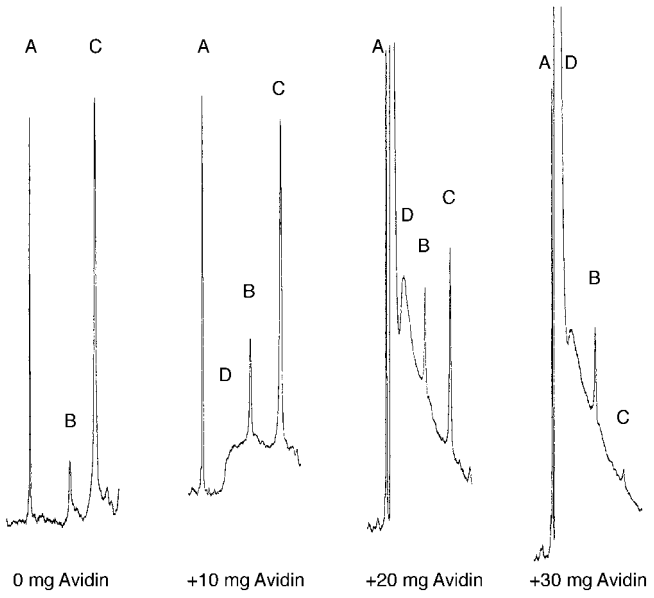

Figure 1. Affinity capillary electrophoresis of product **13** (C) from Reaction (4). A = catalyst complex, B = Pro(*p*-I-Phe)bradykinin (**10**), D = avidin – product complex. See text for further details.

this new method for the construction of structurally well-defined protein conjugates with nonnatural functions can be easily foreseen.

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[1] In principal, known procedures include, among others, total synthesis, peptide-segment condensation,[2] in vitro protein biosynthesis,[3] and reverse proteolysis.[4]

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A Self-Replicating Peptide under Ionic Control**

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The self-replicating abilities of DNA, in addition to providing a genetic transfer mechanism for reproducing species, have played a significant role in the widespread development of biotechnology strategies.[1] DNA is unique in its self-replication, and species that rely on DNA for replication can adapt to environmental changes through natural selection. Recent examples of designed molecular systems capable of self-replication include nucleotide-based oligomers,[2] conjugates of adenine and Kemp's triacid,[3] peptides,[4] and micelles.[5] The production of a self-replicating molecule from a large molecular pool has been a more elusive target.[6] Recent work of Lee et al. demonstrated that peptides from the GCN4 leucine zipper domain self-replicate in an autocatalytic cycle.[4] We sought a peptidic self-replicating system that would be sensitive to environmental conditions and reproduce only under extreme conditions. We now describe a peptide that reproduces autocatalytically in an environmentally dependent manner.

The peptide K1 K2 (Figure 1) was designed based on the sequence of the KK peptide of Zhou et al.[7] and on our peptide E1 E2.[8] The K1 K2 peptide contains Lys residues at

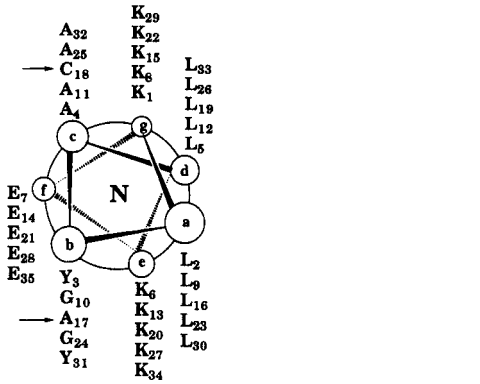

Figure 1. a) Helical wheel diagram of K1 K2 showing the positions of the coiled-coil, heptad repeat (a – g). b) Peptide sequences employed in the study [R = $(CH_2)_2CONH_2$]. Ligation residues Ala and Cys are located at the solvent-exposed b and c positions, as indicated by the arrows.

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the e and g positions of the leucine repeat, thus preventing formation of a stable coiled-coil conformation at acidic and neutral pH due to electrostatic repulsion. Under highly basic conditions or neutral conditions with high concentrations of shielding counterions, the repulsive forces would be minimized, thus allowing appropriate aggregation of the coiled-coil peptide. Ion-assisted nucleation of secondary structure has been demonstrated; however, a simultaneous nucleation in the presence of ions with concomitant autocatalysis has not yet been achieved.[7, 9, 10] In this work it was envisioned that the K1 K2 peptide would self-replicate from smaller fragments only under those conditions that promoted formation of a stable coiled-coil conformation and, therefore, template formation.

