former even though its concentration was as low as 10% (F. Leveiller, unpublished results).
18. L. Addadi and S. Weiner, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4110 (1985).
19. G. J. Warren, *Biotechnol. Genet. Eng. Rev.* **5**, 107 (1987); H. Mizuno, *Proteins* **5**, 47 (1989).
20. We thank E. Shavit and I. Müller for participation in the experiments. We acknowledge a preindustrial grant from Yeda and financial support from the U.S.–Israel Binational Foundation and the Petroleum Fund of the American Chemical Society. M.G. thanks the Feinberg Graduate School of the Weizmann Institute of Science for a postdoctoral fellowship.

31 May 1990; accepted 28 August 1990

Chiral Symmetry Breaking in Sodium Chlorate Crystallization

DILIP K. KONDEPUDI,* REBECCA J. KAUFMAN, NOLINI SINGH

Sodium chlorate ($NaClO_3$) crystals are optically active although the molecules of the compound are not chiral. When crystallized from an aqueous solution while the solution is not stirred, statistically equal numbers of levo (L) and dextro (D) $NaClO_3$ crystals were found. When the solution was stirred, however, almost all of the $NaClO_3$ crystals (99.7 percent) in a particular sample had the same chirality, either levo or dextro. This result represents an experimental demonstration of chiral symmetry breaking or total spontaneous resolution on a macroscopic level brought about by autocatalysis and competition between L- and D-crystals.

IN NATURE WE FIND STATES OF BROKEN chiral symmetry at all levels, from elementary particles to the biosphere (*1*). Processes that lead to transitions from a chirally symmetric state to a totally asymmetric state are thus of general interest. Although many mechanisms that produce totally asymmetric macroscopic states exist in theory (*2–4*), none have been experimentally realized. The difficulty has been that these models require both autocatalysis and competition between the left- and the right-handed products. Here we report how so simple a process as constant stirring of the solution generates both autocatalysis and competition and thus can produce totally asymmetric macroscopic states of crystals that all have the same handedness.

The nucleation of achiral $NaClO_3$ molecules to form an optically active chiral crystal is an example of symmetry breaking, but at a microscopic level. In a typical crystallization, however, the chiral symmetry is restored, in that statistically equal numbers of L- and D-crystals are obtained, as was shown by the experiments of Kipping and Pope almost 100 years ago (*5*).

More recently, Pincock *et al.* (*6–8*) performed a careful statistical study on 1,1′-binaphthyl. For the proportion of molecules that crystallized in one of the two enantiomeric forms, they obtained a distribution that is close to a Gaussian with a mean of 50%. These experiments clearly showed that the two enantiomorphs will be found in equal proportion; any deviation from chiral symmetry is a result of statistical fluctuation. As Pincock *et al.* note, "From this distribution it is apparent that obtaining binaphthyl with an optical purity above 90 percent by this method would be a very exceptional event (observable about one in 150 tries)" (*6*, p. 1019). In order to produce total asymmetry of close to 100% in the product crystals in every try, autocatalysis and competition between the L- and the D-crystals are needed.

We note that in crystallization experiments unequal numbers of L- and D-crystals are often encountered due to the presence of impurities (*9, 10*). On the other hand, numerous studies [see, for example, (*11–13*), and references therein] have investigated enantioselective crystallization induced by the presence of optically active compounds, seeds, or other asymmetric influences. In these cases, the predominance of one chirality is not due to an intrinsic mechanism of symmetry breaking and it does not occur spontaneously.

We first performed the crystallization without stirring and found results similar to those found by Kipping and Pope (*5*): in a total of 1000 crystals obtained from 17 different crystallizations, 525 were found to be levo and 475 were dextro. Of the 17 crystallizations, 8 produced slightly more L- than D-crystals because of statistical fluctuations.

The above crystallizations were performed as follows. A solution of $NaClO_3$ was prepared by dissolving $\sim$100 g of $NaClO_3$ (of 99.9% purity) in $\sim$120 ml of distilled water. The resulting solution was constantly stirred and heated to $\sim$50°C. The stirring was continued for $\sim$30 min to ensure complete desolution of the solute. The solution was then filtered and cooled to room temperature ($\sim$25°C). Samples of $\sim$25 ml of this solution were transferred to several petri dishes and left for crystals to grow. The initial concentration was such that cooling alone did not produce any crystals; evaporation of the solution was necessary. Temperature was not controlled, and it varied about ±3°C. After 4 to 5 days, the crystals that were large and optically transparent enough for the detection of optical rotation were separated. The smallest of these crystals were $\sim$1 mm in size. We used a pair of polarizers to separate and count the L- and D-crystals. When white light is used, the crystals appear blue when the polarizers are crossed and turn red on rotating the analyzer clockwise or anticlockwise depending on the crystal's handedness. For a 1-mm crystal, the change in color from blue to red occurs for a rotation of about 7°.

