CHIRALITY 11:343–348 (1999)

Kinetics of Chiral Resolution in Stirred Crystallization of D/L-Glutamic Acid

THOMAS BUHSE, DILIP K. KONDEPUDI,* AND BRIAN HOSKINS
Department of Chemistry, Wake Forest University, Winston-Salem, North Carolina

ABSTRACT Stirred crystallization of racemic (D/L)-glutamic acid (Glu) in the presence of small amounts of L- or D-lysine (Lys) was studied for the effect of transient chiral resolution by monitoring the time evolution of optical rotation and the concentration of the solution. The presence of a small amount of L- or D-Lys retards the crystallization rate of the corresponding enantiomer of Glu in a chirally selective manner, giving rise to transient optical resolution of racemic Glu during crystallization. The optical rotation of the Glu solution was found to increase from zero to a value corresponding to an enantiomeric excess (ee) of 22–35% and subsequently decreases to zero over a period of many hours. During this process, the ee of the crystallized Gu is nearly 100% during the first 35 min and then it decreases slowly to zero. Our results indicate that the time at which the ee of the solution reaches its maximum and the maximum value of the ee show a nonlinear dependence on the initial mole fraction of the chiral impurity. The effect of the impurity is highly chirally selective, indicating "molecular recognition." *Chirality 11: 343–348, 1999.*

KEY WORDS: amino acids; crystallization kinetics; secondary nucleation; chiral resolution

As shown by extensive studies at the Weizmann Institute,[1–3] the presence of small amounts of "tailor-made" impurities drastically influence the crystallization in the way of retarding the crystal growth rate and altering the crystal habit. Of particular interest is the effect of chiral impurities on the crystallization of chiral compounds that crystallize as conglomerates (i.e., the enantiomers crystallize separately). In the presence of optically active impurities that are structurally similar to one enantiomeric form of the compound that crystallizes as a conglomerate, crystallization may result in transient chiral resolution:[4–7] an initially optically inactive solution may develop optical activity that reaches a maximum and then decreases to zero. This is due to chirally selective interaction between the optically active impurity and one enantiomeric form of the crystallizing compound. As a result, this interaction leads to a slower crystallization rate of one enantiomer while the rate of the other—that is, of opposite handedness—remains almost unaffected. For instance, in the crystallization of D-glutamic acid (D-Glu) from solution, D-lysine (D-Lys) retards the formation of D-Glu crystals but L-Lys has no effect (the same is true for the opposite enantiomers). Thus, if we crystallize racemic D/L-Glu in the presence of L-Lys, for example, the crystallization rate of L-Glu is retarded whereas D-Glu crystallizes unaffected by the presence of the L-Lys. As the crystallization proceeds, the optical activity increases from zero to a maximum value and eventually decreases to zero when both enantiomers crystallize and the system reaches equilibrium. In this article we present our experimental study of this phenomenon in stirred crystallization in which the crystallization rate is enhanced due to secondary nucleation caused by stirring.

Change in the crystallization rate of a compound due to an impurity can be so highly stereospecific to the molecular structure of the impurity that it may be used for detecting the amount of active impurity in a sample containing several other compounds;[8–10] for example, the presence of L- or D-Lys in a sample can be detected through its influence the crystallization of L- or D-Glu. For the observed inhibitory effect of the impurity on the crystallization of a compound, Addadi et al.[1] suggest a mechanism involving two steps: first, binding of the impurity molecule at the surface of the crystal and second, inhibition of the regular growth of the crystal by the adsorbed impurity molecule through lattice distortion. The impurity molecule will be incorporated into the crystal only if it is similar in structure to the crystallizing compound—hence the stereospecificity of the effect. Once incorporated into the crystal, however, the lack of total structural identity between the impurity and the crystallizing compound results in crystal defects that retard the crystal growth rate. In stirred systems, a crystal can generate a large number of nuclei (an autocatalytic process) through a complex and poorly understood mechanism called "secondary nucleation."[11–13] It is very likely that the impurity also retards the rate of secondary

Contract grant sponsor: NSF; Contract grant number: CHE-9527095.
*Correspondence to: Dilip K. Kondepudi, Department of Chemistry, Wake Forest University, Winston-Salem, NC 27109. E-mail: dilip@wfu.edu
Received for publication 26 October 1998; Accepted 18 December 1998

nucleation, which in turn contributes to the overall rate of crystallization of the solute from the supersaturated solution. Because secondary nucleation is an autocatalytic process, a small amount of the impurity can produce a large change in the rate of crystallization causing the two enantiomers to crystallize at significantly different rates. However, despite the fundamental importance of this effect and its possible application for highly effective optical resolution of conglomerates, kinetic studies performed in suspension crystallizers are still scarce. In this study, we establish the time scales and the basic nature of the transient optical resolution that can be accomplished by the addition of an appropriate impurity.

