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ARTICLE TYPE

The Growth of Hexagonal Bipyramids, X- and Polycrystal-shaped Twins of Cholic Acid during Acidic Hydrolysis of the Hydrogel of its Ester

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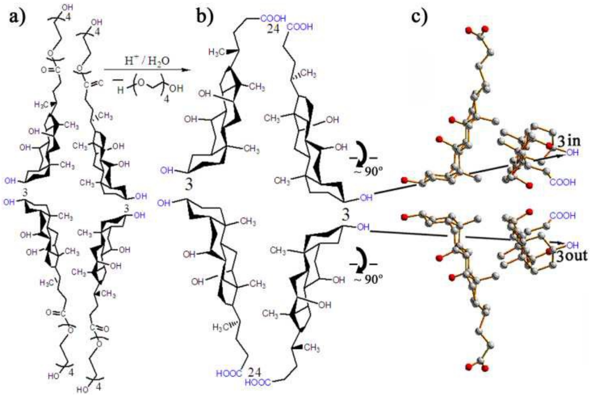
In this paper, hexagonal bipyramids, X- and polycrystal-shaped twins of cholic acid are acquired through acidic hydrolysis of the hydrogel of its ester. The growth mechanism is proposed on the basis of the hydrophobic swallowtail-shaped tetramers found in the single-crystal structure.

Cholic acid (CA) belongs to coprostane steroids whose cis connection between rings A and B produces the curved steroid skeleton on which the concave face with three hydroxyl groups is rendered hydrophilic yet the convex face with two methyl groups is hydrophobic.[1] Although CA processes facial amphiphilicity, it is impossible to form directly the self-assembled structure because it is only slightly soluble in water.[2] Many inclusion crystals of CA with different crystal polymorphs have been prepared from organic solvents.[3–11] Besides recrystallization, CA crystals can also be obtained by guest-exchange and absorption methods.[10,11] Recently, to overcome the poor water solubility and obtain the self-assembled structure, we adopted acid-hydrolysis method to prepare the self-assembled crystals of CA from aqueous solutions: the preassembly of tetraethylene glycol mono-CA ester (CA-4EG) was acid-hydrolyzed by dilute hydrochloride acid (Scheme 1a), and long thin crystal prisms of CA were obtained (Fig. S1).[12] The prisms belong to C2 space group. Lattice parameters: a = 40.334(5) Å, b = 7.729(9) Å, c = 15.620(18) Å, β = 90.602(3)°, V = 4869(10) $Å^3$, and Z = 8. Hydrophobic rhombic tetramers of CA are found in the single-crystal structure (Scheme 1b, Fig. S2).



Scheme 1 Proposed rhombic tetramers of (a) CA-4EG and (b) CA, swallowtail-shaped tetramers of CA (c).

In this paper, the hydrogel of CA-4EG is hydrolyzed by the same method: the acidified hydrogel shrinks gradually, and finally the shrunk gel collapsed. Microscopic observation shows that hexagonal bipyramids, X- and polycrystal-shaped twins of CA are formed in the shrinking hydrogel (Fig. 1). The hexagonal crystals belong to $P6_522$ space group. Lattice parameters: a = 13.745(8) Å, b = 13.745(8) Å, c = 85.186(6) Å, $\alpha = \beta = 90°$, γ = 120°, V = 13938(15) $Å^3$, and Z = 12. Hydrophobic swallowtail-shaped tetramers of CA are found in the single-crystal structure (Scheme 1c, Fig. 2). It is obvious that both the crystal morphologies and the space groups are different when hydrolyzing the preassembly of CA-4EG in aqueous solutions and its hydrogel, respectively.