The two components of K1 K2, the thioester-containing K1 and the nucleophilic fragment K2, were designed to produce K1 K2 based on the chemical ligation method developed by Kent et al.[11] We anticipated that the coupling between K1 and K2 to form K1 K2 under acidic or neutral conditions would proceed by a nonautocatalytic pathway, in which K1 K2 exists mostly in a random coil, nontemplating conformation. The addition of certain salts such as $NaClO_4$ has been shown to enhance coiled-coil formation of peptides containing Lys residues in the e and g positions due to reduced electrostatic repulsion.[7, 9] These conditions, therefore, should promote the templating ability of K1 K2 for K1 and K2, leading to autocatalytic formation of K1 K2 at neutral pH.

Circular dichroism (CD) spectroscopy was used to assess the helical content of the designed peptides. The peptides were found to adopt a helical conformation with dependence on the salt concentration (Figure 2 a). The helical content of

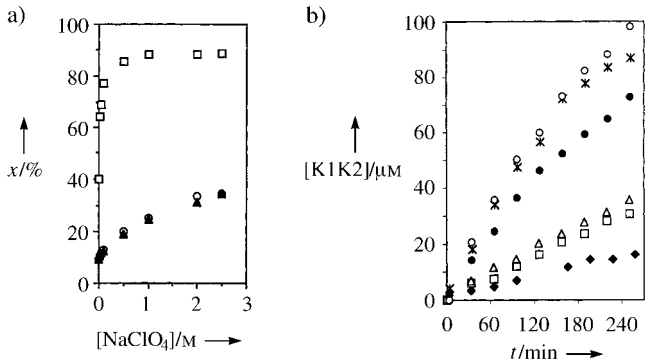

Figure 2. a) The helical content x of K1 K2 (□), K1 (○), and K2 (▲) as a function of $NaClO_4$ concentration. b) K1 K2 production as a function of time in MOPS buffer at pH 7.5 and with different concentrations of $NaClO_4$: 0 M $NaClO_4$ (◆), 0.25 M $NaClO_4$ (□), 0.5 M $NaClO_4$ (△), 0.75 M $NaClO_4$ (●), 1.0 M $NaClO_4$ (*), 2.0 M $NaClO_4$ (○).

K1 K2 reaches a maximum of 82% at 2.5 M $NaClO_4$,[12] presumably due to formation of a coiled-coil conformation when the protonated Lys residues are shielded, as was observed with K K.[7] The ratio of $\theta_{220\,nm}/\theta_{207\,nm}$ has been shown to correlate with the presence of a coiled-coil structure, and a value of 1.03 was obtained for K1 K2 at 1.0 M $NaClO_4$, which is in good agreement with the values for other coiled-coil peptides.[13] Addition of 50% trifluoroethanol, a solvent reported to disrupt interhelical interactions, to K1 K2 at 1.0 M $NaClO_4$ lowered the $\theta_{220\,nm}/\theta_{207\,nm}$ ratio to 0.89, a value indicative of a single-stranded α-helix.[13] The secondary structure of the shorter peptides, K1 and K2, was also dependent on the salt concentration with maximum helical contents of 34 and 35%, respectively, at 2.5 M $NaClO_4$. To ascertain whether K1 and K2 associate at 1.0 M NaClO4, CD spectra of equimolar mixtures of K1 and K2 were compared to the addition spectra of K1 and K2. No increase in helical content was observed with the mixture of peptides over that obtained with the individual peptides alone, suggesting a lack of association between K1 and K2.

Size exclusion chromatography was used to probe the aggregation state of K1 K2 in the presence of 0.5 M $NaClO_4$ at pH 7.5. An apparent molecular mass of approximately 18000 was obtained, which corresponds to a tetrameric structure under these conditions. Without added NaClO4, however, both monomeric and dimeric forms of K1 K2 were observed by size exclusion chromatography; this structural polymorphism has been described for other coiled-coil peptides.[14] The tetrameric form of K1 K2 was denatured with urea, and a two-state transition was observed by CD spectroscopy (Figure 3). A dissociation constant of $2.84 \pm 0.71 \times 10^{-32}\,M^3$ was obtained for K1 K2 at 0.75 M $NaClO_4$ by fitting the denaturation data with equations for a two-state model for the monomer-to-tetramer transition.[15]

