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Reprogramming DNA-Directed Reactions on the Basis of a DNA Conformational Change

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This paper develops a strategy for switching chemical reactions on the basis of a DNA duplex−triplex transition. Three chemical reagents are conjugated with three DNA single strands, respectively. The DNA strands associate with each other to form a complex. Under one solution condition, the DNA complex adopts a conformation that brings two particular reagents together and promotes a chemical reaction between them. When the condition changes, the DNA complex changes its conformation, and the reaction partners change as well.

Nature has selected DNA as the genetic material because of its extraordinary chemical properties. The same properties also stimulate development of nongenetic applications of DNA.[1,2] Recently, DNA has been used as templates to direct chemical reactions.[3,4] Chemicals are conjugated with single DNA stands, and DNA hybridization brings functional groups into proximity for reaction. The linkage between chemicals and DNA strands pre-sets the reaction. To change the reaction scope, a new set of DNA−chemical conjugates must be prepared. Immediately, a challenge arises: could we reconfigure a complex of the same set of DNA−chemical conjugates to direct different reactions between different chemicals, or switch reactions? This is important because it would allow reprogramming chemical reactions in complicated systems. Herein, we report a solution to this challenge based on DNA duplex−triplex transition.

To demonstrate the switching strategy, we have used a model system that forms an amide bond (amine acylation) between one carboxylic acid and one of two identical amines. Here, we differentiate the two amines and allow only one specific amine to react with the carboxylic acid. Under different conditions, different amines will participate in reactions. Two amines and one carboxylic acid are conjugated with three DNA strands, N1, N2, and C, respectively (Figure 1a). Strand C has three segments: C1, C2, and C3. C1 and C2 are complementary to N1 and N2, respectively. C2 and C3 consist of only pyrimidines, and N2 consists of only purines. C3 is a reconfigurable segment, which can participate in two different conformations, a triplex or a compact coil, under two different conditions.

Switching reactions depends on formation and dissociation of a DNA triplex in response to a change of the solution pH (Figure 1b). A DNA triplex containing C^+G-C triplets is stable only in acidic solutions (e.g. pH 5.0) because the formation of C^+G-C triplets requires partial protonation of cytosines (C).[5] At pH 8.0, C3 is unpaired and collapses into a closed random coil conformation.[6] This brings the carboxylate group into proximity with the amino group on strand N2, which promotes formation of an amide bond between them. As a result, strand C will be covalently linked with strand N2. When the solution pH changes to 5.0, segment C3 associates with the C2/N2 duplex and forms a triplex. Consequently, the carboxylate group is brought into proximity with the amino group on strand N1, and an amide bond will be formed between strands C and N1.

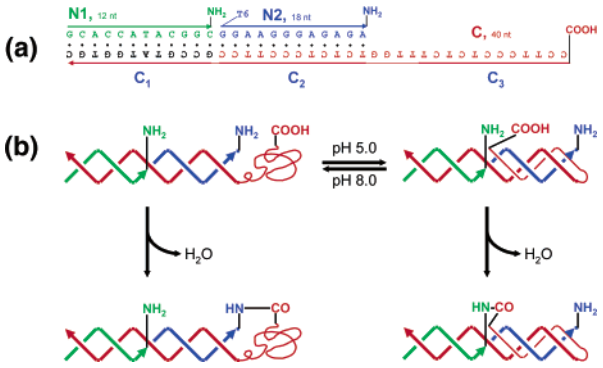

Figure 1. Scheme of switching chemical reactions. (a) DNA sequences and the positions of the interested amino groups and carboxylate group. Note that there is a string of unpaired T6's at the 5′ end of strand N2. Addition of the extra six bases to strand N2 makes strands N1 and N2 have different molecular weights and electrophoretic mobilities, which allows identification of strands N1 and N2 on polyacrylamide gel electrophoresis (PAGE). (b) Switching chemical reactions by switching the location of the carboxylate group. This behavior is triggered by a change of the solution pH value. Note the formation and dissociation of a DNA triplex.

