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The Effect of Additives on Glycine Crystal Growth Kinetics

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The effect of α-amino acids ("tailor-made" additives) on the morphology and nucleation of glycine crystals was studied in batch experiments, and the effect of L-leucine on glycine crystal growth kinetics was investigated in flow cell experiments at constant supersaturation. From dissolution and growth experiments in a flow cell system, L-leucine was found to adsorb onto the $(0\bar{1}0)$ face of glycine crystals and to inhibit the growth of that face, modifying the crystal habit. At high L-leucine concentrations (>8 mg/ml), the growth rate of the (010), (011), and $(01\bar{1})$ faces was faster than that in the absence of additive. This may be the result of the effect L-leucine has on the crystal/solution interfacial tension and on the aggregation of glycine in solution. Oriented nucleation and growth of glycine crystals on the $(0\bar{1}0)$ face were observed. This may be a consequence of multilayer L-leucine adsorption onto the $(0\bar{1}0)$ face, which provides a template for nucleation and growth of glycine twinned crystals about the $(0\bar{1}0)$ basal plane.

1. INTRODUCTION

The growth rate of a crystal face is determined by the interactions among the molecules in the crystal as well as by the solvent–solute interactions at the growing interface and in the bulk of the solution. These may be affected by the degree of supersaturation, nature and concentration of additives, and temperature. The shape of a crystal is determined by the relative growth rates of its faces. Faces with the slowest growth appear as large developed faces. Additives can affect the crystal growth rate by adsorbing onto the surface of a growing crystal or by altering solution properties, crystal/solution interfacial tension, and aggregation of solute molecules in solution. Anisotropic effects of additives on the growth of faces of a crystal may cause changes in its crystal habit.

Adsorption of an additive onto a crystal face may inhibit its growth depending on the growth mechanism and on the interaction of additive molecules with the crystallizing substrate. The relationship between adsorption of additives and inhibition of growth for the Burton–Cabrera–Frank (BCF) growth mechanism has been studied by Cabrera and Vermilyea (1). If the structure of an additive molecule is very similar to that of the host molecule, the additive is often referred to as a "tailor-made" additive. When additives are tailor-made, the stereoselectivity will determine favorable adsorption sites (2–4). In this case, one may be able to predict the effect an additive will have on the habit. Weissbuch and co-workers (3) calculated the binding energy at a surface site in which glycine is replaced by the additive, alanine. They found that growth inhibition of a crystal face is dependent on the chirality of the α-amino acid. L-Amino acids primarily inhibit the growth of the $(0\bar{1}0)$ face of glycine crystals, whereas D-amino acids primarily inhibit the growth of the (010) face. These predictions have been confirmed by experimental results (5). This enantioselectivity may be used to generate and amplify optical activity.

Previous work (2, 3) emphasizes the effect of α-amino acids on morphology. In the study presented here, we investigated the effect that α-amino acids have on glycine crystal growth, particularly the effect L-leucine has on the growth kinetics of glycine. We have shown that glycine crystals grow by the screw dislocation mechanism in the absence of additives (6). A flow cell system was designed to measure the growth rate of faces of single crystals as a function of supersaturation under constant conditions (temperature and solute concentration). Additive incorporation in glycine crystals was examined by measuring the release of radiolabeled L-leucine during dissolution of glycine crystals.

2. EXPERIMENTAL

Materials. Glycine and L-amino acids were obtained from Sigma, and L-[^{3}H]-leucine from Amersham.

Batch experiments. All batch experiments were conducted at room temperature (~23°C). A supersaturated solution was prepared by dissolving appropriate amounts of glycine and additives in water by heating (~80°C). The hot solution was then cooled to room temperature. Glycine nucleated and crystals grew from the unstirred solutions. The

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supersaturation ($\sigma = \ln(c/s)$) range studied was between 0.02 and 0.3, and the concentration of α-amino acids used was between 0.5 and 15 mg/ml of water. The additives studied included hydrophobic amino acids, L-alanine, L-valine, L-leucine, and L-phenylananine, and hydrophilic amino acids, L-serine and L-histidine.