In a previous paper,[14] we reported on the crystallization kinetics of enantiomerically pure L-Glu in the presence of L-Lys and D-Lys as the impurity. We proposed a kinetic model that enabled us to describe the highly chirally selective inhibitory effect of L-Lys on the crystallization of L-Glu that was experimentally observed as a nonlinear function of the impurity concentration. The model also accounted for the essential stochastic nature of crystallization time that showed increased fluctuations with increasing Lys concentration. In this article, we present the results of our study of the chiral interaction between D/L-Glu as the racemic starting compound and D-Lys as the impurity. Our experiments focused in this case on the kinetics of chiral resolution phenomena in stirred conglomerate crystallization. Herein we studied the transient increase of optical rotation in the solution phase and monitored the overall crystallization rate. These two variables enable us to identify the enantiomeric excess (ee) in the solution and the crystalline phases.

MATERIALS AND METHODS

The crystallization experiments were conducted in a thermostated 250 mL jacketed beaker at T = 30.0°C. A Teflon-coated stirbar was used for magnetic stirring (25 × 8 mm), which was kept at a constant stirring rate of 630 RPM to ensure that the crystals were well distributed in the solution. An aqueous solution of 20 g D/L-Glu hydrate (Aldrich, St. Louis, MO) (99%) in 1,000 mL of water was prepared as stock.

A typical experimental run was performed as follows: After filtering, 80 mL of the D/L-Glu stock solution was placed into the reactor and different total amounts of D-Lys hydrate (D-Lys); 0.02, 0.04, 0.06, or 0.08 g, Aldrich, 97% were added and completely dissolved. To achieve supersaturation, 120 mL of 2-propanol (Fisher, Pittsburgh, PA) (HPLC grade) was added to the aqueous solution and the experiment was started by turning on the stirrer. Mixing of the aqueous solution and the added 2-propanol resulted in a completely transparent and monophasic solution within about 3 min. Due to contraction, the total volume of the solution became about 190 mL. Depending on the amount of Lys added, crystallization began to occur at times ranging from 8 to 17 min. Additionally, control experiments were run by using 0.04 g of L-lysine hydrate (L-Lys); Aldrich, 97% and 0.04 g of D-phenylalanine, Aldrich, 99+% as the impurity (Fig. 1).

For the determination of optical rotation in the solution phase, aliquots of about 3 mL were withdrawn from the crystallizing mixture at time intervals from 2.5 to 15 min, depending on the stage of crystallization. Subsequently, the crystalline and the solution phases of the sample were rapidly separated in order to obtain the clear solution phase. This was accomplished by taking the aliquots with 5 mL disposable syringes and forcing the samples through syringe filters equipped with a nylon membrane of 0.45 μm in pore size. Then, 2.0 mL of this transparent solution was combined with 5 mL of 5 N HCl (made from concentrated HCl; Fisher, ACS grade). HCl was added because optical rotation increases with decreasing pH. After that, the combined solutions were transferred into a polarimeter cell of 10 cm path length and the optical rotation (α) was measured at λ = 365 nm in a Rudolph Autopol IV automatic polarimeter. This procedure was repeated for about 20 times during each crystallization. The optical rotation was identified within an accuracy of $\Delta\alpha = \pm 0.005°$. A calibration curve of α vs. [D-Glu] in 2:3 (v:v) water/2-propanol was recorded in order to identify ($[\text{D-Glu}]_S - [\text{L-Glu}]_S$), i.e., the excess in the molar concentration of D-Glu over L-Glu in the solution phase (subscript s).

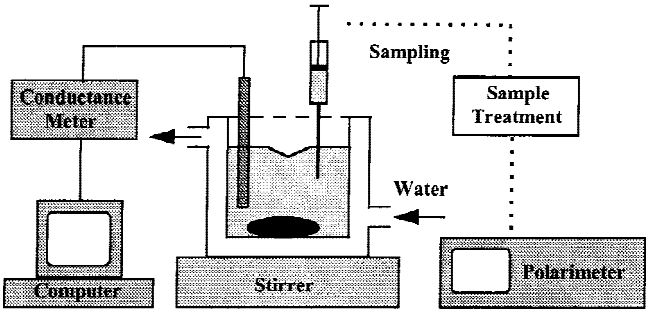


Fig. 1. Schematized set-up of stirred crystallization experiments in 250 mL double wall reactor. Data has been collected by continuous conductance reading of the crystallizing mixture and by discontinuous sampling of the solution phase to determine its optical rotation.

