Homochirality

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Achiral Nucleobase Cytosine Acts as an Origin of Homochirality of Biomolecules in Conjunction with Asymmetric Autocatalysis**

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The homochirality of biomolecules such as L-amino acids and D-sugars is one of the essential features of life and creates a puzzle about the chemical origin of life.[1–5] There have been debates on whether amino acids or sugars[1,6–8] were the earlier class of chiral compounds. Proteins and enzymes containing an irregular mixture of L- and D-amino acids and nucleic acids composed of random D- and L-deoxyribose units do not function. Therefore, the origin of the homochirality of the key organic molecules in nature is a mystery to be solved. Several theories have been proposed as to the origin of homochirality in organic compounds,[1–5] for example, theories involving circularly polarized light,[9,10] chiral inorganic crystals such as quartz,[11] or absolute asymmetric synthesis.[2,4,12,13] Small enantiomeric imbalances induced by these mechanisms have been amplified significantly by asymmetric autocatalysis to afford highly enantioenriched compounds.[12–23]

Certain achiral organic compounds are known to form chiral crystals,[24] and stereospecific reactions[25] with these crystals as substrates have been reported. We recently reported that chiral organic crystals formed from achiral organic compounds[26,27] such as *N*-benzoylglycine can act as chiral inducers in asymmetric autocatalysis to afford highly enantioenriched products.

Cytosine, a constituent of DNA and RNA, is a base of cytidine and deoxycytidine and is an essentially flat achiral molecule. Cytosine plays a crucial role in pairing, through hydrogen bonds with the guanine base of guanosine and deoxyguanosine. Cytosine is involved in the genetic codon of 17 amino acids and controls the essential features of life. It is conceivable that cytosine was formed under prebiotic conditions[28] and already existed before the RNA world emerged.

Here we show that achiral cytosine, a nucleobase, spontaneously forms enantioenriched crystals when stirring is applied during crystallization[29] and that a chiral crystal of achiral cytosine acts as a chiral initiator for asymmetric autocatalysis with amplification of chirality to provide a near enantiopure compound (Figure 1).

We discovered that achiral cytosine, when crystallized from methanol with stirring and without adding any seed crystals, affords powderlike crystals that exhibit either a plus or minus Cotton effect at approximately 310 nm in the solid-state CD spectra (Scheme 1).[30] Out of 55 stirred crystallizations, (+)-cytosine was obtained 21 times, (−)-cytosine was formed 24 times, and 10 samples were below the detection level (Scheme 1, Table 1). When the crystallizations were performed with the same apparatus, (+)- and (−)-crystals were generated preferentially in different runs (Table 1, entries 1–4, 5–8, and 9–12 respectively). The crystalline chirality is spontaneously generated under the stirring

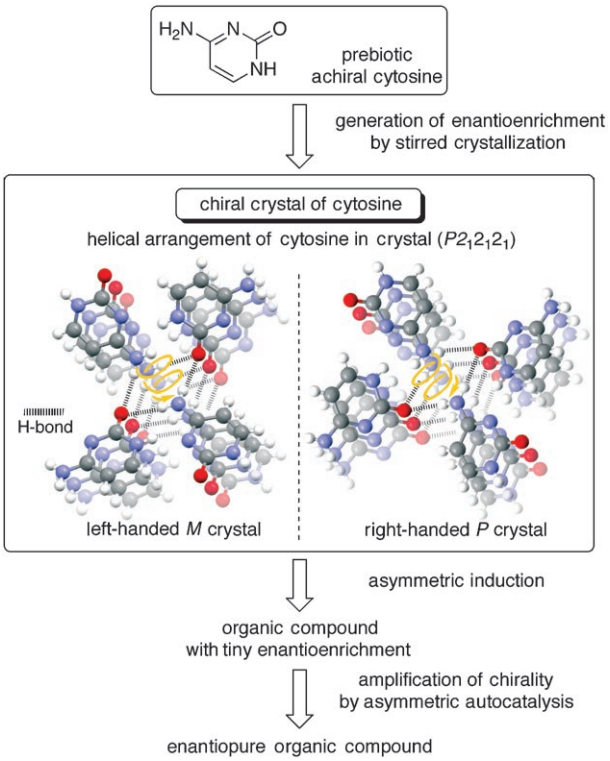
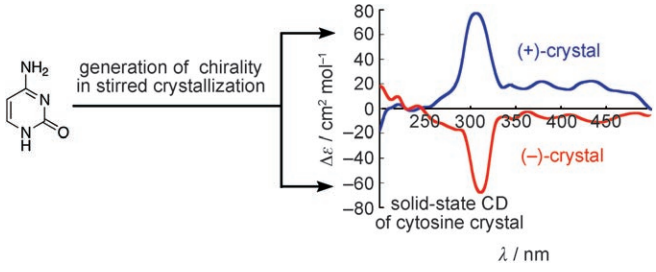