Growth experiments. Glycine crystal growth was studied in aqueous solutions, in the presence of L-leucine at concentrations ranging from 1 to 12 mg/ml and at glycine supersaturation levels of 0.02 and 0.10 in the flow cell system (Fig. 1). Growth rates of crystal faces were measured by monitoring the increase of crystal size under an inverted microscope. Crystal size was measured from the image displayed on a monitor, and the growth rate perpendicular to a face was calculated. A detailed description of a flow cell system has been presented elsewhere (6). Growth experiments were carried out at 20°C. The growth solution was pumped at a flow rate of 10 ml/h corresponding to a velocity of 0.14 cm/s. The concentration of glycine was measured during the growth experiments by UV spectrophotometry (Perkin–Elmer) and by high-pressure liquid chromatography (HPLC) (Beckman).

A procedure for seed crystal preparation was developed that produced the desired size (10–50 μm) and number (<10) of seeds in a cell. In this manner, local concentration gradients in the cells were avoided. Seed crystal preparation was as follows: A 40% ethanol–water solution saturated with glycine was mixed with a saturated glycine water solution (at ~23°C) in a 4:1 ratio. This solution was then placed into the cells. After glycine crystals nucleated, and the seeds grew to a desired size, the cells were flushed with the growth solution for approximately 15 min prior to initiating growth rate measurements.

Dissolution experiments. The distribution of additive in glycine crystals grown in flow cells was determined by monitoring the amount of additive released during dissolution. L-[^{3}H]-Leucine was used in order to provide a more sensitive assay for quantifying the small amount of L-leucine incorporated in the crystals. Crystal seeds were nucleated from a growth solution, at a supersaturation of 0.04. After the growth experiment was completed, the flow cell was flushed with a saturated glycine solution. Water was then pumped into the cells, and the solution was collected and assayed. The crystal size was monitored during dissolution. L-Leucine concentration was determined using a scintillation counter (Beckman).

Solubility measurements. Glycine solubility in water at 25 ± 1°C was determined as a function of leucine concentration. An excess amount of glycine crystals was added to solutions with a known concentration of leucine. The suspension was stirred for at least 24 h until equilibrium was reached. Aliquots of the suspension were filtered through 0.2-μm filters (Acrodisc LC13, Gelman), and the concentrations of glycine and leucine were measured by HPLC. The same procedure was used to measure the leucine solubility as a function of glycine concentration.

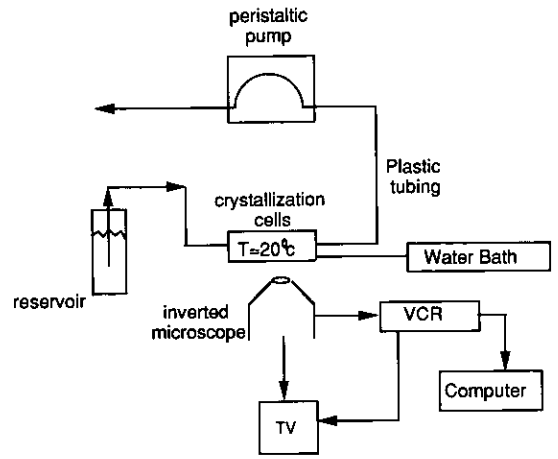

FIG. 1. Schematic diagram of the flow system used to measure growth rates of glycine crystals.

HPLC analysis. A Beckman 110B pump, with a 406 analog interface, and UV detector were used. The mobile phase (0.1 *M* NaH_2PO_4, pH 4.2) was pumped at room temperature through an ASTEC C_{18} column (5 μm spherical, 250 × 46 mm) at a flow rate of 2 ml/min and the absorbance measured at 210 nm (7).

X-ray diffraction. A Weissenberg camera was used to produce an oscillation and zero-level Weissenberg photograph. A Nickel filter was used for the zero-level Weissenberg photograph. The radiation source was Copper $K\alpha$ (λ = 1.5418 Å). A glycine crystal was mounted parallel to the *c*-axis. A two-circle optical goniometer was used to align the crystal for the photographs and to measure the interfacial angles used to determine the Miller indices of the crystal faces.