Complementary to the determination of optical rotation in solution phase, the conductance of the bulk crystallizing mixture was monitored continuously. As the crystallization proceeds, the conductivity of the mixture decreases proportionally to the concentration of solute remaining in the solution phase. Conductivity was measured with a YSI 35 conductance meter equipped with a platinum electrode (YSI 3417) placed into the reactor. The signal was recorded digitally by connecting the instrument to a Pasco digital interface attached to a Macintosh computer. To obtain the concentration of Glu in the solution phase during crystallization, the conductance was calibrated by using standard solutions of D/L-Glu in 2:3 (v:v) water/2-propanol. The first stable conductance reading was normalized so that its maximum value (before the onset of crystallization) equals $[\text{D/L-Glu}]_0$ = 0.051 M

The saturation or equilibrium concentration of D/L-Glu in the solvent mixture at the experimental temperature of T = 30°C has been determined in a number of independent crystallizations without the presence of impurity and by

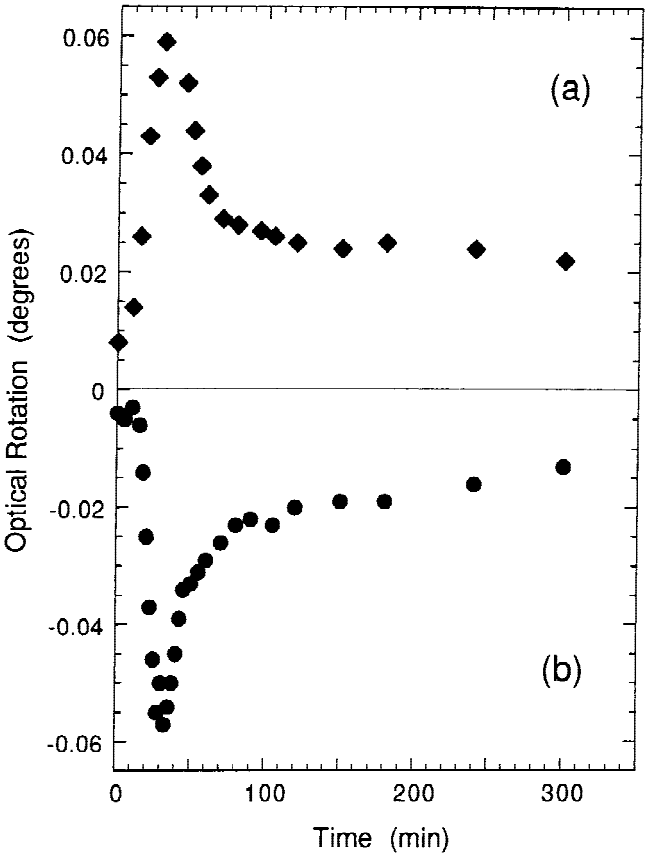


Fig. 2. Optical rotation (α) of solution phase vs. time for the stirred crystallization of 1.6 g D/L-Glu (rac) in 190 mL water/2-propanol, $([D\text{-Glu}] + [L\text{-Glu}])_0 = 0.051$ M. Curve (a), presence of 0.04 g L-Lys, [L-Lys] = 1.44×10^{-3} M and curve (b) presence of the same amount of D-Lys as the impurity.

weighing the solute that has crystallized after about 24 h. The concentration at equilibrium was found to be $([D\text{-Glu}]_s + [L\text{-Glu}]_s)_\infty = 0.013$ M.

RESULTS AND DISCUSSION

In Figure 2, the time evolution of optical rotation during two typical stirred crystallization experiments of D/L-Glu as the racemic starting compound is shown. As clearly indicated, the presence of small amounts of (+)L-Lys or (–)D-Lys gives rise to significant optical rotation in the solution phase with maximum values of $\alpha = 0.059°$ (with L-impurity) and $\alpha = -0.057°$ (with D-impurity). This corresponds to an ee in the solution phase (ee_s) of approximately 30%. Since the D-Lys interacts only with D-Glu, it is appropriate to characterize its effect by its mole fraction with respect to D-Glu. Accordingly we define:

$$m_D = M_{imp}/(M_{imp} + M_{SD}) \quad (1)$$

in which M_{imp} is the number of moles of the impurity D-Lys, and M_{SD} the number of moles of D-Glu in the solution phase. Similar mole fraction, m_L, is defined when L-Lys is added instead of D-Lys. For the data shown in Fig. 2, the value of $m_D = m_L = 0.0535$.