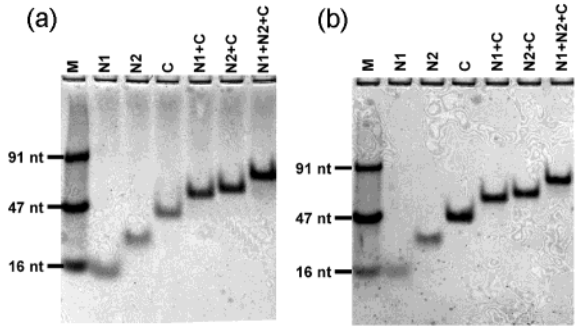

Figure 2. Native polyacrylamide gel electrophoresis (PAGE, 12%) analysis of the formation of the designed DNA complex at both solution pH values: 5.0 (a) and 8.0 (b). DNA contents are indicated above each lane. Lane M contains three single-stranded DNA size markers.

The designed DNA complex is stable at both pH 5.0 and 8.0 (Figure 2). DNA complexes were formed by cooling mixtures containing equimolar amounts of the three individual DNA strands from 90 °C to room temperature over 20 min. The samples were then analyzed by native polyacrylamide gel electrophoresis (PAGE). Each complex appeared as a sharp, single band with expected mobility, which indicates that the DNA complexes are stable. All DNA complexes exhibit similar electrophoretic mobility under both solution conditions. This experiment directly proved that the DNA strands bring the chemicals (two amines and one carboxylic acid) into a single complex, which is essential for directing chemical reactions.

Amine acylation was promoted by addition of a condensation agent after DNA complexes formed.[3e] Reaction mixtures were analyzed by denaturing PAGE. Experimental results clearly show

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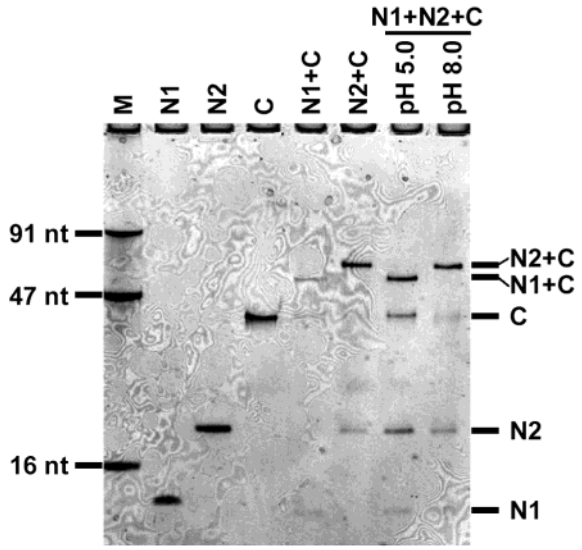

Figure 3. Denaturing PAGE (20%) analysis of the switching chemical reactions. In the right four lanes, DNA samples are reaction mixtures. Lanes N1 + C and N2 + C are separately prepared controls. Lane M contains three single-stranded DNA size markers.

that the switching strategy is very effective (Figure 3). Only one product is formed by acylation at either pH 5.0 or 8.0, and the products from the two conditions have different electrophoretic mobilities. Comparison with controls (separately prepared N1 + C and N2 + C conjugates) allows the identities of the reaction products to be unambiguously assigned. At pH 8.0, the amino group on strand N2 is close to the carboxylate group on strand C, and a chemical reaction happens between them. The formed amide bond joins strands N2 and C covalently (lane N1 + N2 + C/pH 8.0) into an N2 + C conjugate. To confirm that the reaction indeed happens between strands N2 and C, we have performed a control experiment (lane N2 + 3), where only strands N2 and C are present in the solution and strand N1 is absent. Acylation only can happen between strands N2 and C. As expected, these two conditions give products with the same mobility. At pH 5.0, the DNA triplex forms and the carboxylyate group is brought close to the amino group on strand N1. Acylation happens between strands N1 and C, and these two strands become covalently joined (lane N1 + N2 + C/pH 5.0), which is confirmed by comparison with a control experiment (lane N1 + C). Note that the reaction selectivity is extremely high and there are no observable misreaction products. The reaction yields are 88% at pH 8.0 and 67% at pH 5.0, as estimated with OptiQuant (Packard Instruments), an image analysis and processing software.

In summary, we have demonstrated switching two otherwise incompatible chemical reactions by a DNA conformational change. Specific control of reactions is one of the most common problems for synthetic chemistry. A traditional strategy is protection−deprotection. However, it is often difficult to specifically protect some particular functional groups without affecting others, especially when the compounds are large. Protection−deprotection also adds steps and additional cost to syntheses. The strategy developed here overcomes these problems. It allows only one of two similar reactions to take place and easy switching between them. This strategy should be applicable to other reactions, and could potentially be adapted for complicated syntheses.

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Supporting Information Available: Experimental methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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