3. RESULTS

3.1. High Modifications

Weissbuch *et al.* (5) studied the effect of amino acids on the morphology of glycine crystals in batch experiments. Two types of interfaces exist in a batch experiment, a glass/solution interface and an air/solution interface. Weissbuch and co-workers (5) found that in the presence of hydrophilic additives, the majority of the crystals grew at the glass/solution interface. In the presence of hydrophobic additives, crystals nucleated and grew at both the air/solution interface and the glass/solution interface. Our results are in agreement with their observations. Glycine crystals grown from water in the absence of additive were bipyramidal as shown in Fig.

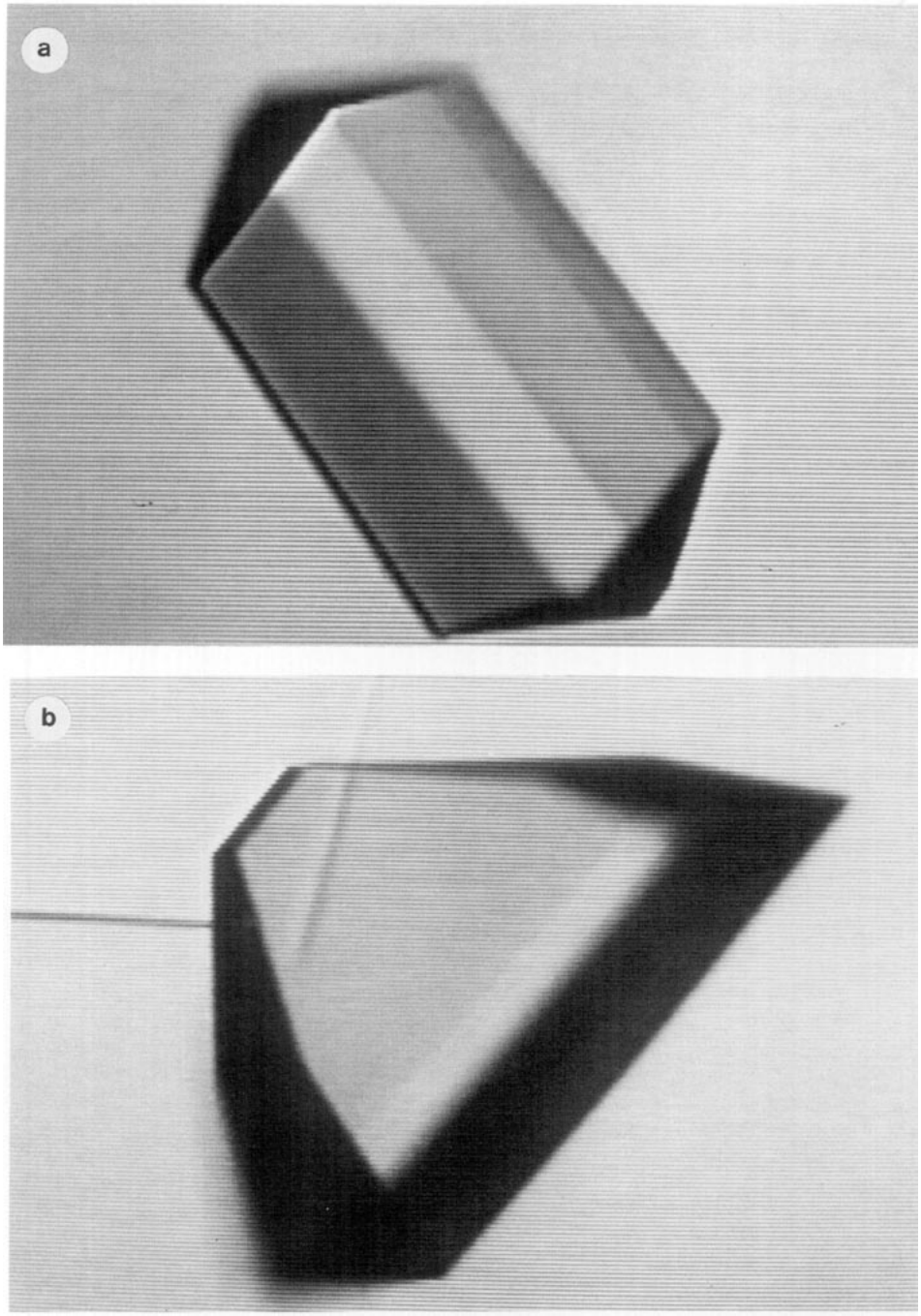

FIG. 2. Morphology of glycine crystals grown from aqueous solutions: (a) in the absence of additive, (b) at L-leucine concentrations less than 8 mg/ml, and (c) at L-leucine concentrations greater than 8 mg/ml.