The optically active impurity does not significantly contribute to the measured optical rotation. Nevertheless, the initial reading has been normalized to $\alpha = 0°$ by subtracting the small amount of optical rotation due to the impurity. When crystallization starts to occur after about 15 min, the optical rotation rises sharply in a typically S-shaped manner and reaches its maximum value after around 30 min. It is obvious, that during this time period an excess of (+)L-Glu or (–)D-Glu accumulates in the solution phase, depending on the presence of L-Lys or D-Lys, respectively. After passing its maximum, the optical rotation starts to decrease more slowly, taking about 5 h to reach ~30% of the maximum value. The decrease is due to the eventual crystallization of the enantiomeric form of Glu that was inhibited by the impurity. It is interesting to note that it took more than 20 h under continuous stirring for the optical rotation α to return to zero—which indicates the completion of crystallization that results in a racemic solution in equilibrium with a conglomerate solid phase.

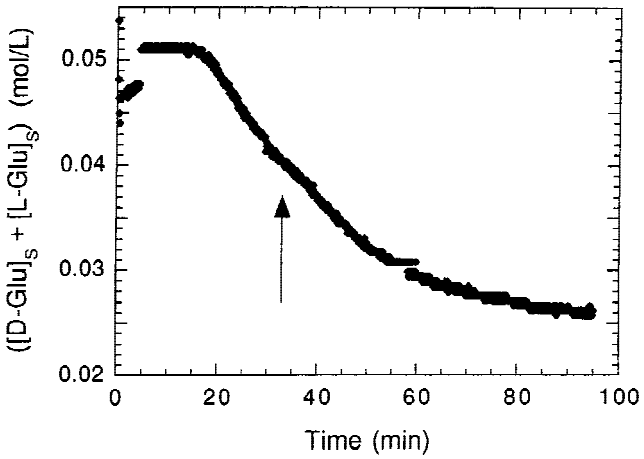


Fig. 3. Time evolution of ([D-Glu] + [L-Glu]) in the solution phase as obtained by monitoring the conductance during crystallization of D/L-Glu in the presence of D-Lys (referring to Figure 2, curve b). The arrow indicates the time when optical rotation reaches its maximum.

An illustration of the high selectivity in this kind of process is given by the result of a control experiment using 0.04 g of D-phenylalanine (D-Phe) as the impurity. In this case, the optical rotation rested at its zero level during the whole crystallization, clearly indicating that D-Phe has no effect on chiral resolution phenomena in D/L-Glu crystallization.

The conductivity of the crystallizing mixture (Fig. 3) gives a picture of the crystallization kinetics of the solute, D/L-Glu, within the first 100 min of the experiment corresponding to the curve (b) in Figure 2. After an induction period of about 15 min in which practically no crystallization occurs, the concentration of D/L-Glu in solution phase slowly drops down from its initial value of 0.051 M to 0.026 M after 100 min. At this point, one still finds $[D\text{-Glu}]_s > [L\text{-Glu}]_s$ in the solution phase ($\alpha < 0$). Hence, the system did not reach its final equilibrium value of $([D\text{-Glu}]_S + [L\text{-Glu}]_S)_\infty = 0.013$ M.

A fairly accurate estimate of the enantiomeric excess in the solution phase, ee_S, can be given by considering that,

$$[L\text{-Glu}]_s - [D\text{-Glu}]_s \propto \alpha \quad (2)$$

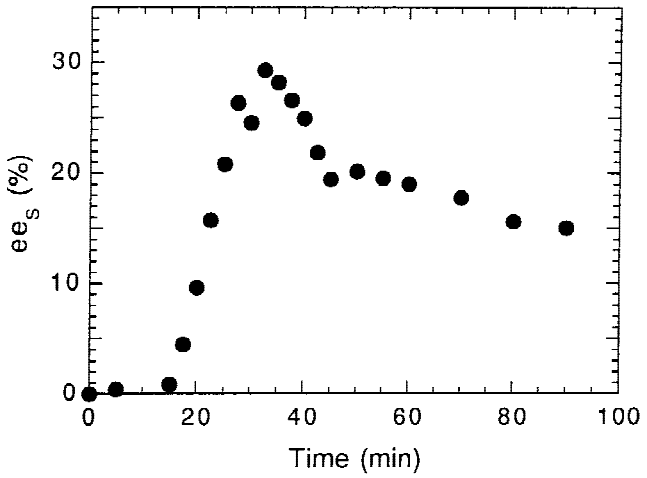


Fig. 4. Enantiomeric excess of solution phase (ee_s) vs. time in stirred crystallization of D/L-Glu in the presence of 0.04 g D-Lys. The maximum value at t = 32.5 min is ee_s = 30%–the calculated theoretical maximum is ee_{smax} = 59%.

