Electronic Supporting Information

Amino Acid Directed Mirror Symmetry Breaking and Chiral Amplification of Ethylenediammonium Sulfate Crystals

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Ethylenediammonium sulfate (EDS) was synthesized as previously reported.[1]

Preparation of saturated ethylenediammonium sulfate (EDS) solution

From a 250 mL separatory funnel, a sulfuric acid solution (3 M, 250 mL) was slowly added dropwise to an ethylenediamine solution (3 M, 250 mL) in a 1 L beaker with continuous stirring under ice cooling. The appearance of precipitate near the end of the addition indicated that the solution was saturated. Concentrated sulfuric acid (96.8%) or ethylenediamine (99%) was added slowly with constant stirring and under ice cooling to adjust the EDS saturated solution to pH 7. The prepared solution often becomes dark yellow with aging thus causing difficulty in determining the optical rotatory dispersion. In this case, activated charcoal was added to the decanted EDS solution and the solution was vacuum filtered through Celite® filtering agent (Celite® 545; Sigma-Aldrich) on a coarse glass frit. To ensure optimal crystal growth, the saturated solution was shaken vigorously one day prior to use so that the solution remained at equilibrium.[2] The ethylenediammonium sulfate solution was centrifuged prior to use.[1]

Closed system setup for abrasion/grinding experiments

A home-made stirrer equipped with a speed controller (OE Motor Controller) was used to fix the stirring rate at *ca.* 1500 rpm. A Buchler Instruments' *Evapo-Mix* vortex shaker operating at *ca.* 3100 rpm under water cooling was used for the rotary shaking

experiments. The abrasion/grinding experiments were performed with the addition of one of the 19 natural chiral amino acids or its enantiomer. The amount of chiral amino acid used was arbitrarily chosen to be between the half maximum and the maximum solubility in water at 25 °C.

Abrasion/grinding with ceramic beads: *ca*. 0.25 g of EDS powder, chiral amino acid (an amount between the half maximum and the maximum solubility in water – Table 1), 1.0 mL of saturated EDS solution, *ca*. 3.0 g of ceramic beads (0.80 mm diameter), and a magnetic stir bar (10 mm x 6 mm) were added to a 5 mL round bottom flask and sealed with a glass stopper. For glucose and tartaric acid, an amount between the half maximum and the maximum solubility in water was added to the abrasion/grinding system (Table 1).

The sample mixture was stirred or shaken at a controlled rate in the presence of ceramic grinding media. In each experiment, seeds were sampled after *ca.* 4 days of abrasion/grinding and were allowed to grow to a larger size in EDS saturated solution for subsequent analysis.

Ten control experiments were performed with EDS in the absence of additive using the *Evapo-Mix* vortex shaker. Based on our experiments, abrasion/grinding with either stirring or shaking both give the same results with respect to chiral symmetry breaking and chiral amplification. In this work, both techniques were used in order to speed up the data collection process. Five different amounts of EDS powder (0.25 g, 0.18 g, 0.125 g, 0.08 g, and 0.05 g) were used in the 185 experiments. In some cases, a smaller amount of EDS powder was added in the closed system setup to increase the ratio of chiral additive to EDS. It is believed that increasing the ratio of chiral additive to EDS may increase the

probability of enantioselective adsorption on the EDS crystal surface. Moreover, a slight increase in temperature to *ca.* 40 °C was observed during the abrasion/grinding process which is caused by the constant stirring or shaking in the presence of grinding media. Therefore, a larger amount of EDS powder (0.25 g and 0.18 g) was chosen when using the high speed vortex shaker to ensure that the solid was not completely dissolved.

The solubility of all L-amino acids, D-glucose and L-tartaric acid was studied in EDS saturated solution at *ca.* 40 °C to simulate the internal temperature of the closed system under experimental conditions. The specified amount of chiral additives used in the abrasion/grinding experiments was added to 1 mL of EDS saturated solution (pH 7) and stirred with a magnetic stir bar (10 mm x 6 mm) in a closed 5 mL round bottom flask on a digital hotplate stirrer at 40 °C (OptiCHEM CHEMGLASS). The results show that most of the chiral additives are soluble or slightly soluble in EDS saturated solution, except for valine, leucine, isoleucine and phenylalanine (Table 2).

The minimum amount of chiral additive required for directed chiral symmetry breaking to occur was studied with decreasing amount of L-asparagine monohydrate in six abrasion/grinding experiments. The concentration of L-asparagine monohydrate varied from 1.2×10^{-1} M to 3.3×10^{-3} M.