2a. At α-amino acid concentrations less than 8 mg/ml, the glycine crystals were truncated into pyramids as shown in Fig. 2b. The hydrophobic amino acids were more effective in modifying the habit of glycine crystals. The α-form of glycine was obtained ($P2_1/n$; $a = 5.10$, $b = 12.0$, $c = 5.46$ Å; $\beta = 111.7°$; $Z = 4$) in both the absence and the presence of L-leucine; this was corroborated from single-crystal X-ray analysis (6).

At L-leucine concentrations greater than 12 mg/ml, the resulting crystals were bipyramidal (Fig. 2c), similar to those grown in the absence of additive (Fig. 2a). At an L-leucine concentration between 8 and 12 mg/ml, the occurrence of

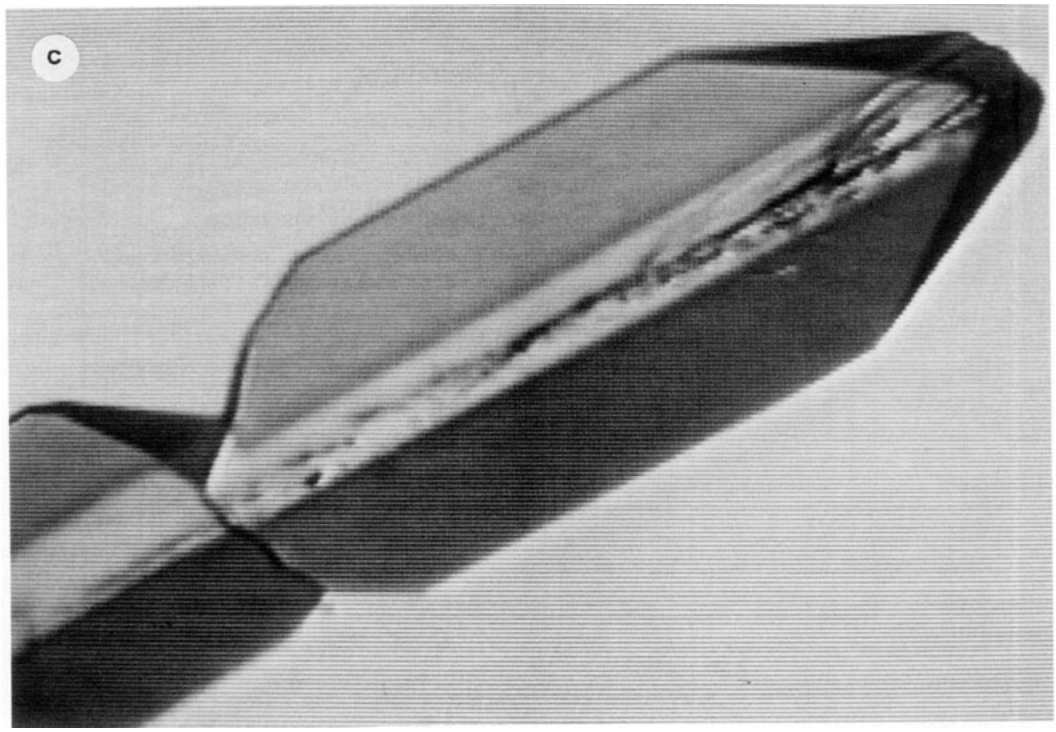

FIG. 2—*Continued*

bipyramidal crystals was dependent on the glycine supersaturation. A larger number of bipyramidal crystals was observed at higher glycine supersaturations for the same concentration of L-leucine (>8 mg/ml). There is a critical concentration of L-leucine above 8 mg/ml where a transition from a pyramidal to a bipyramidal shape occurs (Fig. 3c). This may be a consequence of the following events: (a) adhesion of two pyramidal crystals, and (b) oriented nucleation of glycine onto the $(0\bar{1}0)$ face.