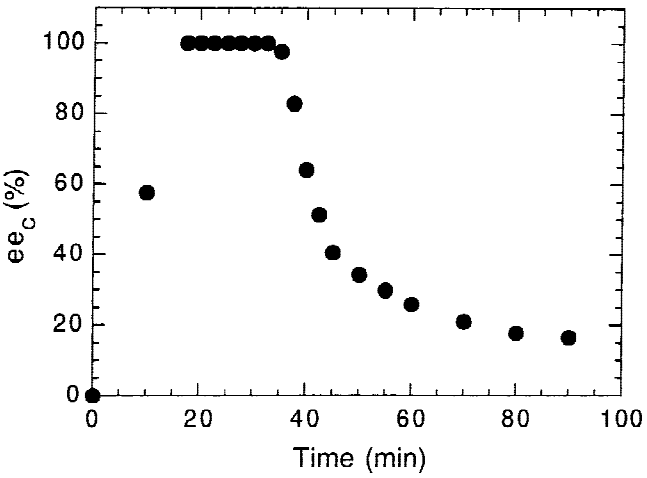


Fig. 5. Enantiomeric excess of crystalline phase (ee_c) as a function of time in stirred crystallization of D/L-Glu (corresponding to Figure 4). The calculated values for ee_c were normalized to ee_c = 100% in cases in which the calculation resulted in $ee_c > 1$ (due to experimental error).

$$[\text{L-Glu}]_s + [\text{D-Glu}]_s \propto \text{conductance} \quad (3)$$

and obtaining appropriate calibrations that relate the sum and difference of the concentrations of the two enantiomers to the conductivity and optical rotation respectively. The enantiomeric excess is then:

$$ee_s = \frac{|[\text{L}-\text{Glu}]_s - [\text{D}-\text{Glu}]_s|}{|[\text{L}-\text{Glu}]_s + [\text{D}-\text{Glu}]_s|}. \quad (4)$$

Figure 4 shows ee_s as a function of time. The maximum of ee_s here corresponds to the maximum of α, as shown in Figure 2b. Its maximum value at t = 32.5 min is ee_s = 30%. As shown below, this is about 50% of the maximal obtainable enantiomeric excess in solution phase, ee_{smax}, under our experimental conditions. In the presence of D-impurity, ee_{smax} is reached when all the excess (above the equilibrium value) L-Glu has crystallized while all of the initial D-Glu remains in the solution. In our experiments, the initial concentrations were: $[\text{L-Glu}]_{s0} = [\text{D-Glu}]_{s0} = (0.0510/2)\text{M} = 0.0255\text{M}$. If L-Glu drops to its equilibrium value, $[\text{L-Glu}]_{s\infty} = (0.013/2)\text{M} = 0.0065\text{M}$, due to crystallization while D-Glu remains at its initial value, the crystalline phase will contain 100% L-Glu in the amount 0.0189 moles per liter of the solution. In the solution phase, we will have:

$$[\text{D-Glu}]_{s0} - [\text{L-Glu}]_{s\infty} = 0.0189\ \text{M} \quad (5)$$

Thus, if all the L-Glu that can crystallize has crystallized but none of the D-Glu did, the enantiomeric excess of the solution phase would be:

$$\frac{[\text{D}-\text{Glu}]_{s0} - [\text{L}-\text{Glu}]_{s\infty}}{[\text{D}-\text{Glu}]_{s0} + [\text{L}-\text{Glu}]_{s\infty}} = \frac{0.0189}{0.0321} = 0.59. \quad (6)$$

Thus, even in the presence of a small amount of impurity, corresponding to an impurity mole fraction m_D = 0.0535, the experimentally obtained ee is up to 50 % of its theoretical maximum. If no D-Glu crystallized, the enantiomeric excess of the crystalline phase, ee_c, should be 100%.

