"Structured Nuclei" of 4-(Octadecyloxy)benzoic Acid Monolayer for Induced Nucleation of 4-Hydroxybenzoic Acid Monohydrate As Determined by Grazing Incidence X-ray Diffraction on the Aqueous Solution

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Received: July 25, 1994; In Final Form: December 19, 1994[®]

A monolayer of 4-(octadecyloxy)benzoic acid at the air-solution interface has been used to induce oriented nucleation of three-dimensional (3-D) crystals of 4-hydroxybenzoic acid (HBA) monohydrate.¹ The twodimensional (2-D) crystalline structure of this monolayer in a self-assembled uncompressed state, both on a water subphase and on a solution containing HBA, was determined by grazing incidence X-ray diffraction (GID) using synchrotron radiation. Crystalline domains of large coherence length were formed on water subphase at 14 °C, and the fraction of the monolayer in the ordered state was much increased on HBA solution. The agreement between the observed and calculated GID data was found to be sensitive to the conformation and orientation of the monolayer molecules. These results demonstrate the ordered binding of the HBA solute to the amphiphile benzoic acid head groups to form hydrogen-bonded cyclic dimers between the two carboxylic acid moieties. The GID method was complemented by other surface sensitive techniques such as specular X-ray reflectivity, surface pressure—area isotherms, and nonlinear optics. A resulting model arrangement of the monolayer, based on the molecular packing, has been proposed for the induced nucleation of the 3-D crystals of 4-hydroxybenzoic acid monohydrate attached to the monolayer.

Introduction

Transfer of structural information from two-dimensional (2-D) to three-dimensional (3-D) organic molecular systems at liquid interfaces can probe the arrangement of molecular aggregates at the solution interface.² Recent studies have provided evidence that water-soluble hydrophobic molecules induce fast and oriented crystallization of suitable cosolute molecules at the solution surface. Making use of hydrophobic α -amino acids as additives, to the aqueous glycine solution, made it possible to induce nucleation of the α -form of glycine at the solution surface.³ It was proposed that the additives at the solution surface form ordered aggregates composed of a 2-D net of hydrogen-bonded molecules that match the structure of a-glycine, leading to induced nucleation thereof.⁴ Waterinsoluble α -amino acid amphiphiles were shown by grazing incidence X-ray diffraction (GID) to form 2-D crystalline aggregates arranged in a manner akin to the α -glycine layer structure.^{5,6} The 2-D crystal structure of monolayers of long chain aliphatic alcohols, which promote ice nucleation,⁷⁻⁹ has also been elucidated by GID.

In the bilayer structures of glycine and of ice, the molecules are interlinked by a net of hydrogen bonds. We extended this approach to other systems whereby the molecules in the layer are connected by both hydrogen bonds and van der Waals contacts. Therefore we investigated the role played by additive 4-methoxybenzoic acid for inducing oriented crystallization of 4-hydroxybenzoic acid monohydrate (HBA).¹ This study was complemented by the use of water-insoluble 4-substituted benzoic acid amphiphiles for induced nucleation of HBA. The structural changes were monitored by optical second-harmonic generation (SHG), a surface sensitive technique which can detect the presence and orientation of molecules at interfaces.^{10,11} Here we report the two-dimensional structure of a selfassembled monolayer of 4-(octadecyloxy)benzoic acid on a pure water subphase and on an aqueous solution containing HBA, as determined by grazing incidence X-ray diffraction (GID) using synchrotron radiation and complemented by specular X-ray reflectivity. The GID results provide a clear correlation between the 2-D structure of the monolayer on pure water and bound to HBA solute molecules and the layer structure of the to-be-nucleated HBA crystals. This analysis gives insight into the induced nucleation process of HBA.

Experimental Section

Materials. 4-Hydroxybenzoic acid 99+%, 4-methoxybenzoic acid, 4-hydroxybenylacetic acid, 4-methylbenzoic acid, 4-fluorobenzoic acid, and 4-*tert*-butylbenzoic acid were used as commercially available. The monolayer compounds were synthesized as follows.

4-(Hexadecyloxy)benzoic acid, 4-(octadecyloxy)benzoic acid, 4-(hexadecyloxy)phenylacetic acid, and (4-(hexadecyloxy)phenyl)propionic acid were prepared by slow addition of the alkyl bromide to the corresponding 4-hydroxybenzoic, -phenylacetic, and -phenylpropionic acids disolved in KOH/ethanol in the presence of catalytic amounts of NaI.¹²⁻¹⁴ After 5 h reflux, the reaction mixture was poured into cold water and acidified and the precipitated product filtered and recrystallized three times from ethanol.

