Organogels

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Enzymatically Derived Sugar-Containing Self-Assembled Organogels with Nanostructured Morphologies**

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Control of building-block assembly and phase behavior is crucial for the ultimate design of functional architectures ranging from the nano- to the macroscales, with organogels representing one important example of this functional architecture.[1] An ideal gelator is an amphiphile that induces gel formation through self-assembly into highly ordered structures in which the hydrophilic moieties interact through extensive hydrogen bonding and the hydrophobic moieties interact with the organic liquid. Among the growing list of low molecular weight compounds that induce gelation,[2] sugars appear to satisfy these requirements, and numerous alkyl- and aryl-based monosaccharide gelators have been generated.[3] As opposed to chemical synthesis, enzymatic catalysis is highly selective and has been used to generate low-molecular-weight compounds that can gel organic solvents,[4] generate a wide range of sugar-based esters,[5] and in particular, prepare highly regioselective symmetrical diesters.[6] We reasoned that such a biocatalytic approach would provide an alternative route to the synthesis of disaccharide-based diesters with physical and structural features appropriate for a low-molecular-weight gelator along with a controlled symmetry that may aid in self-assembly. Combining the principles of supramolecular chemistry with the selectivity of biocatalysis may represent a new and powerful strategy to develop new molecularly defined and functional materials.

To that end, we examined the synthesis of sugar-based diesters by using the lipase B from *Candida antarctica* (CALB). Transesterification reactions were performed in acetone that contained either vinyl stearate or vinyl butyrate as highly or moderately hydrophobic ester donors, respectively, and with several common disaccharides including sucrose, maltose, lactose, and trehalose. Interestingly, only the reactions with trehalose, a symmetrical disaccharide with an α-1,1 glycosidic bond, resulted in gel formation during the course of the transesterification reactions (Table 1, gelators **2** and **5**), thereby confirming the importance of monomer structure in gel assembly. Trehalose-6,6′-distearate and trehalose-6,6′-dibutyrate were obtained as the sole products from the respective enzymatic reactions in yields of $>50\%$. Hence, CALB was highly regiospecific in its acylation of trehalose. The purified dieters were tested in a wide range of solvents for their gelation ability (see the Supporting Information). The trehalose distearate was insoluble in water and soluble in chloroform and 1,4-dioxane, whereas trehalose 6,6′-dibutyrate was insoluble in cyclohexane and olive oil and soluble in water. Gels were formed in all other solvents tested.

As a result of these studies, we generated a series of additional diesters with chain lengths of C2 to C14 (**1, 3, 4**, and **6**) and assessed their gelation capacity in several key solvents, ranging from the hydrophilic acetonitrile to the hydrophobic *p*-xylene (Table 1). The minimum gelator concentration ($c_{\min}$) required to induce gelation is strongly dependent on the acyl-chain length. In most of the cases, a shorter chain length promotes gelation at lower gelator concentration, with the exception of gelation in acetonitrile and isopropanol. These results sharply contrast with typical sugar-based amphiphilic organogels, which require long-chain alkyl or aryl moieties to induce gelation.[3a–e] Surprisingly, the trehalose-6,6′-diacetate (**1**) was capable of inducing gelation at a $c_{\min}$ of 0.04% (w/v; 0.84 mM) in ethyl acetate and nearly this low in methyl methacrylate. This represents, to our knowledge, the lowest $c_{\min}$ value reported for a sugar ester gelator.[7] For ethyl acetate, the $c_{\min}$ represents over 12000 solvent molecules being associated per molecule of **1**. This can be translated into a swelling of the weight of the gelator approximately 2500-fold.

The trehalose diesters are excellent gelators over a broad range of organic solvents (see the Supporting Information) and in a mixture of solvents. For example, in the case of a 1:1 binary mixture of ethyl acetate and acetonitrile, the minimum gelation concentration was between the $c_{\min}$ values in the pure solvents (see the Supporting Information), therefore showing no preference for a given solvent. Interestingly, the longer-ester-chain trehalose derivatives could form gels in olive oil (Table 1) with relatively low $c_{\min}$ values. Addition of 5% free oleic acid, which would be present in low-purity olive oil, did not affect the swelling capacity of the gel. Hence, complex, multicomponent solvent systems, such as that found in a natural oil and in the presence of a charged hydrolysis product, could be subject to gelation.