Seeding of crystallites in freshly prepared saturated solution was carried out as previously reported.[1]

Analysis of the chirality of EDS crystals using a polarizing microscope

Crystals were either analyzed individually after removal from the saturated solution[1] or they were visualized directly in the crystallizing dish using a polarizing microscope. Direct analysis of EDS crystals in saturated solution was performed using a circular

plastic grid of the same diameter as the crystallizing dish. This plastic grid with small circles divides the bottom of the crystallizing dish into labeled areas and the chirality of all EDS crystals in each labeled area were examined using a polarizing light microscope (Nikon, model: SMZ1500, integrated with a Nikon DS-Fi1 digital camera). The top polarizing filter was rotated clockwise to determine the optical rotatory dispersion of individual crystals. The optical rotatory dispersion of the crystals was determined as follows: a color change from clear to blue to amber indicates *dextrorotatory* EDS crystals and a color change from amber to blue to clear indicates *levorotatory* EDS crystals. The crystal enantiomeric excess (CEE) for each sample was calculated as: CEE = (N_d – N_l)/(N_d + N_l) x 100; where N_d represents the number of *dextrorotatory* crystals and N_l represents the number of *levorotatory* crystals. 185 experiments were performed and the number of EDS crystals examined in each experiment varied from 20 to 500 crystals. The selection criteria for including data in our final results required that: (*i*) at least 20 crystals were counted in each experiment, and (*ii*) a CEE of greater than 90% was obtained. All 19 pairs of chiral amino acids were examined and multiple experiments for each pair of amino acid were carried out ($n \geq 3$). The results reported in Figure 1 incorporate abrasion/grinding experiments using both high speed stirring and shaking.

Table 1: Concentration of chiral additives incorporated in EDS saturated solution.

Chiral additives	Concentration (M)
D-threonine	3.4E-01
L-threonine	3.2E-01
D-serine	3.4E-01
L-serine	2.7E-01
D-glutamine	2.5E-01
L-glutamine	1.7E-01
D-alanine	8.1E-01
L-alanine	8.0E-01
D-cysteine	8.3E-02
L-cysteine	7.2E-02
D-leucine	1.4E-01
L-leucine	1.1E-01
D-valine	4.7E-01
L-valine	4.3E-01
D-proline	4.4E-01
L-proline	7.5E-01
D-isoleucine	1.9E-01
L-isoleucine	2.2E-01
D-aspartic acid	3.0E-02
L-aspartic acid	2.9E-02
D-glutamic acid	4.1E-02
L-glutamic acid	3.6E-02
D-phenylalanine	1.2E-01
L-phenylalanine	1.2E-01
D-histidine monochloride monohydrate	1.4E-01
L-histidine	1.9E-01
D-arginine	3.1E-01
L-arginine hydrochloride	3.2E-01
D-methionine	1.5E-01
L-methionine	1.4E-01
D-lysine	1.8E-01
L-lysine monohydrochloride	1.9E-01
D-asparagine monohydrate	1.2E-01
L-asparagine monohydrate	1.2E-01
D-tyrosine	2.8E-03
L-tyrosine	3.3E-03
D-tryptophan	3.1E-02
L-tryptophan	4.1E-02
D-glucose	1.2
L-glucose	3.6E-01
D-tartaric acid	9.4E-01
L-tartaric acid	1.0

Table 2: Solubility of chiral additives in EDS saturated solution at 40°C.

Chiral additives	Concentration (M)	Solubility
L-threonine	3.6E-01	Completely dissolved
L-serine	3.0E-01	Completely dissolved
L-glutamine	2.1E-01	Completely dissolved
L-alanine	1.0	Slightly soluble
L-cysteine	7.7E-02	Completely dissolved
L-leucine	1.8E-01	Slightly soluble
L-valine	5.3E-01	Slightly soluble
L-proline	4.4E-01	Slightly soluble
L-isoleucine	2.8E-01	Slightly soluble
L-aspartic acid	3.4E-02	Completely dissolved
L-glutamic acid	4.8E-02	Completely dissolved
L-phenylalanine	1.3E-01	Slightly soluble
L-histidine	2.0E-01	Completely dissolved
L-arginine hydrochloride	4.4E-01	Completely dissolved
L-methionine	1.7E-01	Very soluble, little solid remained
L-lysine monohydrochloride	1.7E-01	Completely dissolved
L-asparagine monohydrate	1.6E-01	Completely dissolved
L-tyrosine	2.8E-03	Slightly soluble
L-tryptophan	3.9E-02	Very soluble, little solid remained
D-glucose	4.6E-01	Completely dissolved
L-tartaric acid	1.0	Very soluble, little solid remained

1 P. S. M. Cheung, J. Gagnon, J. Surprenant, Y. Tao, H. Xu and L. A. Cuccia, *Chem. Commun.*, 2008, 987.
2 A. Holden, P. Singer, Crystals and Crystal Growing, Anchor Books: Garden City, N.Y., 1960.