Figure 1. Proposed scenario for the evolution of chirality in nature by using achiral cytosine as a source of chirality.



Scheme 1.

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Table 1: Generation of chirality by the crystallization of achiral cytosine with stirring and the frequency of formation of each enantiomorph.[a]

Entry	CD[b]	Entry	CD[b]	Entry	CD[b]
1[c]	−	20	−	39	−
2[c]	BDL	21	BDL	40	+
3[c]	−	22	+	41	+
4[c]	+	23	−	42	−
5[d]	−	24	+	43	BDL
6[d]	+	25	BDL	44	−
7[d]	−	26	−	45	+
8[d]	+	27	+	46	BDL
9[e]	−	28	+	47	−
10[e]	+	29	−	48	+
11[e]	−	30	+	49	+
12[e]	−	31	−	50	BDL
13	+	32	+	51	−
14	BDL	33	−	52	+
15	−	34	−	53	−
16	BDL	35	BDL	54	BDL
17	+	36	−	55	+
18	−	37	+		
19	−	38	+		

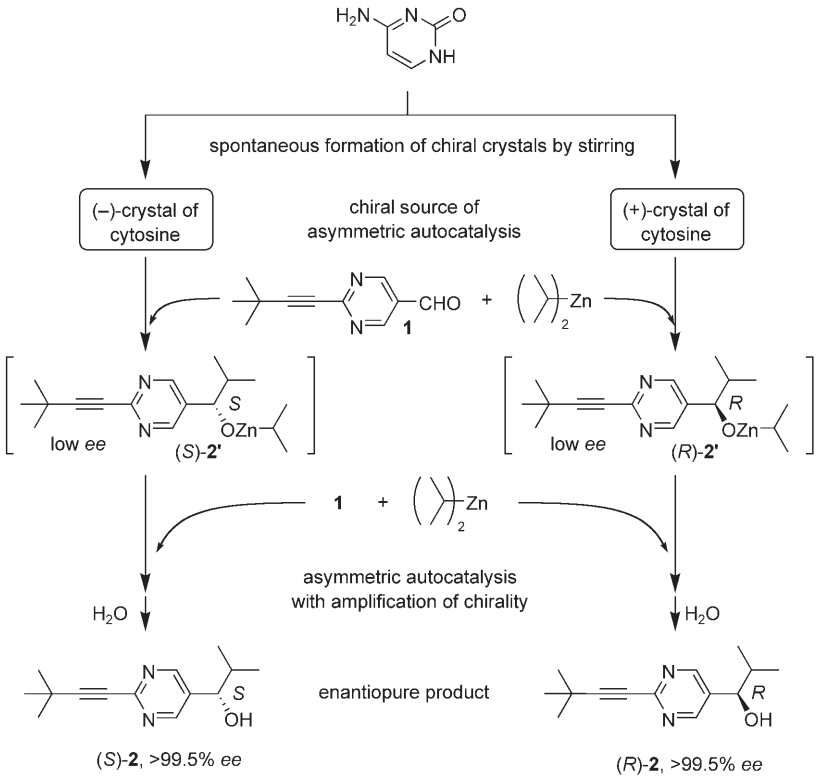
[a] See the Experimental Section for details. [b] Circular dichroism at 310 nm. BDL: Below the detection level. [c, d, e] Crystallizations were performed by using the same apparatuses, respectively.

conditions, and the frequency of generated handedness exhibited an approximately stochastic distribution. When crystallizations were performed 14 times without stirring, the CD spectra of the obtained cytosine crystals were below the detection level. Thus, the stirring is considered to promote the secondary nucleation of the initially generated crystal nuclei to afford a large amount of crystals with the same crystal configuration as the initial nuclei.[29]