The time plot of ee_C, as shown in Figure 5, was obtained by solving the analog of expression (4) for the crystalline phase and by using the relations,

$$(\text{L-Glu})_c - (\text{D-Glu})_c = V([\text{D-Glu}]_s - [\text{L-Glu}]_s) \quad (7)$$

and

$$(\text{L-Glu})_c + (\text{D-Glu})_c = V(([\text{L-Glu}]_s + [\text{D-Glu}]_s)_0 - ([\text{L-Glu}]_s + [\text{D-Glu}]_s)) \quad (8)$$

in which quantities with a subscript "c" denote the total amount of the L-enantiomer in the crystalline phase and V is the solution volume. The quantities on the right hand side of equations 7 and 8 can be obtained from the optical rotation α and the conductivity (and note that ee_c is independent of the volume, V). ee_c starts at its maximum value of 100% at the onset of crystallization of L-Glu (which we could detect, even before crystals becomes visible, by measuring the optical rotation); it remains at this maximum value for about 35 min and then starts to decrease in a first-order-like decay.

Figure 6 summarizes a series of crystallization experiments at different concentrations of initial D-Lys. In all cases, the maximum value for $([\text{D-Glu}]_s - [\text{L-Glu}]_s)$, is readily reached showing S-shaped acceleration kinetics that are typical for stirred crystallization.[14] After passing its maximum value, $([\text{D-Glu}]_s - [\text{L-Glu}]_s)$ decreases slowly in an exponential-like decay, as the inhibited enantiomer slowly crystallizes.

In our earlier article[14] we noted that, being an autocatalytic process that amplifies nucleation events, stirred crystallization exhibits a high degree of stochasticity i.e., repeated runs give significant variation in the concentration-Vs-time curves. Hence, if each of these crystallization experiments were repeated, we should expect variation in both the times at which the maximum is reached and the value of ee_{smax}. These stochastic variations increase with the increasing amount of impurity.[14] Nevertheless we can see some general trends: 1. the obtainable maximal ee_s

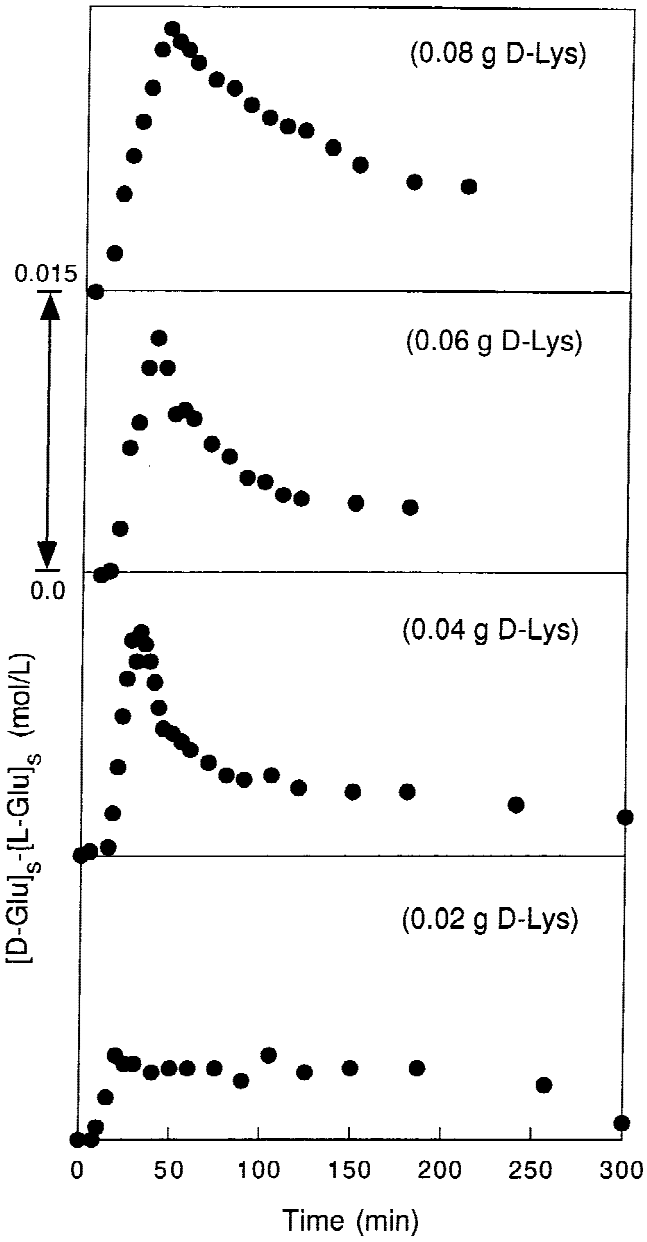
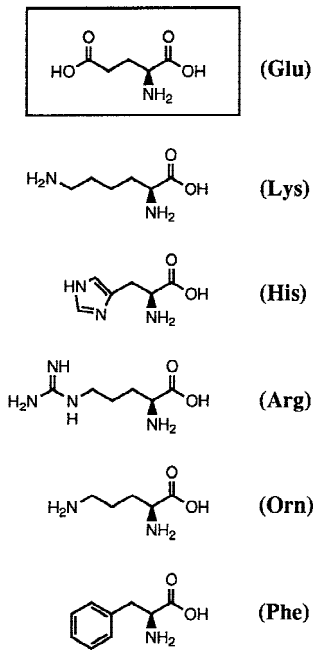