To examine the hydrophobic effect on the nucleation of glycine on the $(0\bar{1}0)$ face of glycine crystals, batch experiments were done at a supersaturation of 0.3 at high concentrations (13–15 mg/ml) of L-leucine, L-phenylalanine, L-histidine, and L-serine. In the presence of L-phenylalanine, nucleation occurred on the $(0\bar{1}0)$ face; however, complete alignment along the $(0\bar{1}0)$ face was not observed. In the presence of L-histidine and L-serine, no nucleation was observed on the $(0\bar{1}0)$ face and glycine crystals were pyramidal.

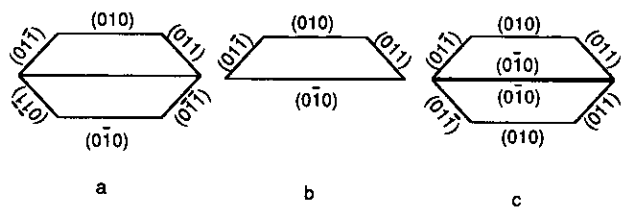

FIG. 3. Faces of glycine crystals grown from aqueous solutions: (a) in the absence of additive, (b) at L-leucine concentrations less than 8 mg/ml, and (c) at L-leucine concentrations greater than 8 mg/ml. Miller indices were obtained from the interfacial angles measured with a two-circle optical goniometer.

3.2. Effect of L-Leucine on the Growth of Glycine Crystals

In flow cell experiments, nucleation always occurred at the bottom of the cells at the glass/solution interface, as there is no air/solution interface in this system. The glycine growth rate of the $\{011\}$ and $\{010\}$ faces (Fig. 3) was measured from the outlines of crystals as a function of time. A typical standard deviation of growth rate is 30%.

The effect of L-leucine on the growth rate of glycine crystals was investigated at a supersaturation of 0.02 (Fig. 4a) and 0.10 (Fig. 4b). At both levels of supersaturation, the growth of the $(0\bar{1}0)$ face was completely inhibited at L-leucine concentrations as low as 1 mg/ml. The growth rates of (010), (011), and $(01\bar{1})$ faces did not change significantly at L-leucine concentrations less than 5 mg/ml. However, at L-leucine concentrations greater than 8 mg/ml, the growth rate of these faces was increased and nucleation was observed on the $(0\bar{1}0)$ face. At a supersaturation of 0.02 the growth rate of the (010) face increased 130%, and that of the $\{011\}$ increased 300% at an L-leucine concentration of 9 mg/ml, compared to the growth rate below 5 mg/ml of L-leucine. At a supersaturation of 0.10 and an L-leucine concentration

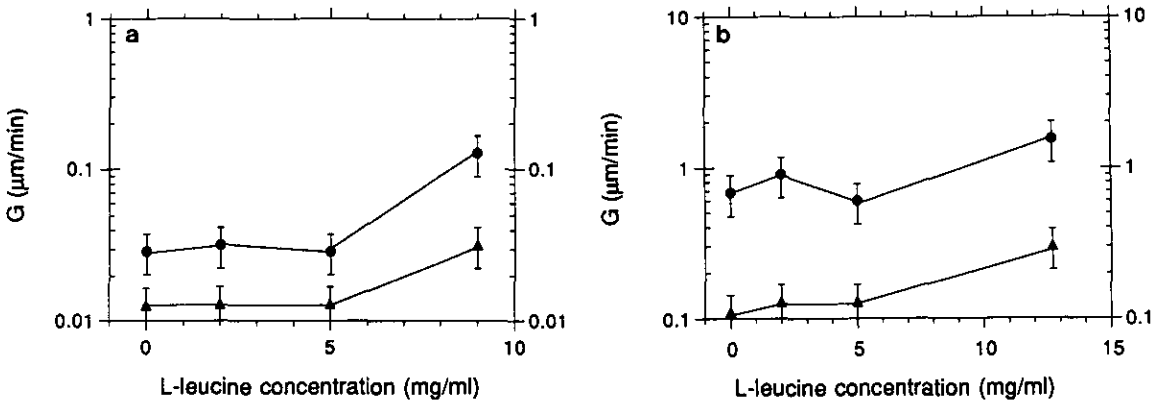

FIG. 4. Growth rate of glycine crystals as a function of L-leucine concentration: (a) supersaturation of 0.02, (b) supersaturation of 0.10. ●, {011} faces, and (▲), (010) face. Error bars represent standard deviations.

of 12.7 mg/ml, the (010) growth rate increased by 70% and the {011} by 110%.

