available for a molecule at the interface [$S_B$ for the cubic structure (Fig. 3) and $S_L$ for the lamellar structure (Fig. 4)] increase.

*Geometry of the sugar chain.* — In the case of **3**, the layer of thickness $d_A$ contains not only the sugar chains, but also the amino acids, the suberyl bridge, and the polar head of the lipid residue. In order to evaluate the space occupied by the sugar chains, it was of interest to divide the "hydrophilic component" A of the phospholiposaccharide into two parts: (a) a part A' having mol. wt. $M_{A'}$ 638, and consisting of the polar head of the lipid residue, the suberyl bridge, and the amino acids, and a part G having mol. wt. $M_G$ 1704 consisting of the sugar chains (see Fig. 2). In the absence of water, one may calculate the respective thickness $d_G$ and $d_{A'}$ of the layers occupied by the sugar chain and the spacer chain by use of Eq. (7) based on simple geometrical considerations, where $G_G$ is the weight fraction of the

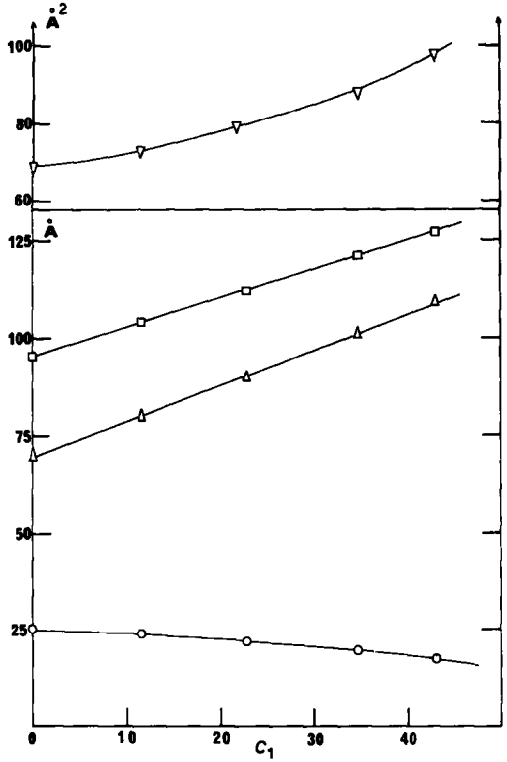



Fig. 4. Variation of the geometrical parameters of the lamellar structure exhibited by **3** *vs.* the water content $c_1$ of the hydrophilic domains: (—□—□—) Intersheet spacing $d$ measured with an accuracy of ±1%; (—○—○—) thickness $d_B$ of the hydrophobic layer containing the paraffin chains; (—△—△—) thickness $d_A$ of the hydrophilic layer containing the polar head group of the lipid residue, the suberyl bridge, and the glycoamino acid **1**; and (—▽—▽—) average surface area $S$ available for a molecule at the interface between the two layers.

$$d_G = d_A \left[ 1 + \frac{(1 - G_G)\, v_{A'}}{G_G\, v_G} \right]^{-1} \tag{7}$$

sugar chain ($G_G = M_G/(M_{A'} + M_G) = 0.728$), $v_{A'}$ the specific volume of the spacer ($v_{A'}$ 0.62), and $v_G$ the specific volume of the sugar chain ($v_G$ 0.62).

In the case of the cubic structure of **3**, the interface may be similarly located between the Asn residue and the first GlcNAc residue (Fig. 1), thus dividing the molecule into a sugar chain having mol. wt. $M_G$ 1704 and the rest of the molecule having mol. wt. $M_{B'}$ 470. Then, the radius $R$ of the spheres containing the paraffin chains and the amino acids may be calculated by Eq. (*8*) based on simple geometrical considerations, where $G_{B'}$ is the weight fraction of the paraffin chains and amino acids ($G_{B'} = M_{B'}/(M_{B'} + M_G) = 0.216$), $v_{B'}$ the specific volume of the paraffin chains and amino acids ($v_{B'}$ 0.913), and $v_G$ the specific volume of the sugar chain ($v_G$ 0.62).