4-(Hexadecylamino)benzoic acid was prepared by the reaction of hexadecyl bromide with ethyl aminobenzoate in hexamethylphosphoramide at 125 °C, followed by basic hydrolysis of the ethyl ester and repeated recrystallizations from ethanol.¹⁵

Octadecyl 4-hydroxybenzoate was prepared by the reaction of 4-hydroxybenzoic acid with stearyl alcohol in the presence of DCC and (dimethylamino)pyridine, followed by separation on an alumina column eluted with hexane/ethyl acetate (4:1).

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[®] Abstract published in Advance ACS Abstracts, March 15, 1995.

SIDE VIEW



TOP VIEW

Figure 1. Side and top view of the grazing incidence X-ray diffraction geometry. The footprint of the grazing incident beam is indicated by the shaded area. The position sensitive detector (PSD) has its axis along the vertical. Only the cross-beam area ABCD contributes to the measured scattering. The Soller collimator consists of thin vertical absorbing foils stacked together to define the horizontal resolution of the detector.

4-(Hexadecanoyloxy)benzoic acid was prepared by the reaction of hexadecanoic acid active ester and 4-hydroxybenzoic acid followed by several recrystallizations from ethanol.

The purity of all the monolayer compounds was checked by TLC, IR, and NMR.

Crystallization Experiments. 4-Hydroxybenzoic acid monohydrate (HBA) was crystallized from aqueous supersaturated solutions (80.5 mM), at room temperature (20 °C), pure and in the presence of the soluble additives (5–10% w/w of HBA). Crystallization under the various monolayers was performed in a thermostated Langmuir trough (Fein Technik, Germany) which allowed the control of the surface pressure. Crystals were collected after about 12 h when they reached a convenient size of about 0.5 × 0.5 × 1–2 mm.

X-ray Measurements of the 4-Hydroxybenzoic Crystals were performed using a Weissenberg camera. Oscillation and zero-level Weissenberg photographs were taken in order to determine the cell dimensions and to assign the (*hkl*) Miller indices of the crystal faces.

II-A Isotherm Measurements. An automatic thermostated Langmuir trough (Lauda, Germany) was used to measure the Π -A isotherms of the various monolayers spread on water and on solutions of different concentrations.

Second-Harmonic-Generation Measurements. The SHG experimental setup used was described elsewere.¹⁶ The dye laser pulses (612 nm, duration 8 ns, energy 5 mJ, diameter 0.5 mm) polarized at 45° to the plane of incidence were directed onto the liquid surface at 65° from the normal. Reflected SHG (306 nm) was detected via gated photon counting and averaged over 2000 pulses.

Specular X-ray Reflectivity Measurements. Specular X-ray reflectivity experiments were performed on a liquid-surface reflectometer^{17,18} constructed at the Risø National Laboratory, Denmark, and mounted on a Rigaku copper rotating anode generator (operating at 10 kW) in the X-ray laboratory at the Weizmann Institute of Science. The reflectometer is equipped with a sealed, thermostated Langmuir trough and a Wilhelmy balance for surface pressure control. The measurements were performed by scanning the incident (α_i), equal to reflected (α_r), beam angles from $0.5\alpha_c$ to $20\alpha_c$, where $\alpha_c = 0.138^\circ$ is the

critical angle for total external reflection. The detection of the reflected radiation was made by a NaI scintillation counter mounted behind a graphite monochromator. The experimental results are given in the form of normalized X-ray reflectivity, R/R_f , as a function of the vertical scattering vector $q_z = (4\pi/\lambda) \sin \alpha_r$, where R_f is the "Fresnel" reflectivity expected for an interface where the electron density changes abruptly from 0 to that of water ($\rho_w = 0.334 \text{ e/Å}^3$).

Grazing Incidence X-ray Diffraction Measurements. The GID measurements on a monolayer spread on water and on 4-hydroxybenzoic acid aqueous solutions were carried out using the liquid-surface diffractometer^{18,19} at the BW1 synchrotron X-ray undulator beam line at Hasylab, DESY, Hamburg, equipped with a sealed and thermostated Langmuir trough having a Wilhelmy balance for surface pressure control (Figure 1). The synchrotron radiation beam, monochromated to a wavelength of $\lambda = 1.376$, by reflection from a Be(002) crystal in Laue geometry, was adjusted for an incidence angle $\alpha =$ $0.85\alpha_c$. The dimensions of the footprint of the incoming X-ray beam on the liquid surface were approximately $5 \times 50 \text{ mm}^2$. The diffracted radiation was collected using a position sensitive detector (PSD) mounted vertically behind horizontally collimating Soller slits (resolution $\Delta(2\theta) = 0.09^{\circ}$), in two modes. Scanning over a range along the horizontal scattering vector $q_{xy} = (4\pi \sin \theta)/\lambda$, where 2θ is the angle between the incident and diffracted beams, as shown in Figure 1, and integrating over the $q_z = (2\pi \sin \alpha_f)/\lambda$ window of the PSD, the scattered intensity yields Bragg peaks. Simultaneously, the scattered intensity recorded in channels along the PSD but integrated over the horizontal scattering vector across a Bragg peak produces q_z resolved scans called Bragg rod intensity profiles.