As described above, although regiospecificity was achieved to give the respective 6,6′-diesters for all disaccharides tested, gel formation only occurred with trehalose, suggesting that the unique symmetry of this molecule, which

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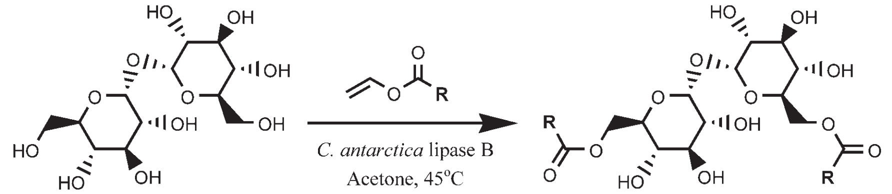
Table 1: Minimum gelation concentration (weight percent) of trehalose-based diesters in different solvents at 25 °C.[a]



Solvent (log P value)	R =	**1** -CH_3	**2** -$(CH_2)_2CH$	**3** -$(CH_2)_8CH_3$	**4** -$(CH_2)_{12}CH_3$	**5** -$(CH_2)_{16}CH_3$	**6** -$CH{=}CH_2$
acetonitrile (-0.34)		G (0.36)	G (0.69)	G (0.18)	G (0.11)	G	G
Acetone (-0.24)		G (0.34)	G (1.0)	G (1.3)	G (1.4)	G	G
isopropanol (0.05)		G (0.54)	G (1.39)	G (2.21)	G (1.31)	G	G
ethyl acetate (0.73)		G (0.04)	G (0.13)	G (0.71)	G (1.1)	G (0.72)	G
methyl methacrylate (1.38)		G (0.05)	G (0.11)	G (0.82)	G (0.85)	G (0.40)	G
p-xylene (3.15)		I	G (0.14)	G (0.18)	G (0.25)	G (0.37)	G (0.72)
olive oil (N/A)		I	I	G (0.09)	G (0.13)	G (0.18)	I

[a] G indicates that the gel formed and I indicates that the gelator could not dissolve in the organic solvent at elevated temperatures.

was maintained by the regioselectivity of lipase-catalyzed acylation, may play the key role in its strong gelation ability. To test this hypothesis, we chemically acylated trehalose with stearoyl chloride to yield a mixture of trehalose esters. Following isolation of the diesters (a mixture of regioisomers among the eight free hydroxy groups, data not shown), gelation studies were performed in ethyl acetate. Gelation did not occur until a diester mixture concentration of approximately 10 % (w/v) was reached; well over 10-fold higher than that required by the 6,6′-distearate. When the 6,6′-distearate was purified from the diester mixture, identical gelation properties to those synthesized enzymatically were obtained. Similar results were obtained with the 6,6′-diacetate and 6,6′-dibutyrate derivatives. These results indicate that gelation is strongly favored with highly symmetrical disaccharide ester derivatives that can be synthesized through regioselective enzymatic catalysis.

Differential scanning calorimetry (DSC) of gels **1**–**4** was performed in ethyl acetate to yield a gel→sol transition temperature as a function of gelator mole fraction and therefore enable calculation of gel melting enthalpy (ΔH_m; see the Supporting Information). Values of ΔH_m for gelators **1**–**4** were determined to be approximately 55, 44, 30, and 22 $kJ\,mol^{-1}$, respectively. The high ΔH_m of **1** indicates that this gelator is the most effective in forming highly stable gels in ethyl acetate, which is consistent with its low c_{min}. Longer acyl chains favor greater solvation of the diester in ethyl acetate and therefore reduce their ability to form strong gels.