Fig. 6. Excess of D-Glu in the solution phase vs. time in stirred crystallization of D/L-Glu (1.6 g in 190 mL water/2-propanol) in the presence of D-Lys. Initial concentrations of D-Lys are 2.88×10^{-3} M (0.08 g), 2.16×10^{-3} M (0.06 g), 1.44×10^{-3} M (0.04 g) and 7.2×10^{-4} M (0.02 g). Data was obtained by monitoring the optical rotation of the solution phase. The reading of the optical rotation has been normalized to $\alpha = 0°$ at the beginning of each experiment (i.e., subtracting the optical rotation due to the initial presence of D-Lys).

(see also Table 2) seems to increase slightly with increasing impurity concentration. The relation between the concentration of the impurity and maximum ee_s seems nonlinear; 2. The time at which ee_s reaches its maximum also with increasing impurity concentration in a nonlinear fashion; and finally 3. The decay kinetics i.e., the rate at which the system reaches its equilibrium, becomes slower with increasing impurity concentration (Table 2).

In other words, the more impurity the longer the phenomenon of chiral resolution lasts. Except for the lowest impurity concentration, in which both crystallization processes are not separated clearly enough, the enantiomeric excess of the crystalline phase (ee_c) was always at the maximal value of about 100% when α was at its maximum. At this point we have the maximum amount of enantiomerically pure L-Glu in the solid phase.

TABLE 1. D- and L-Lys, His, Arg, and Orn inhibit the crystallization of D- and L-Glu, respectively.[1,8–10] Phe has no effect on the crystallization of Glu



*Lys, lysine; His, histidine; Arg, arginine; Orn, ornithine; Glu, glutamic acid.

CONCLUSIONS

Crystallization in stirred solution consists of three distinct processes: primary nucleation, autocatalytic second-

TABLE 2. Stirred crystallization of D/L-Glu in the presence of D-Lys in water/2-propanol. The mole fraction of initial D-Lys is given as $m_D = M_{imp}/(M_{imp} + M_{sD})$, see equation 1. Data refers to single set of experiments and the time when the measured optical rotation of the solution phase is at maximum. The total concentration in solution phase ($[D\text{-}Glu]_s + [L\text{-}Glu]_s$) has been obtained by measuring the conductance during crystallization. Digits in parenthesis refer to the estimated experimental error (not to statistics of several runs) in the last digit of the given values.*

Time (min)	m (D-Lys)	$[D\text{-}Glu]_s - [L\text{-}Glu]_s$ (mol/L)	$[D\text{-}Glu]_s + [L\text{-}Glu]_s$ (mol/L)	ee_s (%)	ee_c (%)
20	0.027	0.005 (±1)	0.023 (±2)	22 (±5)	18 (±4)
32.5	0.054	0.012 (±1)	0.040 (±2)	30 (±3)	96 (±4)
40	0.080	0.013 (±1)	0.040 (±2)	33 (±3)	96 (±4)
45	0.107	0.014 (±1)	0.040 (±2)	35 (±3)	97 (±3)

*Glu, glutamic acid; Lys, lysine; ee, enantiomeric excess.

ary nucleation, and crystal growth. Our experiments illustrate an appropriate chiral impurity can affect one or more of these three processes in a chirally selective way and can produce transient chiral resolution. In the case of stirred crystallization of D/L-Glu in the presence of small amounts of D- or L-Lys, the transient resolution occurs on a time scale that makes it useful for chiral resolution. In our experiments, during the first 30–50 min of crystallization, L-Glu in the crystal phase is nearly 100% enantiomerically pure; the amount of this enantiomerically pure Glu reaches a maximum value of about 0.01 moles per liter of the solution. After that, D-Glu begins to crystallize and ee of the crystalline phase decreases. Since crystallization is generally a slow process (unless one is dealing with very high levels of supersaturation) we may expect crystallization of other compounds to occur on the same time scale showing similar transient chiral resolution. Our measurements of optical rotation and total solute concentration in the solution phase during stirred crystallization, which was the objective of this paper, provide a basis for future theories of the chirally selective effect of impurities on conglomerate crystallization.