The 2θ positions of the Bragg peaks yield the repeat distances $d_{hk} = 2\pi/q_{hk}$, and the peaks were assigned with $\{h,k\}$ Miller indices to calculate the unit cell.

The full width at half maximum (fwhm) of the Bragg peaks, which is resolution corrected,

$$B = [fwhm^2 - \Delta^2(2\theta)]^{1/2}$$

(where fwhm and Δ are in radians)

is associated with the coherence length of the 2-D crystalline



Figure 2. (top) Packing arrangement of 4-hydroxybenzoic acid (HBA) monohydrate crystal with the (401) face viewed edge-on, as delineated by the crystal faces. (bottom) Photograph of an HBA crystal nucleated by the monolayer of 4-(octadecyloxy)benzoic acid amphiphile at the solution surface.

domains CL according to the Scherrer formula:

$$CL = 0.9\lambda/B\cos\theta_{hk}$$

The variation of the intensity in the peak I_{hk} along the Bragg rods as a function of vertical scattering vector q_z is determined by the square of the molecular structure factor $|F_{hk}(q_z)|^2$, which is calculated assuming an atomic coordinate model:

$$F_{hk}(q_z) = \sum f_j \exp\{i(\mathbf{q}_{hk}\mathbf{r}_j + q_z z_j)\}$$
$$I_{hk}(q_z) = KLPA_{ABCD}V(q_z)|F_{hk}(q_z)|^2 \exp(-q^2\sigma^2)$$

where f_j is the scattering factor of atom j, $\mathbf{r} = x_j \mathbf{a} + y_j \mathbf{b}$ is the vector specifying the (x,y) position of the atom j in the unit cell of dimensions a and b; z_j is the atomic coordinate along the vertical direction; $\mathbf{q}_{jk} = 2\pi \mathbf{d}^* = 2\pi (\mathbf{ha}^* + \mathbf{kb}^*)$ is the reciprocal lattice vector, \mathbf{a}^* and \mathbf{b}^* the reciprocal vectors of the unit cell vectors \mathbf{a} and \mathbf{b} ; $V(q_z)$ describes the interference of X-rays diffracted upward with those diffracted down and reflected back up by the interface.²⁰ The Debye–Waller factor DW = $\exp(-q^2\sigma^2)$ accounts for the thermal motion of the atoms in the molecule and also possible ripples on the liquid surface. Corrections for the crossed beam area A_{ABCD} , Lorenz L, and polarization P were also taken into consideration.

The molecular chain orientation in the 2-D crystal can be obtained from the position of the maximum intensity $q_z(\max)$ along each Bragg rod. The tilt angle t between the molecular axis and the normal to the liquid surface is given by

$$\cos \psi_{hk} \tan t_{hk} = q_z(\max)/|q_{hk}|$$

where ψ_{hk} is the azimuthal angle between the molecular tilt direction projected onto the *xy* plane and the reciprocal lattice vector q_{hk} .

Results and Discussion

Crystallization Experiments. 4-Hydroxybenzoic acid monohydrate (HBA) grows from aqueous solutions (usually at the bottom of the crystallizing dish) as $\{100\}$ platelike crystals elongated in the *c* direction. Addition of small amounts of 4-methoxybenzoic acid (MBA) to the crystallizing solution induces fast nucleation of HBA floating at the surface. These crystals exhibit a well-developed (401) face through which they are attached at the interface (Figure 2), and their morphology is very different from the plates growing at the bottom of the dish. Additives such as benzoic acid and 4-methyl-, 4-fluoro-, and 4-*tert*-butylbenzoic acids do not induce the above-mentioned effect.

These results suggested the following correlation: the crystal structure of HBA²¹ belongs to the space group $P2_1/a$ with cell dimensions a = 17.75 Å, b = 6.44 Å, c = 6.73 Å, and $\beta = 105^{\circ}$. The packing arrangement is composed of layers of hydrogen-bonded dimers with the molecules lying parallel to the (401) plane. These dimers are interlinked within this plane by hydrogen bonds involving the phenolic OH groups and water molecules. The induced crystallization of HBA at the air-solution interface can be rationalized by assuming that the additive molecules, which are more hydrophobic than HBA, tend to accumulate at the solution surface and so may facilitate ordered binding of HBA solute molecules, leading to the formation of layered aggregates of structure which mimics that of the (401) face of HBA.