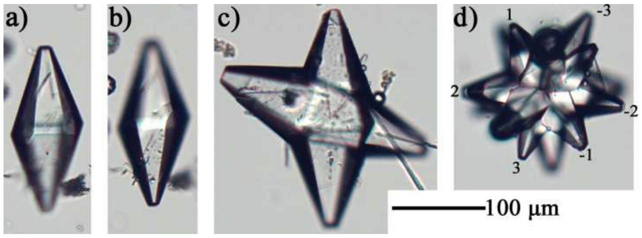


Fig. 1 Optical micrographs of CA crystals in aqueous solutions. a), b) the same hexagonal bipyramid with the top and bottom pyramid in the focal plane, respectively; c) one X-shaped twin; d) one polycrystal-shaped twin. The numbers do not represent the growing sequence.

The refractivity between two hexagonal pyramids indicates the existence of one plate (Fig. 1a, S3, S4). It seems as if the vertical hexagonal bipyramid of the X-shaped twin penetrates the tilt one (Fig. 1c). A few irregular growths appear on the twin plane reentrant edge of the polycrystal-shaped twin (Fig. 1d). Some minerals often show penetration twinning or multiple twinning.[13]

Fig. 2 shows that the hydrophobic swallowtail-shaped tetramers of CA are the basic building blocks to construct the hexagonal crystals rather than the individual CA molecules. This crystal polymorph has been reported by Shibakami et al.[11] They prepared the same polymorph of CA by guest-exchange method: first the inclusion crystals of CA with 2- or 3-fluorobenzyl alcohol ($P2_1$ space group) were obtained by recrystallization from the corresponding alcohols, and then the guest molecules (*i.e.*, alcohols) were exchanged with H_2O (CA: H_2O = 2: 1).

In fluorobenzyl alcohols, the intermolecular H-bonding interactions of CA are the main driving force for the formation of hydrophilic-hydrophobic layer structures, and then water drives the transition to swallowtail-shaped tetramers. However, in aqueous environment, the hydrophobic interaction between the hydrophobic faces of CA-4EG is the initial driving force for the formation of gel fibers; meanwhile the H-bonding interactions also play a key role in stabilizing the fiber structures. After the gel fibers of CA-4EG are partially acid-hydrolyzed, the packing of the hydrophobic swallowtail-shaped tetramers of CA forms these hexagonal crystals in the shrinking hydrogel. Therefore, the acid-hydrolysis method reported here is totally different from the guest-exchange method even though the space group is the same.

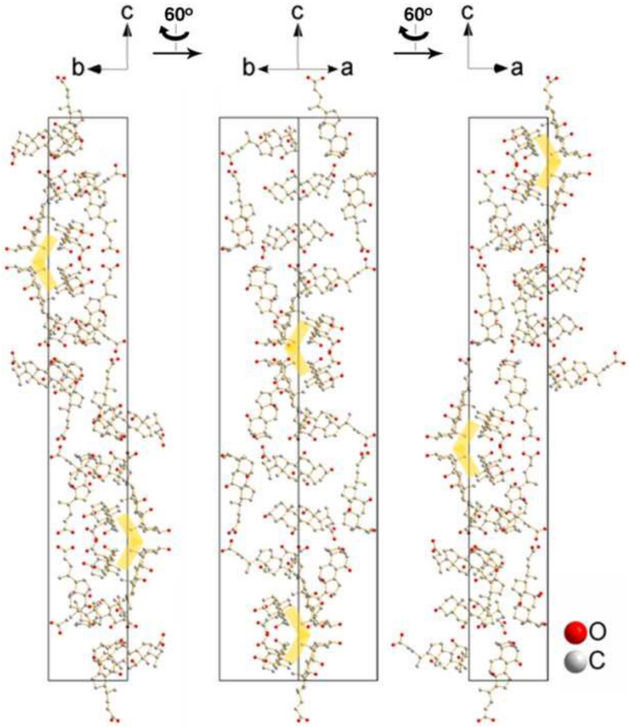
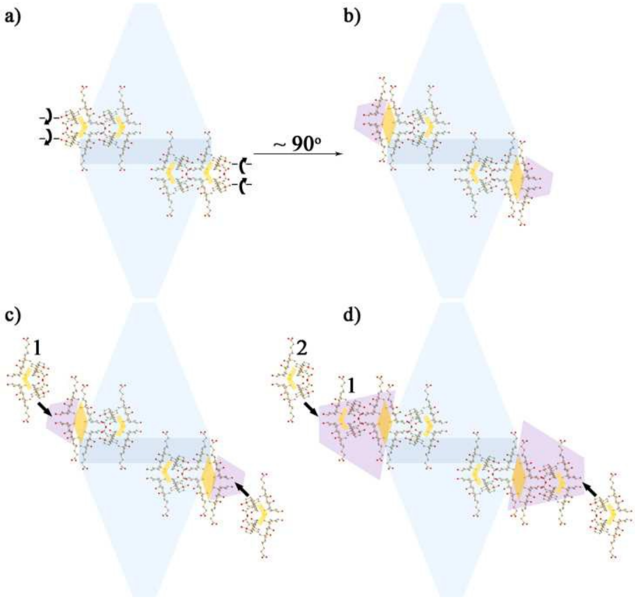