3.3. *L-Leucine Distribution in Glycine Crystals*

The mass ratio of L-leucine to glycine is plotted against the ratio of the crystal equivalent radii during dissolution, r/R, in Fig. 5. At $t = 0$, $r/R = 1$, where R and r are the crystal equivalent radius before and during dissolution. Thus, during dissolution as the crystal size decreases, r/R becomes less than one.

L-Leucine was found on the outer layers of pyramidal crystals, grown at L-leucine concentrations below the critical concentration, as shown by the higher release of L-leucine in the early stages of dissolution. This indicates that L-leucine is mainly adsorbed on the $(0\bar{1}0)$ face since the growth rate of the (010), (011), and $(01\bar{1})$ faces was not inhibited. During dissolution of bipyramidal crystals, obtained at high L-leucine concentration, L-leucine release was constant as the crystals decreased in size. This suggests that L-leucine was mainly distributed at the center of the bipyramidal glycine crystals (interface of the two crystals). These results substantiate the concentration-dependent effect of L-leucine on glycine growth rate inhibition and heterogeneous nucleation.

3.4. *Solubility in the Presence of Additive*

The glycine solubility was not changed significantly ($P < 0.5$) by the presence of L-leucine at concentrations less than 12 mg/ml. However, L-leucine solubility decreased as the glycine concentration increased (Fig. 6). In the saturated glycine solution, the L-leucine solubility was experimentally determined to be 17.5 ± 0.8 mg/ml of water. Thus, the system studied was undersaturated with respect to L-leucine.

4. DISCUSSION

The effect of L-leucine on the glycine growth rate is twofold: (i) inhibition of the growth rate of the $(0\bar{1}0)$ face, and (ii) enhancement of the growth rate of the (010), (011), and $(01\bar{1})$ faces at L-leucine concentrations greater than the

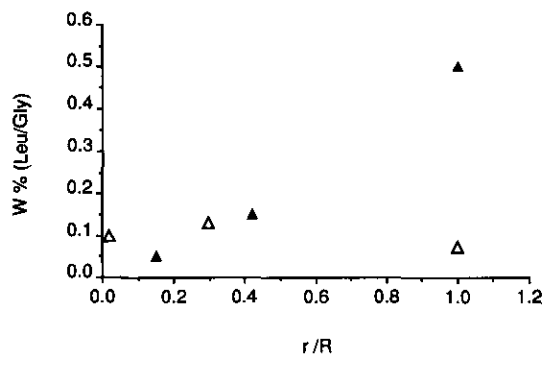

FIG. 5. L-Leucine distribution in glycine crystals obtained from dissolution experiments. Glycine crystals were grown in the prsence of L-leucine: ▲, 2 mg/ml; and △, 10 mg/ml.

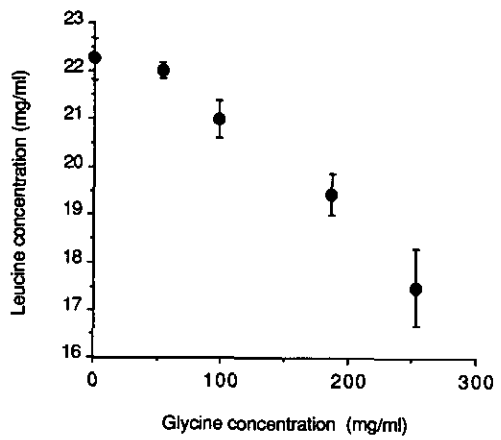

FIG. 6. Solubility of L-leucine in the presence of glycine.

critical concentration. In addition, at high L-leucine concentrations, pairs of glycine crystals were frequently found attached to each other at the $(0\bar{1}0)$ face.