In order to elucidate the mechanism by which the nucleation of HBA occurs, long chain insoluble amphiphilic molecules 1-6 were designed with the XC₆H₄COOH moiety (X = O, NH) as their hydrophilic head group and used as Langmuir films (Table 1). The amphiphiles 1, 2, or 3, when spread on supersaturated HBA solutions, also induced (401) oriented nucleation of HBA crystals at the air-solution interface. But amphiphiles of slightly different structures 4, 5, or 6 did not induce HBA crystallization at the interface. Similarly, crystallization experiments under palmitic acid monolayers, $CH_3(CH_2)_{14}COOH$, did not show any induction.

These results indicate that only those amphiphiles containing the $-XC_6H_4COOH$ head group moiety similar to that of HBA are efficient nucleators of the latter, consistent with the proposed mechanism.



Figure 3. Π -A isotherms of 4-(hexadecyloxy)benzoic acid (1a) monolayer spread on various subphases: (a) pure water; (b-d) HBA solutions of concentrations (b) 18 mM, (c) 32 mM, and (d) 57 mM.



Figure 4. Π -A isotherms of 4-(hexadecylamino)benzoic acid (2) monolayer spread on various subphases: (a) pure water; (b-f) HBA solutions of concentrations (b) 14 mM, (c) 20 mM, (d) 28 mM, (e) 57 mM, and (f) 80.5 mM (solution for crystallization).

II-A Isotherms. The difference in crystallization behavior of the two groups of amphiphiles is also reflected in their Π -A isotherms. All the amphiphiles, when spread over pure water, form monolayers consisting of molecules oriented almost perpendicular to the surface, as determined from the limiting area per molecule. When amphiphiles 1, 2, or 3 are compressed over a supersaturated solution of HBA, more expanded isotherms with a larger limiting area per molecule were obtained. This effect is dependent on the subphase concentration, and Figures 3, 4 show the gradual change in the isotherms of the amphiphiles 1 and 2, as induced by increased concentrations of HBA solution. In contrast, there is no change in the shape of the isotherms of the amphiphiles 4, 5, or 6, when spread on HBA solutions, in keeping with the fact that their hydrophilic head groups behave differently from the amphiphiles that are efficient nucleators of HBA (Figure 5).

Moreover, the isotherms of the amphiphiles 1, 2, and 3, when spread on solutions containing 4-hydroxyphenylacetic acid (a solute molecule somewhat different in structure from HBA), remain unchanged compared with the pure water subphase. These results indicate again that the changes induced in the isotherms are specific to the nature of the solute molecules.

Second-Harmonic-Generation Measurements. In order to independently examine the specific interactions between the amphiphiles 1a and 2 and the HBA solute molecules, we have

phenylacetic acid (4) and (b) (4-(hexadecyloxy)phenyl)propionic acid (5) spread on pure water (--) and on 80.5 mM HBA solution (---). TABLE 2: SHG Signals and Orientations for Monolayers

Spread on Various Subphases ^a										
		on water		on HBA						
monolayer	I45-s	L _{45-p}	$\langle \theta \rangle$	I_{45-s}	I45-p	$\langle \theta \rangle$				
bare subphase	1	0.6		0.4	0.8					
1a -	10	40	24	b						
1a , 80% cov	7	30	24	7	30	24				
2	450	150	42	с						
6	150	40	47	150	40	24				

^{*a*} I_{45-s} and I_{45-p} are the SHG signals polarized perpendicular (s) and parallel (p) to the plane of incidence generated by an input laser polarized at 45^{*c*}. ^{*b*} Very weak signal (see text). ^{*c*} Over time scales of minutes, fluctuations of SHG signals were observed; however, the time average signal was still very weak (see text).

studied these systems with second-harmonic generation (SHG). This is a surface specific technique which can determine the presence and orientation of surface adsorbates on centrosymmetric and isotropic substrates. Amphiphiles **1a**, **2**, and **6** spread on water all exhibited SHG signals considerably stronger than that of the bare water subphase. From the ratio of the s- and p-polarized SHG outputs, the average angle $\langle \theta \rangle$ between the surface normal and the long axis of the XC₆H₄COOH group (X = 0, NH) was calculated, taking a value of n = 1.5 for the refractive index of the monolayer (Table 2). These $\langle \theta \rangle$ values refer to the orientation of the polar XC₆H₄COOH head group, but not necessarily to that of the hydrocarbon chain.

The bare HBA solution had an SHG intensity similar to that of water. When a monolayer of **6** was spread on the HBA solution, its SHG signal and $\langle \theta \rangle$ values were identical to those found on the pure water subphase, in agreement with the isotherm and the crystallization experiments. When a monolayer of **1a** was spread over the saturated HBA solution at room temperature (65 mM), the SHG signal was generally very weak, equivalent to, at most, a few percent of its signal on water. When a monolayer of **2** was spread over saturated HBA solution at room temperature, large fluctuations of the SHG signal were observed over time scales of minutes. The SHG signal varied between the background level and (for short periods of time) the monolayer signal. However, the time average signal was still very weak. Spreading a monolayer of **2** over a less



Figure 6. Measured (points) and calculated (full line) reflectivity curves expressed as normalized R/R_f (where R_f is the Fresnel reflectivity) for the 4-octadecyloxybenzoic acid monolayer (**1b**), in an uncompressed state at 14 °C, spread on (a) water subphase (\Box) and (b) 32.6 mM HBA solution (Δ). Note that the reflectivity measurements were carried out in conditions represented by the arrow on the isotherms shown in Figure 7.