The morphological properties of the trehalose-based organogels were obtained through scanning electron microscopy (SEM). Figure 1 depicts selected SEM images of the xerogels of **1** in ethyl acetate (Figure 1 a,b) and in isopropanol (Figure 1 c,d), as well as **6** in ethyl acetate (Figure 1 e,f). These xerogels consist of 3D entangled fiber-like aggregates with diameters of 10–500 nm and lengths in the micron scale. The high aspect ratios of the gel fibers clearly indicate that the intergelator interactions are highly anisotropic. Most likely, the fibers observed in the electron micrographs consist of bundles of gelator aggregates, similar to the structures observed in other gel systems. The solvent clearly influences the gel structure, for example, the ethyl acetate gel of **1** is transparent and shows extended fibrous structures (Figure 1 a), whereas the isopropanol gel of **1** is opaque and consist of larger and more crystalline fibers (Figure 1 c). Furthermore, the fact that low gelator concentrations can yield gels with the weak hydrophobicity of the C2 acyl moiety suggests that H-bonding is the predominant mechanism for gel assembly, although hydrophobic interactions may also contribute to gelation at longer acyl-chain lengths. The ability of H-bonding to dominate gel assembly is supported by the extremely high hydroxy-group density in a disaccharide like trehalose, as depicted in Figure 2 e (for more detail, see the Supporting Information).

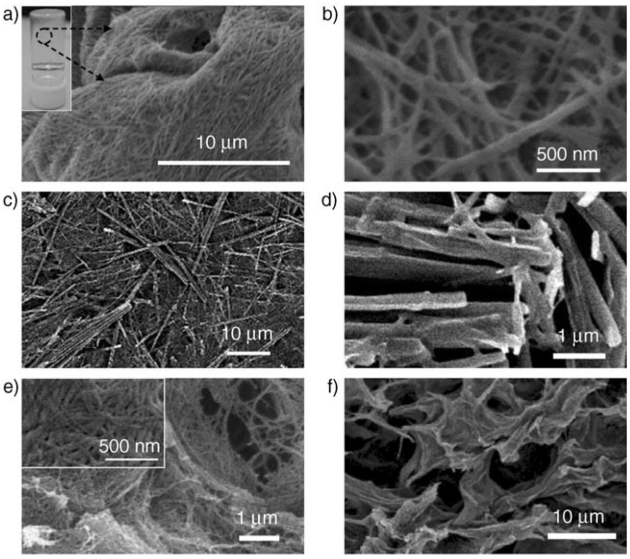


Figure 1. FE-SEM images of the organogels from a) **1** in ethyl acetate, b) **1** in ethyl acetate at a higher magnification, c) **1** in isopropanol, d) **1** in isopropanol at a higher magnification, e) **6** in ethyl acetate; inset shows higher magnification of **6** in ethyl acetate, f) **6** self-supporting and porous scaffold after UV polymerization in ethyl acetate.

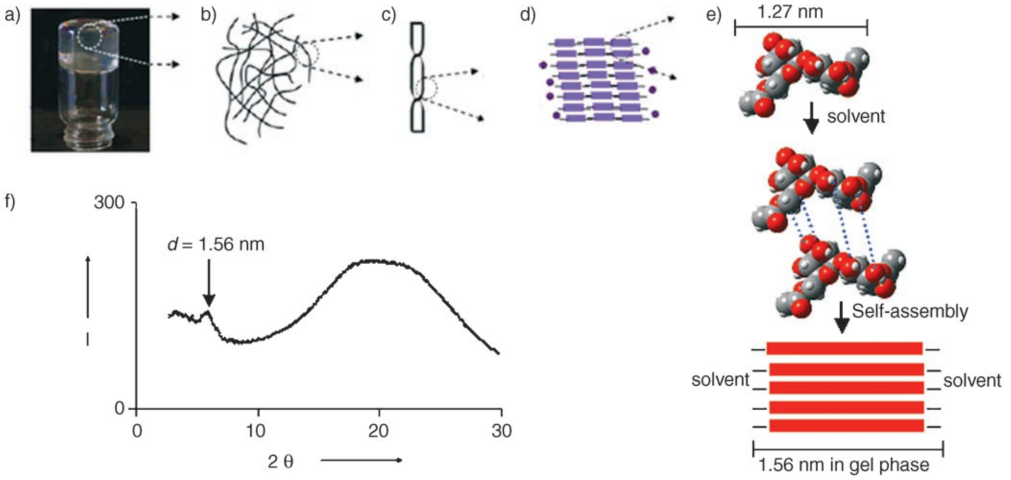


Figure 2. Proposed scheme of molecular packing. a) Gel formed by **1** in ethyl acetate, b) 3D network, c) fibers, d) multilayers, and e) modeled molecular packing. f) SAXS data for ethyl acetate gel of **1**.