Glycine crystal growth can be described by the screw dislocation mechanism (6). In the BCF model, kink sites are growth sites, and crystals grow by the advancement of the steps. Adsorption of additives onto kink sites and on the ledge may retard growth by blocking the advancement of steps (1). If additive concentration on a crystal surface is high, the distance between additive molecules will be less than the diameter of the two-dimensional critical nucleus at a given supersaturation, and this may result in cessation of crystal growth. At very low additive concentrations on a crystal surface, the distance between additive molecules will be greater than the diameter of the two-dimensional critical nucleus and greater than the distance between kink sites along the step. Thus, the additive will be expected to have little effect on the growth rate. At a given additive concentration in a solution, the amount of the additive adsorbed onto a crystal face is determined by the number of sites favorable for additive adsorption on that face and by the strength of the bonds formed between additive and substrate.

The growth rate of the $(0\bar{1}0)$ face of glycine was completely inhibited at L-leucine concentrations above 1 mg/ml as a consequence of L-leucine adsorption. This resulted in a shape change of glycine crystals in the presence of additive from bipyramidal to pyramidal. Weissbuch and co-workers (3) predicted from binding energy calculations that L-alanine adsorbs onto the $(0\bar{1}0)$, $(0\bar{1}1)$, and $(0\bar{1}\bar{1})$ glycine crystal faces. They found that L-alanine primarily adsorbs at two of the four symmetry-related sites on the $(0\bar{1}0)$ face. Even though L-leucine has a bulkier group $-(CH_3)_3$ than L-alanine, adsorption seems to primarily occur at the $(0\bar{1}0)$ face, and the $(0\bar{1}1)$ and $(0\bar{1}\bar{1})$ faces do not exist, or they are very small, for pyramidal crystals. In the flow cell experiments, there was no effect of additive adsorption on the growth of the (010), (011), and $(01\bar{1})$ faces, at L-leucine concentrations lower than the critical value (8 mg/ml). However, the growth rate of these faces increased at L-leucine concentrations greater that the critical concentration value.

The increase in growth rate can be explained by examining the changes in solution properties caused by the additive. Since glycine solubility did not change significantly at the L-leucine concentrations used in this study, less than 13 mg/ml, the enhancement of growth may be a result of changes in the interfacial tension (8) and the influence L-leucine has on glycine aggregation in solution (9–12). As the amount of clusters in solution increases, the growth rate will increase, provided the structure of a growth unit is similar to that of the cluster. According to Myerson and Lo (9), dimers exist in a saturated glycine solution, and dimer concentration increases with an increase in the concentration of hydrophobic amino acids. The dimerization of compounds structurally similar to glycine in aqueous solution (*N*-acetyl-L-alanine and diketopiperazine) has been examined by Asakura *et al.* (10) and Gill and Noll (11). They found that molecules form dimers by head-to-head hydrogen bonding. Moreover, in crystalline α-glycine, the dimer has been suggested to be the growth unit. This was based on a comparison of the predicted morphology from attachment energy calculations to the morphology of α-glycine crystals obtained by sublimation (12). As shown in Fig. 7, glycine molecules are linked by head-to-head hydrogen bonds, forming bilayers parallel to the *ac* plane. Thus, the glycine dimer in solution may participate in the growth process. The presence of L-leucine increases glycine dimerization in solution and increases the growth rate of the faces that do not have favorable sites for L-leucine adsorption.

Nucleation of glycine occurred on the $(0\bar{1}0)$ face at L-leucine concentrations higher than the critical concentration value. The close attachment and orientation of the pairs of crystals may be a result of the adsorption of multiple layers of L-leucine. The effect of L-leucine on the oriented nucleation of glycine crystals at the air/solution interface has been studied by Weissbuch *et al.* (5). Nucleation of glycine crystals occurs at this interface with the $(0\bar{1}0)$ face toward the air. The complete oriented nucleation of glycine at the air/solution interface indicates that the L-leucine molecules at the interface may be ordered and that head groups of glycine and L-leucine are linked by hydrogen bonds to form a heterogeneous bilayer (13). Further evidence of this phenomenon is provided by a study on the induction of oriented nucleation of glycine crystals by compressed Langmuir