Figure 7. Π -A isotherms of 4-(octadecyloxy)benzoic acid monolayer (1b) spread on various subphases: (a) pure water; (b) 32.6 mM HBA solution. The arrow represents the point on the isotherms where the reflectivity and GID measurements (Figures 6, 10, and 11) were performed.

TABLE 3: Fitted Parameters for the4-(Octadecyloxy)benzoic Acid Monolayer Spread on Waterand on 32.6 mM 4-Hydroxybenzoic Aqueous Solution in aThree-Box Model

monolayer (subphase)	cov	$A_{m}{}^{a}$	N_1	L_1	N_2	L_2	N ₃	L_3	σ	t°
1b (water)	0.7	23	43	4.0	40	3.5	153	20	2.9	29
1b (solution)	0.9	27	72 ^b	6.5	40	3.0	153	18	2.7	43

 ${}^{a}A_{m}$ is in Å², N in electron units, L in Å, and σ , the surface roughness parameter, in Å². b Considering 70% bound solute molecules.

concentrated HBA solution (32.6 mM) yielded more stable SHG signals, quite similar to the signals on pure water. Lowering the temperature to 9 °C did not produce significant changes.

The absence of significant SHG from the monolayer of 1a on HBA solution may be interpreted as consistent with either of two possibilities: (i) the XC_6H_4COOH groups of amphiphiles 1a lie almost flat ($\langle \theta \rangle \sim 90^\circ$) on the surface of the solution with random domain orientations in the xy plane, or (ii) the XC_6H_4 -COOH moiety forms a pseudo-centrosymmetric hydrogenbonded dimer with HBA. Since the nonlinearities per molecule of 1a and 2 differ by more than a factor of 3, the latter proposal should not lead to cancellation of the SHG signals in the case of amphiphile 2. The fluctuating SHG signals from the monolayer of amphiphile 2 on saturated HBA solution may reflect the dynamic behavior of the monolayer over domains comparable in size to the laser diameter (several hundred microns). Interpreting the instances of low average SHG as representative of stable domains of monolayer of amphiphile 2 on saturated HBA solution leads to the conclusion that the molecules lie flat on the surface. If, however, the fluctuations



Figure 8. Measured (points) and calculated (full line) reflectivity curves, R/R_t , for the 4-(octadecyloxy)benzoic acid monolayer (1b), in an uncompressed state at 14 °C, spread on (a) water subphase (\Box) and (b) 30 mM 4-hydroxyphenylacetic acid solution (\triangle). Note that the reflectivity measurements were carried out for the monolayer spread for an area per molecule of 24 Å² and zero surface pressure for both subphases.



Figure 9. Measured (points) and calculated (full line) reflectivity curves, R/R_t , for the 4-(octadecyloxy)phenylacetic acid monolayer (4), in an uncompressed state at 14 °C, spread on (a) water subphase (\Box) and (b) 32.6 mM HBA solution (Δ).

TABLE 4: Fitted Parameters for the4-(Octadecyloxy)benzoic Acid Monolayer Spread on Waterand on 30 mM 4-Hydroxyphenylacetic Aqueous Solution in aThree-Box Model

monolayer (subphase)	cov	Am	N_1	L_1	N ₂	L_2	N ₃	L_3	σ	ť°
1b (water)	0.96	23	23	2.5	40	3.5	153	20	2.7	29
1b (solution)	0.96	23	65	8.0	40	3.0	153	20	3.4	29

arise due to monolayer instability, the SHG results are insufficiently conclusive to distinguish between the two possible monolayer structures.

The drastic decrease in the signal generated from the monolayer of **1a** is very specific to the nature of the solute molecules, as was demonstrated by an experiment in which HBA was replaced by 4-hydroxyphenylacetic acid. SHG measurements showed the same signal as on water. Since the second order nonlinearity per molecule of 4-hydroxyphenylacetic acid is very small, we infer from the absence of change in SHG that the molecular orientation in the monolayer is unchanged. We shall show in the next section that 4-hydroxyphenylacetic acid molecules are bound to the monolayer.

