New Evidence for Template Effects in a Self-Replicating System

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In a recent paper in this Journal,[1] Menger, Eliseev, and Khanjin (hereafter MEK) present experiments purported to bear on our self-replicating system.[2,3] None of the data reported from MEK's experiments were obtained under our published conditions, or even in our range of concentrations; rather, the "catalysts" are 4–90 times more concentrated. Even so, MEK conclude that template effects are superfluous in our system. Instead, they offer a "syllogism" based on the premise that "amides accelerate acylations." [1] They contend that the amide formed in our reaction is responsible for the catalysis observed, even though we had earlier excluded this possibility.[3] We describe here our own experimental evidence that shows that (1) MEK's core observations are not reproduced under our published conditions; (2) the premise of MEK's syllogism is false; (3) MEK's experiments contradict their own arguments; and (4) template-catalyzed replication is more consistent with the observations.

The reaction involves the coupling of pentafluorophenyl ester **1** (Chart 1) with the amine **2** in $CHCl_3$ to give the self-complementary **3a**. The reaction was originally run at 1.6, 8.2, and 16 mM initial concentrations of starting materials, and the initial rates of coupling were determined by following the appearance of product **3a** using HPLC. The reaction shows modest but reproducible autocatalysis: adding the product to the reaction mixture increases the coupling rate. For example, at 8.2 mM, adding 0.5 equiv of **3** increases the initial rate by 70%.[3]

At issue is neither how efficient this autocatalytic process is nor whether other, more effective catalysts can be found. Rather, the question is, What structural features in **3a** are responsible for the autocatalysis which we observed? A most informative experiment, reported earlier,[3] involved the N-methylated imide **3b**: no rate enhancement is seen (within the 5% experimental error) when 0.2 equiv of **3b** is added to the reaction of **1** + **2** at initial concentrations of 1.6–16 mM. We interpreted this result as evidence that imide–adenosine recognition was required for rate enhancement, and we proposed the termolecular complex **4** (Chart 2)[4] as a mechanism for the observed autocatalysis.

Given the sweeping claims of amide catalysis made by MEK, we undertook a series of overlapping control experiments. These were designed to isolate the potentially catalytic functional groups of **3a**, present them individually and in combinations *in the structural environment of 3a*, and test their contributions to the observed autocatalysis. The results, obtained at 2.2 mM concentration, are summarized in Table 1.[5]

Table 1. Effect of Various Additives on the Reaction of **1** + **2** in $CHCl_3$, 1% TEA Base Added, 22 ± 1 °C, 2.2 mM Initial Concentrations of **1** and **2**

entry	additive (0.5 equiv)	av init rate of prod. formatn (μM/min), ±5%	% of base-line rate
1		0.55	
2	**3a**	0.82	149
3	**3b**	0.55	100
4	**5**	0.56	104
5	**6**	0.50	91
6	**7**	0.52	95
7	**8**	0.56	102
8	**9**	0.56	102
9	**10**	0.57	104

Chart 1

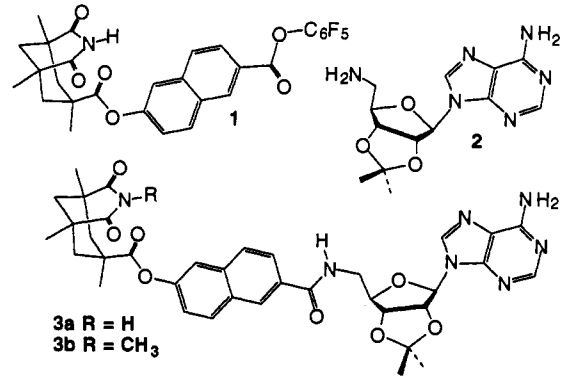
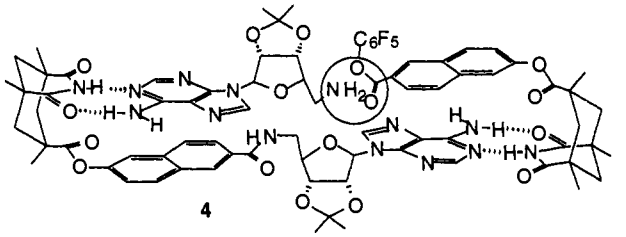