To gain additional insight into the structures that comprise the gel formed from **1**, small-angle X-ray diffraction (XRD) was employed. XRD of the wet gel gave a weak Bragg reflection at 1.56 nm, indicating that the sugar diester assembled into a well-ordered structure (Figure 2 f). This reflection is approximately the same as that of the molecular length of **1**, which was confirmed by crystalline-sample measurements (1.25 nm) and molecular modeling studies by using energy-minimized calculations.[8] XRD measurements of the gels from **2**–**6** also gave similar diffraction patterns showing that, in a suitable organic solvent, the trehalose diesters self-assemble into ordered structures. Based on these results, we propose a molecular arrangement of the trehalose diesters in the organic liquid (Figure 2 e). Molecular stacking of the multilayers (Figure 2 d) leads to the formation of gel fibers (Figure 2 c) followed by further growth into fibrous 3D networks (Figure 2 b) and finally formation of the gel (Figure 2 a). The transparency of the resulting ethyl acetate gel of **1** attests to a low-gelator-volume fraction along with a nanoscale fiber size that does not interfere with light transmission. Additional information on the packing arrangement of the trehalose-6,6′-distearate (**5**) gelators through H-bonding was further obtained by temperature-dependent ^{1}H NMR spectroscopic measurements in isopropanol and acetone (see the Supporting Information). The gelators exhibit peaks in both the sol and gel states, and as expected, the peak width becomes sharper when the temperature increases above the T_{gel} (46 °C). The peak width at 2.17–2.21 ppm ($COOCH_2$ adjacent to the sugar) decreases below T_{gel} but remains constant at $T > T_{gel}$. This result is consistent with the loosening of H-bonds that occur at the gel→sol transition temperature. Interestingly, the organogels retain visible ^{1}H NMR spectroscopic peaks in the gel state, suggesting that the gelator molecules maintain sufficient thermal motion[3d,e,9] in contrast to other gelating systems.[10]

Although the gel structure can be dissociated in several ways, such as by adding a good solvent of the gelator, another route to degradation of these particular trehalose diester gels is through selective ester-bond hydrolysis catalyzed by lipase in the presence of a small amount of added water. Indeed CALB (0.5 mg mL^{-1}) in the presence of 2 % (v/v) water caused the ethyl acetate gel of **1** to undergo disintegration with concomitant formation of free trehalose and some trehalose 6-acetate.

Gels containing acrylate esters (for example, **6**) can be further subjected to post-gelation cross-linking[11] in the presence of 2,2-dimethoxy-2-phenylacetophenone (5 mol %) as the photoinitiator and subsequent polymerization through UV irradiation. Following solvent evaporation, the cross-linked organogel from **6** was lyophilized to yield a highly porous material (Figure 1 f). This material has a far-larger pore structure than the gel from **6**, which is not cross-linked (Figure 1 e). This suggests that it is suitable as a porous scaffold. No gel shrinkage occurred during lyophilization. Following drying into the aerogel, the cross-linked material remained intact as a self-supporting scaffold (Figure 3 a). This material was capable of behaving as a modest hydrogel; within 5 h the gel absorbed its weight 12-fold in water to give a self-supporting transparent material (Figure 3 b). We believe that this is the first example of the generation of nanostructures from self-assembled precursors in organic solvents with hydrogel function.