Specular X-Ray Reflectivity Measurements. In order to explain the observed changes in the Π -A isotherm of the 4-(octadecyloxy)benzoic acid amphiphile (1b) spread on the 4-hydroxybenzoic acid solutions of various concentrations and



Figure 10. GID measurement of a monolayer of 4-(octadecyloxy)benzoic acid (1b), in an uncompressed state, at 14 °C, on the water surface. (a) Bragg peaks along the horizontal scattering vector q_{xy} . (b,c) Observed (points) and calculated (continuous curve) Bragg rod intensity profiles along the vertical scattering vector q_z for each of the two reflections $\{1,1\} + \{1,\bar{1}\}$ and $\{0,2\}$, respectively.

TABLE 5: Fitted Parameters for the4-(Hexadecyloxy)phenylacetic Acid Monolayer Spread onWater and on 32.6 mM 4-Hydroxybenzoic Aqueous Solutionin a Three-Box Model

monolayer (subphase)	cov	Am	N_1	L_1	N ₂	L ₂	N ₃	L_3	σ	ť°
4 (water)	0.96	23	33	3.5	48	4.5	139	18	2.9	38
4 (solution)	0.96	23	33	4.0	48	4.5	139	18	2.9	38

to correlate such changes with specific binding of the solute, we have performed X-ray reflectivity experiments. All the measurements were carried out at 14 °C and on the monolayer in an uncompressed state at $\Pi = 0$ mN/m.

Figure 6 shows the X-ray reflectivity curves of the monolayer **1b** spread on water and on a 32.6 mM 4-hydroxybenzoic acid aqueous solution for a surface area per molecule of 32.5 $Å^2$, corresponding to an uncompressed state but with expected interaction between the monolayer head groups and the solute molecules (see isotherm, Figure 7). The calculated curves (full



Figure 11. GID measurement of a monolayer of 4-(octadecyloxy)benzoic acid (1b), in an uncompressed state, at 14 °C, spread on the surface of a 32.6 mM HBA solution. (a) Bragg peaks along the horizontal scattering vector q_{xy} (b,c) Observed (points) and calculated (continuous curve) Bragg rod intensity profiles along the vertical scattering vector q_z for each of the two reflections. Note that $\{1,1\}$ + $\{1,\overline{1}\}$ Bragg rod profiles (lines) were calculated assuming various amounts of bound HBA solute molecules, namely, 0% (curve with lowest maximum), 40%, and 100% (curve with highest maximum).

line) were obtained assuming a three-box model (Table 3). For the case of the monolayer spread on water, the first box (of length L_1 and containing N_1 electrons) models the carboxylic acid head group of the monolayer to which water molecules are bound, the second box (of length L_2 and containing N_2 electrons) models the rigid aromatic ring ($-C_6H_4-$), and the third one models the chain ($C_nH_{2n+1}O-$). The reflectivity curve measured on water was modeled assuming close packed domains of the amphiphile with a molecular surface area of 23 Å² (derived from the GID measurements described below) and a coverage of 70%. The fitted parameters, L_1 , L_2 , and L_3 , and surface roughness, σ , are given in Table 3 together with the number of electrons of each molecular section. The molecular tilt (t) of 29°, derived from the monolayer thickness ($L_2 + L_3$), is in good agreement with the SHG measurement. The fit was found to be sensitive in box length L to changes greater than 0.3-0.4 Å; thus Table 3 shows these lengths to the nearest 0.5 Å.

Reflectivity curves measured for the monolayer **1b** spread on the solution were best modeled for 70% of the carboxylic acid head groups bound to the HBA solute molecules and an area per molecule of 27 Å². As a consequence, the monolayer molecules were found to increase their tilt angle from 29° to 43°.

The reflectivity curve was found to be dependent on the nature of the solute molecules bound to the carboxylic acid head groups of the monolayer. The Π -A isotherm of the same monolayer 1b spread on a solution of 4-hydroxyphenylacetic acid (30 mM) did not change as compared to the isotherm on water. Therefore, the reflectivity measurements were carried out with the monolayer spread for an area per molecule of 24 $Å^2$, corresponding to a point on the isotherm where a zero surface pressure was still measured. The experimental and calculated reflectivity curves for the monolayer 1b spread on water and on the solution of 4-hydroxyphenylacetic acid are shown in Figure 8. Both curves could be modeled assuming the threebox model with the molecule occupying an area of 23 $Å^2$, and a tilt of about 29° (Table 4), in agreement with the same position of the first minimum at $q_z = 0.22 \text{\AA}^{-1}$, which is dependent on the total thickness of the monolayer. This result is also compatible with the SHG measurements on this subphase. The lower maximum at $q_z = 0.33$ Å⁻¹ for the reflectivity curve measured on the 4-hydroxyphenylacetic acid solution can be attributed to partial binding (40-50%) of solute molecules (Table 4).

The role played by the $-OC_6H_4COOH$ moiety for strong binding to the HBA solute molecules could be probed by X-ray reflectivity measurements of a monolayer of 4-hexadecyloxyphenylacetic acid (4) spread on water and on HBA solution (Figure 9). The reflectivity curves, which were modeled by assuming the three-box model, are the same, proving insignificant binding of HBA to the monolayer (Table 5).