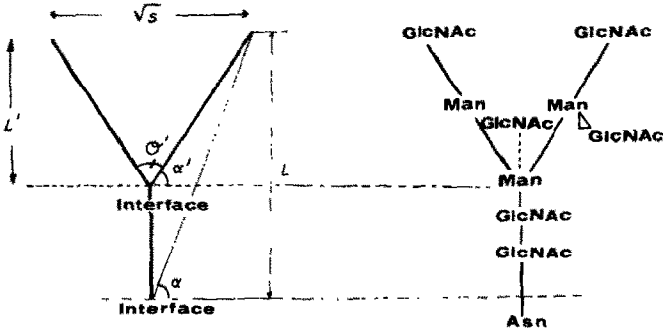



Fig. 5. Schematic representation of the sugar chain **1** in the Y-shaped conformation.

Fig. 6. Molecular model of the glycoamino acid **1** in the "Y-shaped conformation".

$$R^3 = \frac{3a^3}{4\pi}\left[1 + \frac{(1 - G_{B'})\nu_G}{G_{B'}\nu_{B'}}\right]^{-1} \qquad (8)$$

In order to compare easily the geometrical shape of the sugar chains in the cubic structure of **2** and in the lamellar structure of **3**, another parameter was used, *i.e.*, the anisotropy $A_n$ of the sugar chains defined by the ratio of the extension $L$ of the sugar chains in the direction perpendicular to the interface (see Fig. 5) *vs.*

Fig. 7. Molecular model of the glycoamino acid **1** in the "T-shaped conformation".

the square root of the specific surface ($A_n = L/\sqrt{S}$), where $L = d_G/2$ for the lamellar structure, and $L = (a - 2R)/2$ for the cubic structure. The anisotropy $A_n$ is related to the angle $\alpha$ that defines the general direction of the sugar chain (see Fig. 5) by Eq. tg $\alpha = 2\,A_n$. In the absence of water, the lamellar structure of **3** had $A_n$ 3.1 and $\alpha$ 81°, but the cubic structure of **2** had $A_n$ 0.7 and $\alpha$ 54°.

If one considers that the sugar chains of *N*-glycoprotein may be divided into a rigid core (GlcNAc$^1$→GlcNAc$^2$→$\beta$-Man$^3$) and branches that are able to rotate around the linkages $\alpha$-Man$^4$→$\beta$-Man$^3$ and $\alpha$-Man$^{4'}$→$\beta$-Man$^3$, the interface may be located between GlcNAc$^2$ and $\beta$-Man$^3$ (see Fig. 5) and the anisotropy $A'_n$ of the branches, the angle $\alpha'$ between the branches and the interface, and the angle $\theta'$ between the branches may be calculated (see Fig. 5). For the lamellar structure $L' = d_G/2$, $S' = S$ and $d_G$ was calculated by Eq. (*7*), but with $G_G$ 0.454, $v_G$ 0.62, and $v_{A'}$ 0.62. For the cubic structure $L' = (a - 2R')/2$, $R'$ was calculated by Eq. formula (*8*), but with $G_{B'}$ 0.403, $v_{B'}$ 0.777, $v_G$ 0.62, and $S' = 2M'v'_B/NR'$ with $M'$ 876. In the absence of water, the following values were obtained: For the lamellar structure of **3**, $A'_n$ 2.35, $\alpha'$ 78°, and $\vartheta'$ 24°, which suggest "Y-shaped conformation" for the sugar chain, as illustrated by Fig. 6; for the cubic structure of **2**, $A'_n$ 0.14, $\alpha'$ 16°, and $\theta'$ 148°, which suggest a slightly deformed "T-shaped conformation" for the sugar chain, as illustrated by Fig. 7. Thus, by changing the nature of the "hydrophobic moiety" linked to the sugar chain of hen ovotransferrin, it was possible to modify the conformation of the sugar chain.

## DISCUSSION

The results presented herein indicate that liposaccharide **2**, in which the sugar chain of hen ovotransferrin is linked through a peptidic bond to palmitic acid, exhibits a mesomorphic cubic structure where the paraffin chains are "liquid" and the sugar chains adopt a slightly deformed "T-shaped conformation (Fig. 7), whereas the phospholiposaccharide **3**, in which the same sugar chain of hen ovotransferrin is linked to the polar head of dipalmitoylphosphatidylethanolamine through a suberyl bridge, exhibits a lamellar structure where the paraffin chains are in crystalline form and the sugar chains adopt a "Y-shaped conformation" (Fig. 6). Therefore, by changing the nature of the "hydrophobic residue" linked to the sugar chain of hen ovotransferrin, it was possible to modify the structure adopted by the model glycoconjugate and the conformation of the sugar chain. Whether this effect of the nature of the "hydrophobic residue" on the conformation of the *N*-glycoprotein glycan chains is general or not will require further investigations.

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