Next, in order to show that the chiral crystal of achiral cytosine can generate a large amount of enantiopure organic compound, we performed an asymmetric autocatalysis with amplification of chirality[14,15] by using a chiral crystal of cytosine as the chiral initiator (Scheme 2, Table 2). In series A, the chiral crystals that are spontaneously crystallized with stirring are used as chiral triggers for asymmetric autocatalysis. As shown in entry 1 in Table 2, when pyrimidine-5-carbaldehyde **1** and diisopropylzinc (iPr$_2$Zn) reacted in the presence of a crystal of (+)-cytosine, which was obtained by spontaneous crystallization with stirring (Table 1, entry 4), enantioenriched pyrimidyl alkanol (*R*)-**2** was obtained with 89% *ee* after the subsequent asymmetric autocatalysis, through addition of **1** and iPr$_2$Zn (Table 2, entry 1). On the other hand, the (−)-cytosine crystal (Table 1, entry 3) induced the production of enantioenriched alkanol (*S*)-**2** with 86% *ee*. The reproducibility is shown in entries 3 and 4 of Table 2, respectively. In series B, crystals prepared by a seeding method that used chiral crystals of cytosine were used as the initiators in the asymmetric autocatalysis. The correlation between the crystal configuration and the absolute configuration of the resulting alkanol **2** is the same as that for the asymmetric autocatalysis with spontaneously generated crystals (Table 2, entries 5–16). It should be noted that additional consecutive asymmetric autocatalysis afforded near enantiopure (*R*)- or (*S*)-**2** with >99.5% *ee* (Table 2, series C). It is apparent that the crystalline chirality of the achiral cytosine is responsible for the enantioselective iPr$_2$Zn addition to aldehyde **1**. In these reactions, the crystalline chirality, which comes from the helical arrangement of cytosine molecules ($P2_12_12_1$),[31] induces a tiny enantiomeric imbalance during the formation of the zinc alkoxide in the initial stage of the reaction. During the subsequent asymmetric autocatalysis, this tiny enantiomeric imbalance is amplified to afford the near enantiopure alkanol product.

This sequence of reactions represents one of the chemical processes in which the scenario for the evolution of chirality from the achiral nucleobase cytosine was achieved in real chemical reactions. The sequential process of asymmetric induction in the organic product with an asymmetric carbon atom and the amplification of chirality through asymmetric autocatalysis indicates the possibility of cytosine being the origin of chirality.

In summary, we have demonstrated that the origin of the homochirality of biomolecules may have involved the inherently achiral biomolecule cytosine. In conjunction with suitable amplification of chirality by asymmetric autocatalysis, spontaneously formed chiral crystals of achiral cytosine



Scheme 2.

Table 2: Asymmetric autocatalysis with amplification of chirality initiated by chiral crystal of achiral cytosine.[a]

Entry	Crystal of cytosine		5-Pyrimidyl alkanol **2**		
	CD sign[b]	sample	yield [%][c]	*ee* [%][d]	Configuration
		series A[e]			
1	+	Table 1, entry 4	81	89	*R*
2	−	Table 1, entry 3	83	86	*S*
3	+	Table 1, entry 6	81	75	*R*
4	−	Table 1, entry 7	85	88	*S*
		series B[f]			
5	+	crystal no. 1	88	94	*R*
6	+	crystal no. 1	88	95	*R*
7	−	crystal no. 2	88	90	*S*
8	−	crystal no. 2	88	93	*S*
9	+	crystal no. 3	90	95	*R*
10	+	crystal no. 3	91	96	*R*
11	−	crystal no. 4	86	89	*S*
12	−	crystal no. 4	93	94	*S*
13	+	crystal no. 5	83	88	*R*
14	+	crystal no. 5	94	95	*R*
15	−	crystal no. 6	91	91	*S*
16	−	crystal no. 6	93	95	*S*
		series C[g]			
17	+	crystal no. 1	88	>99.5	*R*
18	−	crystal no. 2	85	>99.5	*S*