We found that constant stirring of the solution has a dramatic effect on the enantiomorphic ratio of the $NaClO_3$ crystals: in each sample the crystals are almost all dextro- or all levo-rotatory. This process occurred without the need for careful control of temperature or other conditions.

Samples of the $NaClO_3$ solution (25 ml) prepared as described above were transferred to several 100-ml beakers and stirred constantly with a magnetic stirrer and a 12 mm by 4 mm by 4 mm Teflon stirbar. The beakers were covered with tissue paper to prevent dust particles from entering the system and to slow the rate of evaporation. All of the stirrer speeds were set at the same level ($\sim$100 rpm). As before, L- and D-crystals were separated and counted. These crystals were in general smaller and more numerous than those found in unstirred experiments; they were also more rounded.

In a total of 32 different crystallizations, 18 were predominantly dextro while 14 were predominantly levo. No sample that produced crystals was discarded. In Table 1 the crystal counts are shown for each of the 32 crystallizations. Stirrers, with fixed direction of stirring, produced both L- and D-dominated crystallizations in different runs. A total of 11,829 crystals were counted. As can be seen from the data, amidst predominantly L-crystals a small number of D-crystals grew and vice versa. Similar asymmetric crystallization was also obtained when an overhead stirrer was used.

In the 18 D-dominated crystallizations in Table 1, the fraction of D-crystals is found to

---

Department of Chemistry, Box 7486, Wake Forest University, Winston-Salem, NC 27109.

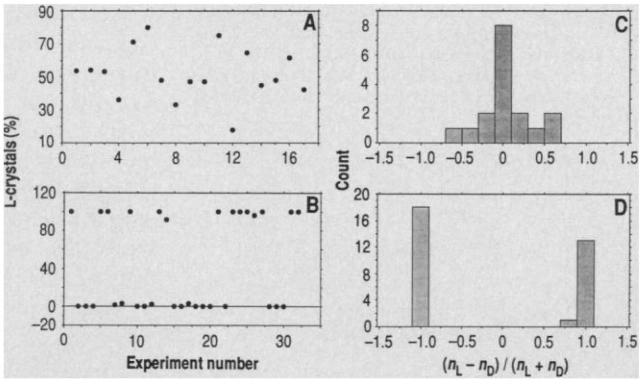

Fig. 1. Comparison of statistics of stirred versus unstirred samples. The number of crystals in the unstirred experiments were generally much lower than those in the stirred experiments. The experiment numbers are in the order in which the data were acquired. Scatter plot of the percentage of L-crystals is shown in (**A**) for the unstirred and in (**B**) for the stirred experiments. Frequency distribution for crystal enantiomeric excess is shown in (**C**) for the unstirred and in (**D**) for the stirred experiments; n_L and n_D are the number of L- and D-crystals, respectively, in a particular experiment.

be 0.9973; for the 14 L-dominated crystallizations the fraction is 0.9970. This process is an example of total spontaneous resolution: $NaClO_3$ crystals of optical purity greater than 99% were produced in every try with high certainty. In one particular run (173 L- and 15 D-crystals), however, a large fluctuation did arise. A comparison of the scatter plots for the percentage of L-crystals and histograms for the stirred and unstirred experiments are shown in Fig. 1.

As for the mechanism, the rapid production of secondary nuclei from a primary nucleus in stirred systems [a process much studied by researchers of continuous crystallization (*14–16*)] must be involved. This process is chirally autocatalytic. Empirical rates, R_s, for secondary nucleation of the form

$$R_s = k(c - c_0)^\alpha \qquad (1)$$

in which k is a factor that depends on the rate of stirring, c is the concentration, and c_0 is the concentration at saturation, have been suggested (*14*). The exponent α is found to have a value greater than 1 (*14, 17*).

Table 1. Number of L-crystals (n_L) and D-crystals (n_D) counted in 32 different crystallizations performed while the solution was constantly stirred. (For the first seven samples, counting was stopped at 100 or 150. When effect of stirring became clearer, as many crystals as possible were counted in each sample to accumulate good statistics).

L-dominated		D-dominated	
n_L	n_D	n_L	n_D
150	0	0	150
100	0	0	150
150	0	0	150
100	0		
500*	0	0	450
86	0	3	100
173	15	2	841
335	0	0	291
651	0	6	263
642	0	0	405
800	0	0	297
26	1	2	73
865	0	0	401
758	0	0	781
563	0	1	487
		1	480
		0	457
		0	371
		0	580
		2	120
5399†	16	17	6397

*Subtotals. †Totals.

