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Self-Organization of Adenine and Thymine in the Solid State[†]

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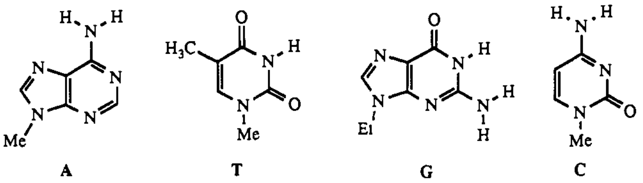
In the last several years we have demonstrated that hydrogen-bonded cocrystals can be formed simply by grinding two or more components together in the solid state.[1] The driving force often is the formation of stronger hydrogen bonds in the cocrystal than those present in the starting crystals of either component. The rates of phase transformation from a mixture of two phases to a pure cocrystal phase vary from minutes to weeks depending on grinding efficiency and vapor pressure of the components. A typical example involves the formation of cocrystals of 2-aminopyrimidine with carboxylic acids where the acid and pyrimidine molecules form hydrogen bonds to one another.[2] Here we report that 9-methyladenine (A) and 1-methylthymine (T) also form cocrystals in the solid state by grinding pure A and pure T together and that the A and T molecules in the cocrystal are associated as Hoogsteen base pairs. Moreover, AT cocrystals form selectively when A and T are ground together in the presence of a third competing purine or pyrimidine. Cocrystals of 9-ethylguanine (G) and 1-methylcytosine (C), which have been cocrystallized from solution and characterized crystallographically,[3] do not form in the solid state, however, and remain as a G, C mixture despite intermittent grinding and heating at 50 °C for more than 7 days.

In Table I, a summary of the base pairing experiments is given. Stoichiometric amounts of the crystalline bases totalling about 100 mg were placed in a 2.5-cm steel capsule containing a 3-mm ball bearing and ground using a mechanical amalgamator for 20 min. X-ray powder patterns run on the product phases were compared to the patterns of the reagents and analyzed for new peaks corresponding to base pair cocrystals (Figure 1). The standard powder pattern spectrum of the AT base pair was generated by computer simulation (POWD)[4] on the basis of known single crystal structural data.[5] The experimental and calculated X-ray powder diffraction data are presented in Table II. The match between the experimental and calculated powder pattern indicates that the crystalline AT phase has the same Hoogsteen hydrogen-bonding pattern as observed in the AT crystal structure.

In all cases the only new peaks found in the product X-ray powder patterns were those corresponding to the complementary base pair combination AT. In ternary mixtures, peaks due to the noncomplementary component (e.g., G in a mixture of A, T, and G) remained unchanged during the grinding process. In other words, the base pairing of AT occurs selectively in the presence of the noncomplementary purine or pyrimidine bases, G and C, as demonstrated by grinding A + T + C or A + T + G (Figure 2). We have also seen that noncomplementary base pairs, such as GT, will not form when independently ground together.

Table I. Solid-State Grinding Experiments with Nucleotide Bases



component in reaction mixture[a]	product composition (··· indicates cocrystal pairs)
A, T	A···T
G, C	G, C
G, T	G, T
A, T, C	A···T, C
A, T, G	A···T, G
C, G, A	C, G, A
C, G, T	C, G, T

[a] The mixtures were ground for 20 min at room temperature in a mechanical amalgamator. Mixing conditions for G and C are given in the text.

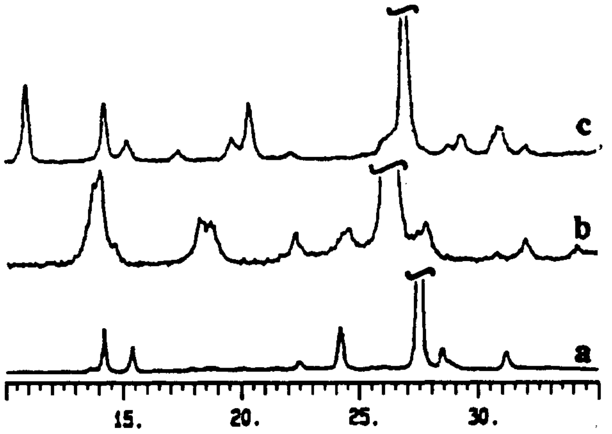


Figure 1. Solid-state complexation of A and T is demonstrated by X-ray powder pattern analysis of (a) A, (b) T, and (c) the cocrystal A···T formed by grinding A and T together.