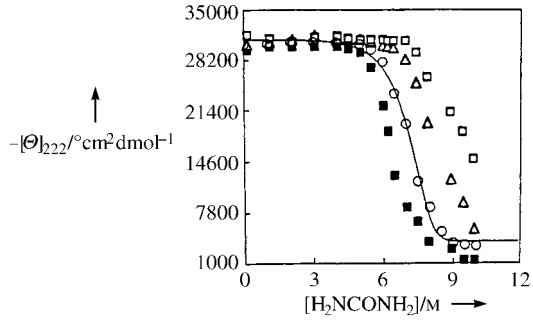

Figure 3. Urea denaturation of 50 μM K1 K2 at 0.5 M $NaClO_4$ (■), 0.75 M $NaClO_4$ (○), 1.25 M $NaClO_4$ (△), 1.5 M $NaClO_4$ (□), The data for 0.75 M $NaClO_4$ (○) are shown fit to a curve for a monomer–tetramer equilibrium.[15] [θ_{222}] is the mean residue ellipticity at 222 nm.

Since the conformation of K1 K2 can be controlled by the addition of salt, the production of K1 K2 from K1 and K2 was next investigated over the concentration range of 0 to 2.0 M $NaClO_4$. As the concentration of $NaClO_4$ in the reaction mixture was decreased from 2.0 M there was a concomitant decrease in the formation of K1 K2 (Figure 2 b). The significantly higher rate of K1 K2 formation at 1.0 and 2.0 M $NaClO_4$ as compared to that at other salt concentrations is presumably due to the coiled-coil and templating ability of the product K1 K2 under these conditions. As seen in Figure 3, the stability of the aggregate increases with increasing $NaClO_4$ concentration. At lower salt concentrations electrostatic repulsion from the protonated Lys residues likely results in uncoiling of K1 K2 and leads to a diminishing of its templating abilities for K1 and K2.

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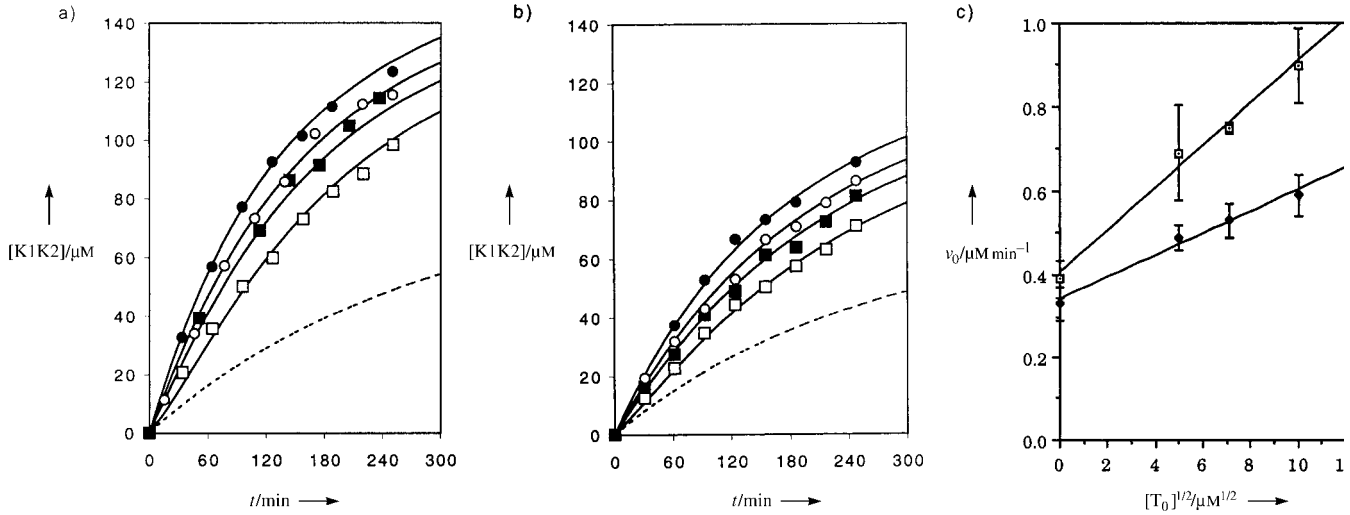