Chart 2



Under these conditions—within our published concentration range[3]—a 49% increase in initial rate was observed when the reaction was seeded with 0.5 equiv of product **3a** (entries 1 and 2). Again, experiments with the N-methylated imide **3b** revealed no rate enhancement; N-methylation of the imide shut down autocatalysis[6] (entry 3). Furthermore, addition of other adenines such as 9-ethyladenine (**5**) (Chart 3) or the naphthoylated ribosyl derivative **6** (entries 4 and 5) excluded the purine nucleus and the ribose as the sources of catalysis, as these molecules failed to catalyze the reaction. Addition of other imides such as **8** or **9** (entries 7 and 8) also showed no rate enhancement, excluding the imide as the sole source of catalysis. Finally, when taken together, the control runs with added **6**, **7**, and **9** revealed that a *trans* secondary amide function, presented in the steric environment of **3a**, was unable to catalyze the reaction *by itself or with either the adenosine or the imide end of 3a*. Thus, the full template **3a** is necessary for catalysis.

The isolated, individual features and partial combinations of the functionalities of **3a** are, therefore, unable to account for the autocatalysis observed. Rather, the whole product molecule is more effective than the sum of its parts. These results are nicely accommodated by a model which invokes template-catalyzed coupling as the source of autocatalysis, that is to say, invokes

(1) Menger, F. M.; Eliseev, A. V.; Khanjin, N. A. *J. Am. Chem. Soc.* **1994**, *116*, 3613–3614.
(2) Tjivikua, T.; Ballester, P.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1990**, *112*, 1249–1250.
(3) Nowick, J. S.; Feng, Q.; Tjivikua, T.; Ballester, P.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1991**, *113*, 8831–8839.
(4) The structure in Chart 3 shows only Watson–Crick base pairing at either end of the complex, but Hoogsteen and combinations of Watson–Crick and Hoogsteen base pairs are equally likely.
(5) Relative rates shown are obtained from the slopes of the first 5% of the coupling reaction. All reactions were performed at 2.2 mM initial concentrations of reactants in $CHCl_3$ with 1% TEA base. Formation of product **3a** was followed by HPLC at 270 nm on a Waters 600E instrument equipped with a Waters 717 autosampler and a Waters 490E UV detector. Temperature inside the autosampler was constant at 22 ± 1 °C in an individually thermostated room. Separation was achieved using a Beckman Ultrasphere SI column, 4.6-mm i.d. × 25-cm length, with gradient elution from 1% to 5% MeOH/$CHCl_3$.
(6) If the purine of **3b** competes with base pairing as suggested by MEK, then so must the purine of **3a**; yet the latter is a catalyst and the former is not.

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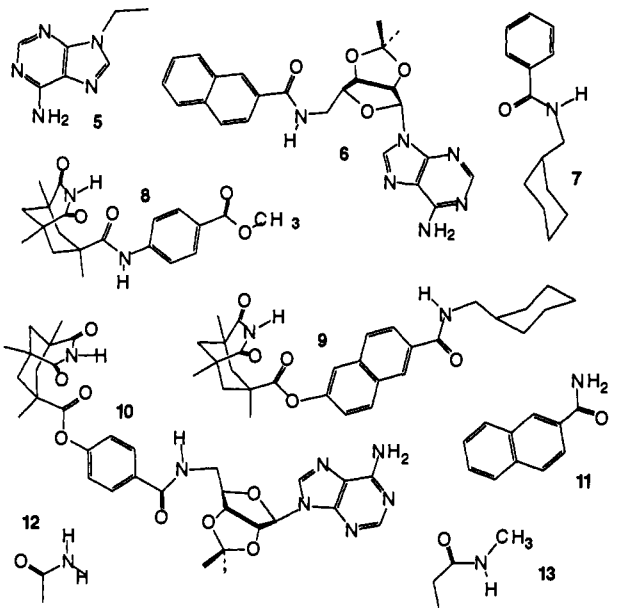
Chart 3



replication through a productive termolecular complex as proposed in Chart 2. There is considerable precedent for catalysis by such termolecular template effects in the literature of both molecular recognition[7,8] and nucleic acid replication.[9] That there is no need to revise this explanation in light of MEK's paper is supported by the following:

Firstly, MEK claim some catalysis of the reaction by the shorter secondary amide structure **10** (Chart 3). However, we were unable to reproduce this result in three separate attempts under our conditions (Table 1, entry 9). While the experimental details for MEK's claim of catalysis by **10** are missing, perhaps the problem lies in their use of ^{19}F NMR to follow a reaction in which the product contains no fluorine. While MEK followed starting materials and released pentafluorophenol, we followed formation of the product **3a** in all of our published experiments.

Secondly, MEK's premise that "amides accelerate acylations" is false; not all amides do so. The data in Table 1 show that the five secondary amides **3b**, **6**, **7**, **9**, and **10** all fail to catalyze the reaction under conditions where (auto)catalysis by **3a** is observed. MEK's proposed mechanism of amide catalysis in our system is thus refuted. The problem lies in the choice of analogy. MEK assert that 2-naphthamide (**11**) and acetamide (**12**) catalyze the coupling of **1** and **2**, and then they argue that any amide will do likewise. This is not sound reasoning; **11** and **12** are primary amides, functions which do not appear in **3a**.

Thirdly, when MEK examine the secondary amide **13** at our concentrations (footnote 12 of their publication), they find that **13** fails to catalyze the acylation reaction. This result contradicts their own arguments.

Why do MEK find significant amide catalysis by molecules **11**, **12**, **13**, and even **10** under their conditions? To begin, neither **11** nor **12** is a trans secondary amide, and **11** and **12** are therefore more sterically and electronically inclined toward catalysis, especially through the proton donor–acceptor capability of a primary (or cis) amide.[10,11] In the case of **10** and **13** *at concentrations 27 times higher than our own*, it is likely, perhaps inevitable, that the process of general base catalysis, which has a third-order rate constant,[11] overtakes the template catalysis of **3a**, which is of order $2^1/_2$. As shown by von Kiedrowski,[9] the lower rate constant of template catalysis is due to template dimerization.

In summary, we have shown that *replication*—autocatalysis based on molecular recognition—best accommodates the facts observed in the reaction of **1** with **2**, and that simple amides of the type present in **3a** are ineffective as catalysts under these conditions. Whether amide catalysis applies to reactions beyond those established in the work of Su and Watson[11] will be addressed in the sequel.

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(7) For a timely review, see: Anderson, S.; Anderson, H. L.; Sanders, J. K. M. *Acc. Chem. Res.* **1993**, *26*, 469–475.

(8) Kelly, T. R.; Bridger, G. J.; Zhao, C. *J. Am. Chem. Soc.* **1990**, *112*, 8024–8034. Terfort, A.; von Kiedrowski, G. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 654–656.

(9) von Kiedrowski, G.; Wlotzka, B.; Helbing, J.; Matzen, M.; Jordan, S. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 423–426. von Kiedrowski, G. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 932–935.

(10) For bifunctional catalysis with these groups, see: Swain, C. G.; Brown, J. F., Jr. *J. Am. Chem. Soc.* **1952**, *74*, 2538–2543. Rony, P. R. *J. Am. Chem. Soc.* **1969**, *91*, 6090–6096.

(11) Su, C.-W.; Watson, J. W. *J. Am. Chem. Soc.*, **1974**, *96*, 1854–1857. This definitive study of the catalysis of aminolysis of nitrophenyl esters in chlorobenzene is the closest analogy to the case at hand. It was found that hydrogen bond donors assist the slow breakdown of the tetrahedral intermediate. Oxides of amines, phosphines, and arsines and 2-pyridone were found to be the most effective catalysts, while small, unhindered amides (dimethyl acetamide) were moderate catalysts at 10–50 mM. We suggest that the success of modern peptide coupling reagents such as BOP may be due to their generation of such hydrogen bond donors as side products.