Fig. 2 Crystal structure of CA. H atoms and solvent molecules are omitted for clarity.

As mentioned above, we also prepared the long crystal prisms of CA from aqueous solutions.[12] According to the hydrophobic rhombic tetramers and fibers discovered in the single-crystal structure (Fig. S2), we supposed that CA-4EG formed similar tetramers in aqueous solutions (Scheme 1a), and the tetramer structures of CA were preserved after acid hydrolyzing the ester bond of CA-4EG (Scheme 1b). Then the stacking of CA tetramers formed the inner hydrophobic and outer hydrophilic fibers (Scheme 1b can be considered as the top view of the fiber), and their parallel arrangement formed the crystal prisms in aqueous solutions.[12] Therefore, the hydrophobic rhombic tetramers of CA were the basic building blocks to construct the long prisms rather than the individual CA molecules.[12]

It is very likely that the rhombic tetramers of CA also exist in the acidified hydrogel of CA-4EG, and their stacking forms the fibers with rhombic cross-section. But the crystalline arrangement of these CA fibers to form the long crystal prisms is difficult because of the spatially confined environment. It is the crystallization that drives the rhombic-tetramer of CA to swallowtail-shaped-tetramer transformation, even though the intermolecular interactions of the former such as hydrogen bonds and the hydrophobic interactions outweigh those of the latter (Scheme 1b, c). As a result, the hexagonal crystals are formed in the shrinking hydrogel.



Scheme 2 Proposed processes for the formation of X-shaped twins. a) swallowtail-shaped tetramers consisting of the hexagonal bipyramid; b) swallowtail-shaped-tetramer to rhombic-tetramer transformation on the crystal surfaces; c) the stacking of swallowtail-shaped tetramers on the rhombic tetramers; d) the stacking of swallowtail-shaped tetramers forming new hexagonal pyramids.

Suspension (*i.e.*, in gel) is an external condition for centrosymmetric twinning, which guarantees the concentration around the growing crystal is equal. The original concentration of CA-4EG is just 1 % w/v, and the diffusion speed in gels should be much slower than in solutions. Both suggest that the concentration of swallowtail-shaped tetramers around the growing crystal may fall below the critical concentration for crystallization during crystal growth, causing the hexagonal bipyramid to stop growing (Scheme 2a). In this circumstance the CA molecules consisting of the crystal surfaces may rotate back to reduce the exposure of the hydrophobic moieties of their swallowtail-shaped tetramers to the aqueous environment, again forming the rhombic tetramers (Scheme 2b). The concentration of the swallowtail-shaped tetramers around the hexagonal bipyramid may reach again the critical concentration for crystallization with the acidic hydrolysis of the rhombic tetramers of CA-4EG, diffusion and rhombic-tetramer of CA to swallowtail-shaped-tetramer transformation in the shrinking gel, but the rhombic tetramers formed on the crystal surfaces inhibit the hexagonal pyramids from continued growing because the crystal lattice has changed.