Figure 4. a) K1K2 production as a function of time for reaction mixtures of K1 and K2 containing different initial concentrations of K1K2 as the template at pH 7.5 in 250 mM MOPS (1% (v/v) 3-mercaptopropionic acid) containing 2.0 M $NaClO_4$; no K1K2 (□), 25 μM K1K2 (■), 50 μM K1K2 (○), 100 μM K1K2 (●). b) Same as a) except that the concentration of $NaClO_4$ in the solution was 0.75 M. Curves were generated with the program SimFit.[2d, e] The dashed lines represent the calculated production of K1K2 in the absence of autocatalysis. c) Initial rate v_0 of K1K2 formation as a function of the square root of the initial template concentration $[T_0]$ in 2.0 M $NaClO_4$ (□) and 0.75 M $NaClO_4$ (●). We assumed the following reaction equations for data analysis with SimFit: a) K1 + K2 → K1K2 with a rate constant of k_b; b) K1 + K2 + 0.5 K1K2 → 1.5 K1K2 with a rate constant of k_a; c) K1 → K1* with a hydrolysis rate of k_h.

The autocatalytic nature of the K1K2-forming reaction at pH 7.5 in the presence of $NaClO_4$ was unambiguously established by performing the coupling reaction between K1 and K2 at two different concentrations of $NaClO_4$ (0.75 and 2.0 M) in the presence of differing amounts of K1K2 as a template (Figure 4a and 4b). The production of K1K2 was accelerated by the presence of K1K2 as a template at both salt concentrations. In addition, the initial rate of product formation was found to be linearly proportional to the square root of the template concentration (Figure 4c), as is expected for an autocatalytic reaction.

The program SimFit[2d, e] was used to analyze the experimental results at concentrations of 2.0 and 0.75 M $NaClO_4$, and provided apparent rate constants, k_a, for the autocatalytic reaction of $24.6 \pm 1.5\ M^{-3/2}s^{-1}$ and $12.9 \pm 0.8\ M^{-3/2}s^{-1}$, rate constants, k_b, of the uncatalyzed reaction of $0.087 \pm 0.008\ M^{-1}s^{-1}$ and $0.082 \pm 0.009\ M^{-1}s^{-1}$, and rate constants, k_h, for the hydrolysis of the thioester-containing peptide fragment K1 of $2.78 \pm 0.25 \times 10^{-5}s^{-1}$ and $3.5+0.4 \times 10^{-5}s^{-1}$, respectively. These values provided an autocatalysis efficiency ($\varepsilon = k_a/k_b$) of approximately 280 and 160, respectively. While the rate constant for the uncatalyzed reaction was not appreciably affected by increasing concentrations of $NaClO_4$, the rate constant for autocatalytic K1K2 production increased from $12.9\ M^{-3/2}s^{-1}$ to $24.6\ M^{-3/2}s^{-1}$, presumably due to the enhanced coiled-coil and template formation.

Less than 5% of K1K2 was obtained in the ligation reaction upon addition of 50% trifluoroethanol, an agent known to inhibit coiled-coil formation.[13] Similarly the addition of 7 M urea to the ligation reaction in the presence of 1 M $NaClO_4$ reduced product formation to background reaction levels, also providing evidence for the role of K1K2 as a template for the reaction of K1 with K2. Additionally the rates of the reaction between K1 and K2 without added $NaClO_4$, in both the presence and absence of K1K2 at pH 7.5, were found to be identical, confirming that autocatalysis does not occur under conditions that do not promote the formation of a stable coiled-coil structure. Since K1K2 exists as a tetramer the templating species may be a coiled-coil trimer, although we cannot rule out the potential for a monomeric or dimeric template at the very low, initial concentrations of K1K2 during the early stages of the reaction.

We have successfully designed a self-replicating peptide that promotes its own production at high salt concentrations. At low salt concentrations autocatalysis is suppressed and the reaction with added template is indistinguishable from the background reaction. This self-replication reaction demonstrates that ionic control within the peptide autocatalysis regime is feasible, and we are applying this strategy to design self-replicating systems where cross-catalysis is possible under controlled conditions.

Experimental Section

Peptides K1K2 and K2 were synthesized by solid-phase peptide synthesis on the Rink resin[16] using Fmoc-based chemistry.[17] Peptide K1 was synthesized with a MBHA resin functionalized with Boc-Ala-$S(CH_2)_2CO_2H$[18] by using Boc-based chemistry with Kent's in situ neutralization method.[11] Cleavage of the peptide from the solid support with HF/anisole provided the thioester-containing peptide K1 (Figure 1). All peptide were purified to homogeneity by reversed-phase HPLC and characterized by mass spectrometry (plasma desorption) and amino acid analysis.