Grazing Incidence X-Ray Diffraction (GID) Measurements. The GID patterns were measured for the 4-(octadecyloxy)benzoic acid monolayer (1b), spread on water and on a 32.6 mM 4-hydroxybenzoic acid aqueous solution, at 14 °C. The measurements were performed on the monolayer in an uncompressed state, at a nominal area per molecule of 32 Å² and zero surface pressure because, according to the II-A isotherms and the reflectivity measurements (Figures 6-8), this point of the isotherm (Figure 7) corresponds to a state in which the solute molecules are bound to the monolayer head groups. Indeed, this is brought out by a comparison of the GID patterns of the monolayer on the two subphases. The GID patterns of the two systems (Figures 10, 11) are similar in the positions of their observed Bragg peaks and the shapes of their Bragg rod intensity profiles. The lateral extent of crystalline order (i.e. coherence length) as derived from the widths of the Bragg peaks are in the range 600 Å to minimally 1000 Å. The monolayer spread on solution yielded Bragg peaks about 4 times as intense as the monolayer on pure water. Therefore, we could conclude that the structures of the monolayer on both subphases are basically similar and attribute the 4-fold increase in intensity to the effect of ordered binding of the solute molecules on the number of molecules in the crystalline state.

The two Bragg peaks were assigned as $\{1,1\} + \{1,\overline{1}\}$ for the reflection at $q_{xy} = 1.32 \text{ Å}^{-1}$ and $\{0,2\}$ for the reflection at $q_{xy} = 1.69 \text{ Å}^{-1}$, resulting in a rectangular cell of dimensions a = 6.2 Å and b = 7.4 Å and a molecular area of 23 Å². Such an assignment²² implies that the molecules are tilted along the



Figure 12. Effect of the molecular orientation on the calculated $\{1,1\}$ + $\{1,\overline{1}\}$ Bragg rod intensity profile. (a) Measured $\{1,1\} + \{1,\overline{1}\}$ Bragg rod. (b,c) Calculated $\{1,1\} + \{1,\overline{1}\}$ Bragg rod assuming two molecular orientations differing by 180° about the molecular axis. Note that only one of these two molecular orientations fits the local maximum at $q_z = 0.3 \text{ Å}^{-1}$. Drawings of part of the molecule in the two orientations are shown as insets.

a axis by an angle of about 35° from the normal to the liquid surface, in agreement with the intensity profile of $\{1,1\} + \{1,\overline{1}\}$ Bragg rod, which has a maximum intensity at $q_z = 0.7$ Å⁻¹.

The dimensions of the cell as projected along the chain axis (i.e. $a_p = a \cos 35$, $b_p = b$), $a_p = 5.1$ Å, and $b_p = 7.4$ Å, are consistent with the commonly observed orthogonal, OL, packing motif of hydrocarbon chains.²³ Therefore, the plane group of the monolayer structure is p11g,²⁴ with molecules tilted along the *a* axis and the glide plane parallel to the tilt direction. The Bragg rod intensity profiles along the vertical scattering vector q_z were calculated on the basis of a molecular model constructed from the structure of 4-(decyloxy)benzoic acid²⁵ in its 3-D crystal and assuming either gauche or trans conformations about



Figure 13. Packing arrangement of the 4-(octadecyloxy)benzoic acid monolayer on pure water subphase: (a,b) views along the a and b axes; (c) view perpendicular to the water surface.



Figure 14. Packing arrangement of the 4-(octadecyloxy)benzoic acid monolayer with bound HBA solute molecules: (a,b) views along the a and b axes; (c) view perpendicular to the solution surface. For clarity, the head group of one chain was removed.

the alkyl–O–aryl ether moiety. The Bragg rod intensity profiles were best fitted with a molecular model of *trans* conformation and a rotation of the $-OC_6H_4COOH$ moiety by 20° out of the plane of the hydrocarbon chain and a molecular tilt of 35.6° (Figure 10b,c). The molecular packing arrangement is shown in Figure 13.

It is noteworthy that the Bragg rod profiles are very sensitive to the orientation of the molecule about its long axis. Figure 12 shows the calculated $\{1,1\} + \{1,\overline{1}\}$ Bragg rod intensity profile for two molecular models²⁶ differing in orientation by 180° about the molecular axis. Only one molecular orientation gives a calculated profile which fits the intensity modulation observed at $q_z = 0.3$ Å⁻¹.