In conclusion, we have used a biocatalytic strategy to design and synthesize a novel family of highly symmetrical trehalose diesters that self-assemble in a range of organic solvents and form gels at concentrations as low as 0.04 % (w/v). The gel fibers, particularly those obtained from short acyl-chain length, are self-assembled and stabilized most likely through the extensive H-bonding networks that are available in the sugar; although both van der Waals packing and hydrophobic interactions are also expected to contribute to gel stability particularly as the acyl-chain length increases. Combining the principles of supramolecular chemistry and the selectivity of biocatalysis represents a powerful strategy to develop new molecularly defined and functional materials. The organogels reported herein may find potential applications in the food, pharmaceutical, and cosmetic industries in which trehalose is already used routinely.[12] In particular, the

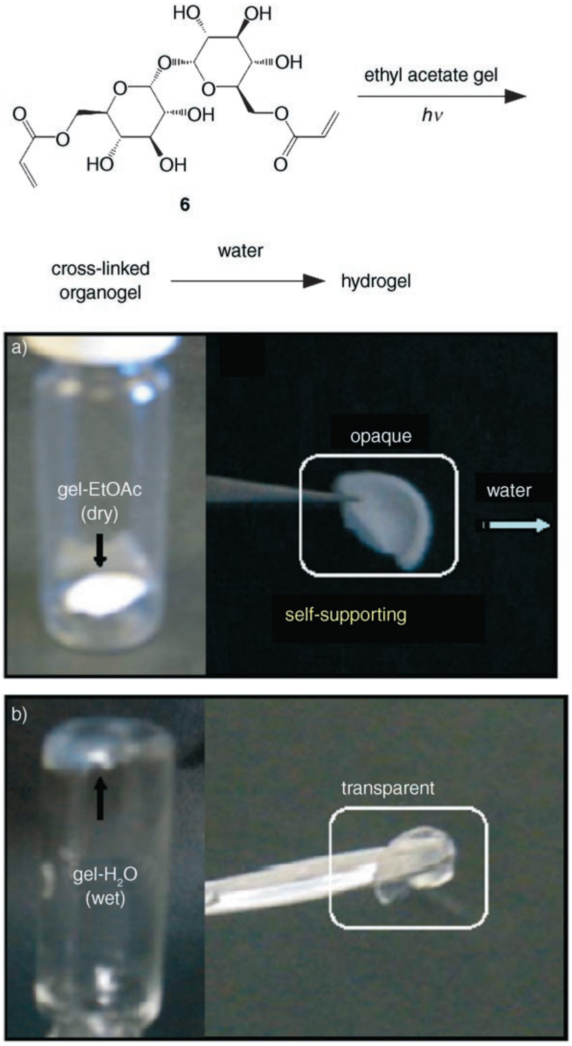


Figure 3. Self-supporting organo a) and hydrogel b) from trehalose 6,6′-diacrylate after polymerization.

ability of the longer-chain trehalose diesters to gel olive oil attests to its potential use as a food or cosmetic additive that can be prepared by using food-approved enzymatic synthesis approaches.[13] Finally, photopolymerization of diacrylate esters results in stable organo- and hydrogels. These porous, self-supporting structures may find use as a scaffold for tissue engineering, templated materials synthesis, nanoreactors for chemical and enzyme catalysis, and controlled-pore, hydrophilic membranes.

Experimental Section

Trehalose diesters were synthesized as follows: Novozyme 435 (1.5 g) was added to acetone (100 mL) containing trehalose dihydrate (0.01 mol) and vinyl ester (0.03 mol). The reaction mixtures were then incubated at 45 °C and agitated at approximately 200 rpm for 48 h. The reactions were terminated by filtering the reaction mixtures to remove the solid enzyme. The crude products were purified by flash chromatography by using an ethyl acetate/methanol/water (17:4:1 v/v) mixture as the eluent. The yields of the isolated products ranged from 50–80 %. The trehalose diesters were analyzed by standard spectroscopic and elemental analysis procedures.

Gel→sol transition temperatures (T_m) were determined by DSC with a Mettler DSC-822 differential scanning colorimeter equipped with a nitrogen-gas cooling system. Field emission (FE)-SEM measurements were carried out with a JEOL electron microscope. A piece of the gel was placed on a carbon-coated copper grid and dried for 3 h under vacuum before imaging. XRD measurements were conducted by using a Bruker axs-D8 Discover with GADDS diffractometer with graded *d*-space elliptical side-by-side multiplayer optics, monochromated $Cu_{K\alpha}$ radiation (40 kV, 40 mA), and imaging plate. The organogel was used as prepared in wet conditions for the analysis. The typical exposure time was 1 min for self-assembled structures with a 100 mm camera length.

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