According to the morphologies of the X-shaped twins (Fig. 1c), we conjecture that the vertical hexagonal bipyramid grows first, and then the two tilt hexagonal pyramids grow at the connections of the two vertical pyramids. The reason why only two pyramids grow simultaneously is that the critical concentration for crystallization cannot maintain all base edges to grow simultaneously into hexagonal pyramids, and growing two hexagonal pyramids in opposite directions is conducive to the spatial diffusion. Furthermore, Scheme 2d shows the swallowtail-

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shaped tetramers can stack on the rhombic tetramers, and then their stacking forms the tile pyramids. Similarly, the stacking of swallowtail-shaped tetramers at the two reentrant obtuse angles of one X-shaped twin forms the polycrystal-shaped twins.

In summary, it is the packing of the hydrophobic swallowtail-shaped tetramers of CA that forms the hexagonal crystals rather than the individual CA molecules. Suspension is an external condition for the centrosymmetric twinning. Crystallization drives rhombic-tetramer of CA to swallowtail-shaped-tetramer transformation, even though the intermolecular interactions of the former outweigh the latter. Once the concentration of the swallowtail-shaped tetramers around the growing crystals falls below the critical concentration for crystallization, the hexagonal crystals stop growing. At this time, the self-adjustment of CA molecules on crystal surfaces takes place. To our knowledge, it is the first time to discuss the tetrameric self-adjustment on organic crystal surfaces. The change in lattice on crystal surfaces inhibits the hexagonal pyramids from continued growing, even though the concentration of the swallowtail-shaped tetramers around the pyramids reaches again the critical concentration for crystallization. The rhombic tetramers formed at the base edges of hexagonal pyramids are the candidate nuclei. The critical concentration for crystallization cannot maintain all base edges to grow simultaneously into hexagonal pyramids and growing two hexagonal pyramids in opposite directions is conducive to the spatial diffusion. Therefore, centrosymmetric twins, such as the X- and polycrystal-shaped twins, are prepared in hydrogels.

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Notes and references

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† Electronic Supplementary Information (ESI) available: The cif file, Cryo-SEM image and crystal structure of the crystal prisms of CA, Optical micrographs of the hexagonal crystals of CA. See DOI: 10.1039/b000000x/

‡ CA-4EG (50 mg) was dissolved in MeOH (0.25 mL), followed by the addition of water (2.25 mL) and an acid-hydrolyzing agent hydrochloric acid (0.2 M, 2.5 mL). A hydrogel formed first, and then the gel-to-sol transition took place in a water bath (55 °C). After 3 h the water bath was cooled down slowly to room temperature. The new hydrogel formed was apparently weaker than the original hydrogel, indicating partially acidic hydrolysis of the gel fibers of CA-4EG. The acidified hydrogel shrunk gradually and expelled transparent aqueous solutions (syneresis), and finally the shrunk gel collapsed. These suggest the remaining gel fibers of CA-4EG were also acid-hydrolyzed gradually at room temperature. Both amorphous solids and hexagonal crystals of CA were found in aqueous solutions (Fig. S4). X-ray single-crystal diffraction measurement was performed on a Rigaku MM-007 diffractometer (λ = 1.54178 Å) radiation at 100 K. The structure was solved by direct methods: SHELXS-97 and refined using the full-matrix least-squares technique with SHELXL-97.

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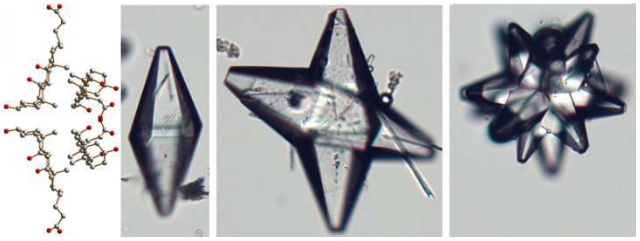

Hydrophobic swallowtail-shaped tetramers of cholic acid crystallize into X- and polycrystal-shaped twins.