However, secondary nucleation alone is not sufficient to produce the observed homochirality. The formation of crystals of a particular handedness, and their proliferation due to secondary nucleation, must be accompanied by suppression of nucleation of crystals of the opposite handedness (a form of competition). Without this suppression, considering the duration of the experiment—which is of the order of 4 to 5 days—one must expect nuclei of the opposite handedness to also form and proliferate. In 4 to 5 days, we do see that primary nucleation produces crystals of both kinds in crystallization performed without stirring.

Effective suppression can come about due to the sensitive dependence of the rate of primary nucleation (nucleation that is not caused by the presence of other crystal nuclei) on the $NaClO_3$ concentration (*18, 19*). Primary homogeneous and heterogeneous nucleation rates are both proportional to $\exp[-A(T)/\ln^2(c/c_0)]$, in which $A(T)$ is a term that depends on absolute temperature T, c is the concentrations, and c_0 the concentration at saturation (*17–19*).

In our experiments, the depletion of the solute from the solution due to the growth of the primary nucleus and the secondary nuclei that are rapidly generated from it could reduce the concentration to a level at which the rate of primary nucleation is virtually zero. Thus, the L- and D-nuclei compete for the solute and the growth of one suppresses all primary nucleation and therefore the formation of the nuclei of the opposite handedness. As a result, the only nuclei that grow are the initial nucleus and the secondary nuclei that are generated from it, all of which have the same handedness.

When the solution is not stirred, there is no rapid autocatalytic production of nuclei; all of the nuclei are produced through primary nucleation, homogeneous or heterogeneous, and their handedness is random. In this case, too, the depletion of the solute due to crystal growth may eventually stop the primary nucleation, but apparently only after a sufficiently large and statistically equal number of L- and D-crystals have been produced.

That crystals of high optical purity can be obtained by appropriate human intervention such as ample seeding, and appropriate fluxing of solution is well known (*11, 13*). However, spontaneous resolution described above, occurring as a consequence of autocatalysis and competition, and in conditions that do not require careful control, has never been demonstrated to our knowledge. Processes such as these may provide us insight into the possible origins of biomolecular chirality.

REFERENCES AND NOTES

1. S. F. Mason, *Nature* **311**, 19 (1984); R. Hegstrom and D. K. Kondepudi, *Sci. Am.* **262**, 108 (January 1990).
2. E. Havinga, *Biochim. Biophys. Acta* **13**, 171 (1954).
3. M. Calvin *Chemical Evolution* (Oxford Univ. Press, London, 1969), p. 150.
4. W. Thiemann, Ed., *Origins Life* **11**, 1 (1981).
5. F. S. Kipping and W. J. Pope, *J. Chem. Soc. Trans.* **73**, 606 (1898).
6. R. E. Pincock, R. R. Perkins, A. S. Ma, K. R. Wilson, *Science* **174**, 1018 (1971).
7. R. E. Pincock, *J. Chem. Ed.* **50**, 455 (1973).
8. K. R. Wilson and R. E. Pincock, *J. Am. Chem. Soc.* **97**, 1474 (1975).
9. G. Wald, *Ann. N.Y. Acad. Sci.* **69**, 352 (1957).
10. K. Harada, *Naturwissenschaften* **57**, 114 (1970).
11. R. M. Secor, *Chem. Rev.* **63**, 297 (1963).
12. K. L. Kovacs, *J. Mol. Evol.* **10**, 161 (1977).
13. A. Collet, M-J. Brienne, J. Jacques, *Chem. Rev.* **80**, 215 (1980).
14. G. D. Botsaris and E. G. Denk, *Annu. Rev. Ind. Eng. Chem.* **1970**, 493 (1972).
15. R. F. Strickland-Constable, *AIChE Symp. Ser.* **121**, 1 (1972).
16. S. J. Jancic and P. A. M. Grootscholten, *Industrial Crystallization* (Reidel, Dordrect, 1984).
17. A. D. Randolph and M. A. Larson, *Theory of Particulate Processes* (Academic Press, New York, 1971).
18. J. W. Mullin, *Crystallization* (Butterworths, London, ed. 2, 1972).
19. A. C. Zettlemoyer, *Nucleation* (Marcel Dekker, New York, 1961).

20. We thank R. Carter and P. M. McBride of Yale University for confirming this effect of stirring. We thank F. Quina for providing the initial impetus for this work and W. Hinze for his constant encouragement. We also thank RECREAC of Wake Forest University and the Z. Smith Reynolds Foundation for providing funding for this work. We thank P. M. McBride for making suggestions for improving the manuscript.

10 August 1990; accepted 23 October 1990