CD spectroscopy: The spectra for K1, K2, and K1K2 were recorded at 20°C in a 10 mM MOPS buffer at pH 7.5 (Buffer A). $NaClO_4$ and urea were added from stock solutions in Buffer A for salt dependence and denaturation studies. To ensure that no reaction took place during the measurements, Cys to Ala variants of K2 and K1K2 were used.

Size exclusion chromatography: A 1.8 cm × 98 cm column packed with Sephadex G-50 at 25°C and a flow rate of 0.34 mL min^{-1} was equilibrated with a buffer containing a) 100 mM phosphate, 0.5 M $NaClO_4$, pH 7.5; b) 100 mM phosphate, pH 7.5. A 75 μM solution of K1K2 (500 μL) was loaded onto the column, and the apparent molecular mass of the eluting

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species was calculated by interpolation of a standard curve of carbonic anhydrase, cytochrome C, and aprotinin.

Reaction conditions: Mixtures of K1 (240 μM) and K2 (240 μM) were incubated at 23°C in 250 mM MOPS (1% (v/v) 3-mercaptopropionic acid), 0 to 2.0 M $NaClO_4$, at pH 7.5. At the indicated time interval 50 μL of the reaction mixture was evaluated by reversed-phase HPLC. The reaction products were identified by direct isolation and characterization by mass spectrometry, or by HPLC coinjection with authentic samples. Concentrations of product were determined by interpolation of peak areas from a standard curve.

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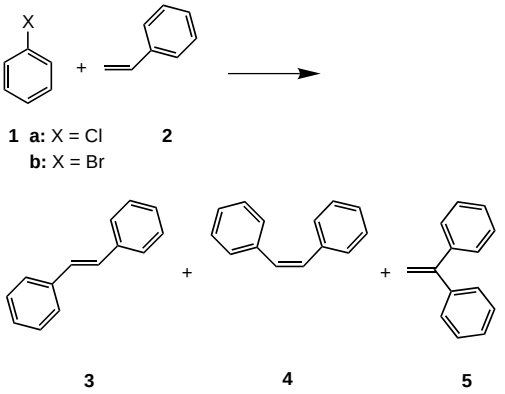
A New Catalyst System for the Heck Reaction of Unreactive Aryl Halides

Manfred T. Reetz,* Gunther Lohmer, and Renate Schwickardi

Although the palladium-catalyzed Heck reaction of aryl halides ArX (X = Cl, Br, I) with olefins is a standard C – C bond-forming reaction,[1] industrial applications are rare,[2] because the reactivity of aryl halides decreases drastically in the order ArI > ArBr > ArCl, which means that the cheap chlorides and even some bromides do not react with sufficiently high yields, turnover numbers (TON), and selectivities. In spite of recent progress, for example, use of certain palladacycles prepared from tris(*o*-tolyl)phosphane as catalysts in Heck reactions of bromo and activated chloro arenes,[3] the activation of unreactive aryl halides such as chlorobenzene remains a real challenge.[4] We report on a new and surprisingly simple catalyst system, which for the first time makes such coupling reactions possible in an unusually efficient manner.[5] We serendipitously discovered that the use of simple Pd salts such as $PdCl_2$ or $Pd(OAc)_2$ in the presence of tetraphenylphosphonium salts Ph_4PX (X = Cl, Br, I) leads to unexpectedly high catalytic activities.[6] Optimization of the reaction of chlorobenzene (**1a**) with styrene (**2**) to form the Heck products **3**, **4**, and **5** [Eq. (1)] showed that a ratio of Pd to Ph_4PX of 1:6 leads to the best results; sodium acetate is suitable as base and *N*,*N*-dimethylformamide (DMF) or *N*-methylpyrrolidinone (NMP) as solvent (Table 1).[7]



(1)

Another surprising discovery is that the use of small amounts of *N*,*N*-dimethylglycine (DMG) as additive leads to a pronounced improvement in regioselectivity.[7] Upon employing six equivalents of DMG relative to Pd and only 2 mol% of the Pd catalyst, the proportion of undesired regioisomer **5** is significantly reduced. Although no chemically engineered optimization was carried out, simple exploratory experiments showed that a further decrease in the amount of catalyst is certainly possible. Using 0.5 mol% catalyst a TON of 130 is reached. The same reaction with

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