Table II. Experimentally Determined and Calculated X-ray Powder Diffraction Data for the AT Complex

exptl		calcd[4]	
2θ	(I)	2θ	(I)
11.1	(41.0)	11.1	(65.1)
14.4	(30.0)	14.4	(34.5)
15.3	(12.0)	15.3	(14.1)
19.8	(13.7)	19.8	(11.7)
20.6	(29.5)	20.6	(36.7)
26.6	(13.2)	26.6	(8.4)
27.1	(100.0)	27.2	(100.0)
29.5	(10.6)	29.5	(10.4)
31.1	(15.1)	31.1	(9.7)

Additional evidence for base pairing was obtained by comparing the solid-state IR spectra of the milled mixtures to those of the individual nucleotide bases. The most profound changes seen in the spectra upon complexation were in the N–H stretching region. Whereas the individual A and T bases showed two N-H stretching bands at 3268 and 3093 cm^{-1} and at 3149 and 3068 cm^{-1}, respectively, the AT complex N-H stretching bands were sharper and appeared at 3395 and 3198 cm^{-1}. IR spectroscopy revealed that at least 90% of the solid sample, including the crystalline and amorphous phases, is composed of the complementary AT base pair. Selective formation of the AT complex in the presence of the noncomplementary base pairs was also verified by the IR

[†] This paper is dedicated to the memory of Professor Margaret C. Etter, deceased June 10, 1992.

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(1) (a) Etter, M. C. *J. Phys. Chem.* **1990**, *95*, 4601. (b) Etter, M. C.; Frankenbach, G. M. *Chem. Mater.* **1989**, *1*, 10. (c) Etter, M. C.; Urbañczyk-Lipkowska, Z.; Zia-Ebrahimi, M.; Panunto, T. W. *J. Am. Chem. Soc.* **1990**, *112*, 8415. (d) Etter, M. C.; Reutzel, S. M. *J. Am. Chem. Soc.* **1991**, *113*, 2586.

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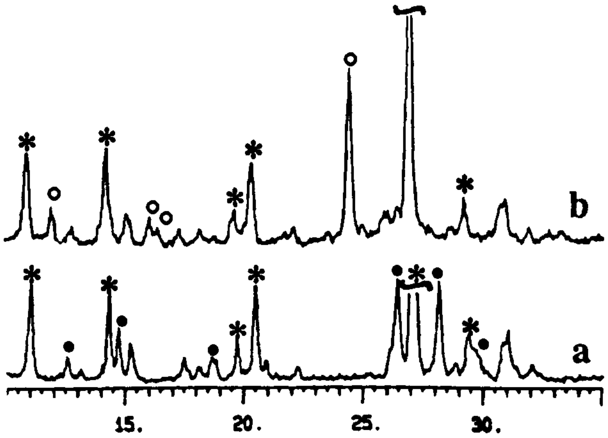
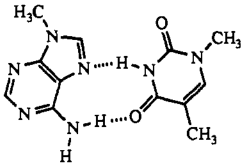


Figure 2. Selective solid-state complexation of A and T in the presence of the noncomplementary bases, C and G, is demonstrated by X-ray powder pattern analysis. (a) The powder pattern of the ternary mixture shows that all peaks can be assigned to two phases: the AT cocrystal phase (*) and pure C (●). (b) The powder pattern of the product formed by grinding A, T, and G shows AT and G (○) phases only.

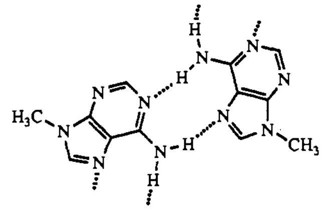
spectra. The resulting spectra of the ternary mixtures consisted of the superimposed spectra of AT and the "extra" nucleotide base.

The results of these experiments clearly show that complementary base pairing occurs when A and T are ground together. The product AT phase that forms not only contains the complementary components in the cocrystal but also has precisely the same Hoogsteen hydrogen-bonding pattern and crystal structure as the analogous cocrystal grown from solution.

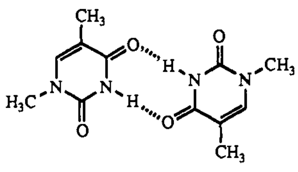
The phase changes observed here involve selectivity of a purine for a particular pyrimidine, or vice versa, as well as selectivity of only one of many possible alternative hydrogen-bonding patterns that could be envisaged for purine–pyrimidine pairs. The mechanism for complementary base pairing in the solid state most likely involves complex cooperative processes between homomeric and heteromeric hydrogen-bond aggregates, not just interactions between individual free molecules as might occur in mobile phases. Thus, it is even more striking that the same selectivity rules for heterodimer formation have been observed by both the solid-state and solution crystallization methods. The starting material hydrogen-bonding patterns, shown below, rearrange to the Hoogsteen AT pattern.



9-methyladenine : 1-methylthymine (AT)
Hoogsteen mode



9-Methyladenine (A)



1-Methylthymine (T)

It is not clear why the GC base pair does not form in the solid state. Further work is needed to understand the intricate processes involved in cooperative hydrogen-bonding rearrangements and solid-state phase transformations in organic solids.

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