Our data on optical activity and concentration of the solution phase give us both qualitative and quantitative features that a theory of kinetics of crystallization in the presence of impurities must explain. In our earlier article[14] we presented a empirical kinetic model that reproduced many of the observed effects but a fundamental theory based on nucleation theory and crystal growth is yet to be formulated. The biggest problem in theoretical modeling is the rate of secondary nucleation and the influence of the impurity on this rate.

According to recent theoretical work of Qian and Botsaris,[11] attractive interparticle forces play an important role for the onset of secondary nucleation.[12–13] For the system investigated, this means that the presence of spontaneously formed nuclei of A induces or accelerates the crystallization of B. According to classical nucleation theory[15] we can expect that there is no difference between A and B in prenuclear clustering or in the cluster–cluster attractive forces in the presence of an impurity. However, the "switch" from a cluster into a crystalline nucleus, the latter expected to be influenced by the impurity, is still a poorly understood process and requires a further dynamical investigation. Indeed, some of our preliminary results indicate that in the presence of the same amount of L-impurity the inhibition effect is more pronounced in a system of L-Glu than in one with D/L-Glu as the crystallizing compound. An equally important problem is kinetic modeling of the effect of the impurity on primary nucleation rate and the crystal growth rate. A crude model[14] seems to indicate that under our conditions these rates decreases exponentially with increasing mole fraction of the impurity.

ACKNOWLEDGMENTS

We thank NSF (grant CHE-9527095) for supporting this work.

LITERATURE CITED

1. Addadi L, Berkovitch-Yellin Z, Domb N, Gati E, Lahav M, Leiserowitz L. Resolution of conglomerates by stereospecific habit modifications. Nature (London) 1982;296:21–26.
2. Addadi L, Weinstein S, Gati E, Weissbuch I, Lahav M. Resolution of conglomerates with the assistance of tailor-made impurities. Generality and mechanistic aspects of the "rule of reversal." A new method for assignment of absolute configuration. J Am Chem Soc 1982;104:4610–4617.
3. Weissbuch I, Addadi L, Lahav M, Leiserowitz L. Molecular recognition at crystal interfaces. Science 1991;253:637–645.
4. Secor RM. Resolution of optical isomers by crystallization procedures. Chem Rev 1963;63:297–309.
5. Collet A, Brienne MJ, Jacques J. Optical resolution by direct crystallization of enantiomer mixtures. Chem Rev 1980;80:215–230.
6. De Min M, Levy G, Micheau JC. Chiral resolutions, asymmetric synthesis and amplification of enantiomeric excess. J Chim Phys 1988;85:603–619.
7. Eliel EL, Wilen SH. Stereochemistry of organic compounds. New York: John Wiley; 1994.
8. Grases F, Costa-Bauzá Forteza R, March JG. Crystallization control by additives: molecular recognition. Application to the determination of L-glutamic acid using L-lysine as substrate. Anal Lett 1994;27:2781–2787.
9. Ballesteros E, Gallego M, Valcárcel M, Grases F. Enantiomer discrimination by continuous precipitation. Anal Chem 1995;67:3319–3323.
10. Ballesteros E, Gallego M, Valcárcel M. Sequential determination of D- and L-glutamic acid by continuous fractional crystallization. Anal Chem 1996;68:322–326.
11. Qian RY, Botsaris GD. A new mechanism for nuclei formation in suspension crystallizers: the role of interparticle forces. Chem Eng Sci 1997;52:3429–3440.
12. Randolph AD, Larson MD. Theory of particulate processes. 2nd ed. San Diego: Academic Press; 1988.
13. Ang HM, Loh PIW. Kinetics of secondary nucleation of alumina trihydrate in a batch crystallizer. In: Myerson AS, Toyokura K, editors. Crystallization as a separation process. Washington, DC: Am Chem Soc; 1990. p 329–343.
14. Kondepudi DK, Culha M. Chiral Interaction and stochastic kinetics in stirred crystallization of amino acids. Chirality 1998;10:238–245.
15. Volmer M. Kinetik der Phasenbildung. Dresden, Leibzig: Steinkopff; 1939.