As alluded to above, the structure of the monolayer **1b** spread on the aqueous solution of 4-hydroxybenzoic acid must be very similar to that on pure water, except for the contribution of the bound solute molecules. We therefore constructed a model in which the monolayer amphiphilic molecules form a planar hydrogen-bonded cyclic dimer with the solute molecules, akin to that found in the 3-D crystal structures of carboxylic acids. The Bragg rod intensity profiles calculated using this model yielded a good fit to the measured profiles for a 40% occupancy of the bound solute molecules and a 36° molecular tilt, as shown in Figure 11b,c. The packing arrangement of the monolayer with bound solute molecules is shown in Figure 14. According to this packing arrangement, more than 50% solute occupancy would be impossible because of interpenetration between neighboring HBA molecules bound to the monolayer.

Proposed Model of Induced Nucleation. On the basis of the two-dimensional crystal structure of the monolayer 1b spread on aqueous HBA solution, we propose the following mechanism for the oriented nucleation of 3-D crystals of 4-hydroxybenzoic acid monohydrate. The monolayer molecules form ordered rows separated by a 6.2 Å translation along the a direction (Figure 15b), a distance similar to the 6.4 Å translation separation of the phenyl rings in the crystal structure of 4-hydroxybenzoic acid monohydrate (Figure 15a). Furthermore, two such neighboring molecular rows in the monolayer, related by glide symmetry along the a axis and separated by 3.7 Å, can be regarded as pairs of rows which bind the solute molecules in an ordered manner. These pairs of monolayer rows with bound solute, extending several cells in the *a* direction, may be considered as "pre-formed nuclei" (Figure 15b) which resemble a corresponding pair of molecular rows on the (401) crystal face (Figure 15a). From the orientation of the crystals nucleated by the monolayer and the structure of the (401) face we must,



Figure 15. Proposed mechanism to explain the oriented nucleation of HBA crystals as induced by the 4-(octadecyloxy)benzoic acid monolayer. (a) Packing arrangement of a layer of HBA molecules in the monohydrate crystal viewed perpendicular to the (401) face. (b) Packing arrangement, as viewed perpendicular to the solution surface, of a pair of rows of cyclic hydrogen-bonded dimers formed between molecules of 4-(octadecyloxy)benzoic acid monolayer and the bound HBA solute. Each row contains three molecular units related by 6.2 Å translation along *a*, and the two rows are related by glide (*g*) symmetry. For clarity, only part of the hydrocarbon chains are shown. (c) Proposed rearrangement of the pair of rows of monolayer molecules to serve as a template for HBA crystal nucleation. Note the similarity to the corresponding pair of rows in (a).

however, assume a molecular reorientation of the "pre-formed nuclei" in the early stages of 3-D crystallization so that the pair of rows best mimic the (401) crystal layer exposed at the solution surface (Figure 15c). This reorientation involves a rotation of the cyclic dimers formed by the amphiphilic head group and bound solute about the $H_2C(chain)-O(C_6H_4)$ bond of 1b so as to bring the cyclic dimers parallel to the solution surface. Such a rearrangement can occur only within the pair of rows along the *a* direction and implies partial destruction of the monolayer structure along the *b* direction. This model is in keeping with the SHG experiments, according to which the monolayer $-XC_6H_4COOH$ head group bound to HBA lies parallel to the solution surface. This model is also in agreement

with the X-ray specular reflectivity results, according to which the HBA molecules are bound to the monolayer.

As mentioned above, this model implies partial destruction of the monolayer structure during the nucleation process of HBA. On this basis nucleation of HBA occurs in a cooperative way with the reorientation of the amphiphilic head groups within the pairs of rows. What is still unknown is whether nucleation of HBA involves one pair of molecular rows or an ensemble of neighboring pairs of rows.

Conclusion

Grazing incidence X-ray diffraction (GID) using synchrotron radiation was used to determine the two-dimensional crystal structure of a monolayer of 4-(octadecyloxy)benzoic acid, including the orientation and conformation of the constituent molecules, on water and on solutions containing 4-hydroxybenzoic acid. Analysis of the GID data proved that the HBA solute molecules are specifically bound to the monolayer head groups to form hydrogen-bonded cyclic dimers. This result is in agreement with the much greater amount of monolayer in the 2-D state over HBA solution than over water. The GID experiments were complemented by X-ray reflectivity, surface pressure-area isotherms, and optical second-harmonic-generation measurements. The derived packing arrangement of the monolayer led to a molecular model to account for the oriented nucleation of three-dimensional crystals of HBA at the airsolution interface. This is the first molecular system where the contribution of the HBA solute molecules bound to the monolayer head group was measured, in situ, although it did not prove possible to monitor the process of 4-hydroxybenzoic acid monohydrate crystal nucleation.

Acknowledgment. We are grateful to Mrs. E. Shavit for synthesis of some of the compounds. We thank the Israel Academy of Basic Science and Humanities, the Minerva Foundation, Münich, Germany, and the Danish Foundation for Natural Sciences for financial support. We thank HASYLAB, DESY, Hamburg, Germany, for beam time.

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JP941918D