[a] The molar ratio of cytosine/**1**/iPr$_2$Zn was 0.075:1.325:2.78. Aldehyde **1** and iPr$_2$Zn were added in four separate portions. [b] See footnote [b] in Table 1. [c] Yield of isolated product. [d] The enantiomeric excess (*ee*) value was determined by HPLC on a chiral stationary phase. [e] Cytosine crystals obtained from spontaneous crystallization with stirring (Table 1), were used as the chiral inducer. [f] Crystals obtained from the seeding method were used as the chiral inducer. By using (+)-cytosine as a seed crystal, the formation of cytosine crystals with the same (+) configuration was observed, with good reproducibility. The analogous production of (−)-cytosine crystals was also observed. [g] After the typical experimental procedure, four additional rounds of consecutive asymmetric autocatalyses were performed.

may have acted as an origin of homochirality in biomolecules. Consequently, our results significantly expand the opportunities for the use of cytosine, not only as a nucleobase but also as a source of homochirality in biomolecules.

Experimental Section

Spontaneous stirred crystallization of cytosine: Cytosine (0.2 g) was dissolved in MeOH (30 mL) under reflux conditions. The resulting solution was cooled gradually to room temperature with stirring. The precipitate appeared at room temperature and was filtered to obtain powderlike crystals of cytosine. The obtained crystals were dried in vacuo and were ground with a pestle and mortar (particle size: 1–5 μm). The solid-state CD spectra (nujol, NaCl, Jasco J-725 spectropolarimeter) were measured and used to determine the crystal configuration.

Preparation of the (+)-cytosine crystal by seeding with a (+)-cytosine crystal: Cytosine (0.2 g) was dissolved in MeOH (30 mL) under reflux conditions with stirring. A crystal of (+)-cytosine (approximately 0.4 mg) was added to the solution as the seed crystal at 60 °C. The temperature of the mixture was gradually lowered to 35 °C for a period of 2 h with stirring, and the mixture was then filtered. The resulting precipitate was dried in vacuo to afford the crystals of (+)-cytosine (approximately 60 mg). After being ground with a pestle and mortar, the enantiomorphs were evaluated by measurement of the solid-state CD spectra. In a similar manner, crystals of (−)-cytosine were formed with good reproducibility by seeding with (−)-cytosine crystals.

General procedure for asymmetric autocatalysis with an enantiomorphous crystal of cytosine (Table 2, entry 16): An enantiomorphous crystal of cytosine was ground into a fine powder by using a pestle and mortar (particle size: 1–5 μm). iPr$_2$Zn (0.08 mmol, 0.8 mL, 1.0 M toluene solution) was added dropwise to the powdered crystal of (−)-cytosine (8.3 mg, 0.075 mmol) and aldehyde **1** (4.7 mg, 0.025 mmol) at 0 °C and the mixture stirred for 12 h. After the addition of toluene (0.75 mL), iPr$_2$Zn (0.3 mmol, 0.3 mL, 1.0 M toluene solution) was then added over a period of 30 min at 0 °C, and the mixture was stirred for 10 min at 0 °C. A solution of **1** (18.8 mg, 0.1 mmol) in toluene (0.75 mL) was added over a period of 1 h at 0 °C, and the reaction mixture stirred at 0 °C for 2 h. Toluene (5.0 mL), iPr$_2$Zn (0.8 mmol, 0.8 mL, 1.0 M tolouene solution), and a solution of **1** (75.3 mg, 0.4 mmol) in toluene (2.0 mL) were then added over a period of 1 h at 0 °C, and the mixture was stirred at 0 °C for 1 h. Once again, toluene (14 mL) and iPr$_2$Zn (1.6 mmol, 1.6 mL, 1.0 M toluene solution) were added successively, and a solution of **1** (150.4 mg, 0.8 mmol) in toluene (4.0 mL) was added dropwise over a period of 1 h at 0 °C. After the mixture had been stirred for 1 h, the reaction was quenched with HCl (1 M, 6 mL) and neutralized with a saturated $NaHCO_3$ solution (18 mL). The mixture was then filtered through celite and the filtrate was extracted with AcOEt (three times). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification of the residue by column chromatography on silica gel (hexane/AcOEt, 3:1 to 2:1) gave the alkanol (*S*)-**2** (284.9 mg, 1.23 mmol, 95 % *ee*) in 93 % yield. The *ee* value was determined by HPLC with a chiral stationary phase.

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