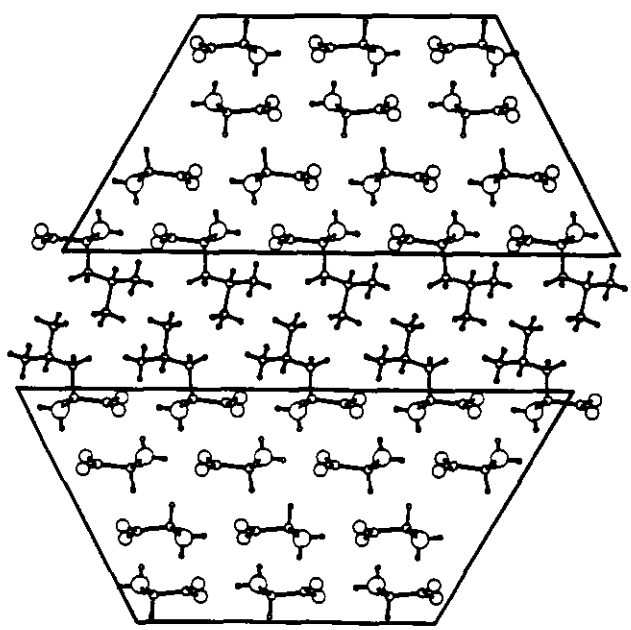

FIG. 7. Packing arrangement of α-glycine viewed along the *a*-axis and proposed orientation of adsorbed bilayer of L-leucine.

monolayers of amino acids with long alkyl side chains (14). The similarities of crystal structures of L-leucine to glycine support the possibility of matching the head group of glycine to the head group of L-leucine.

At the crystal/solution interface, for an adsorbed monolayer of L-leucine on the $(0\bar{1}0)$ face, the hydrophobic tail of L-leucine is toward the solution. In this case, the head-to-head hydrogen bonded bilayer cannot be formed, and nucleation and growth of glycine would be improbable. However, multilayer L-leucine adsorption on the $(0\bar{1}0)$ face, at high L-leucine concentrations, provides a template for the nucleation and growth of glycine crystals (Fig. 7), resulting in the formation of twinned crystals about the $(0\bar{1}0)$ basal plane to yield the crystal shown in Fig. 2c. In order to verify if these crystals were twinned having the $(0\bar{1}0)$ as a basal plane, a crystal was mounted with the long axis parallel to the Weissenberg camera spindle axis, and a zero-level photograph of the X-ray diffraction pattern was obtained. This photograph showed split spots, proving that the crystals are indeed twinned about the long axis of the crystal. In addition, the length of the mount axis was found to correspond to the crystallographic c-axis.

This hypothesis of the ordered structure of multiple adsorbed layers of L-leucine due to its hydrophobicity is supported by two additional observations. Glycine nucleation was observed on the $(0\bar{1}0)$ face of large glycine crystals at high concentrations of L-phenylananine, whereas nucleation was not observed in the presence of hydrophilic amino acids, such as L-histidine and L-serine. During dissolution experiments, L-leucine was found along the center of the bipyramidal glycine crystals, since L-leucine adsorption mainly occurred on the $(0\bar{1}0)$ face of glycine crystals.

5. CONCLUSIONS

Additives may affect crystal growth by adsorbing onto the crystal surface or by altering the solution properties. The effect of L-leucine on the crystal growth of glycine can be explained by these mechanisms, depending on the concentration of the additive. Tailor-made additives selectively inhibit growth of the crystal faces with favorable adsorption sites and effectively change the morphology of the crystal. At L-leucine concentrations lower than the critical concentration value (8 mg/ml), adsorption of L-leucine inhibits the growth of the $(0\bar{1}0)$ face of glycine crystals. This causes the glycine crystal morphology to change from bipyramidal, in the absence of L-leucine, to pyramidal. At higher L-leucine concentrations, the increased dimerization of glycine in solution promotes the growth of the (010), (011), and $(01\bar{1})$ faces. Adsorption of multiple layers of L-leucine on the $(0\bar{1}0)$ face provides a template for the oriented nucleation and growth of glycine twin crystals.

ACKNOWLEDGMENT

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