Molecular Devices

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DNA Logic Gates Based on Structural Polymorphism of Telomere DNA Molecules Responding to Chemical Input Signals**

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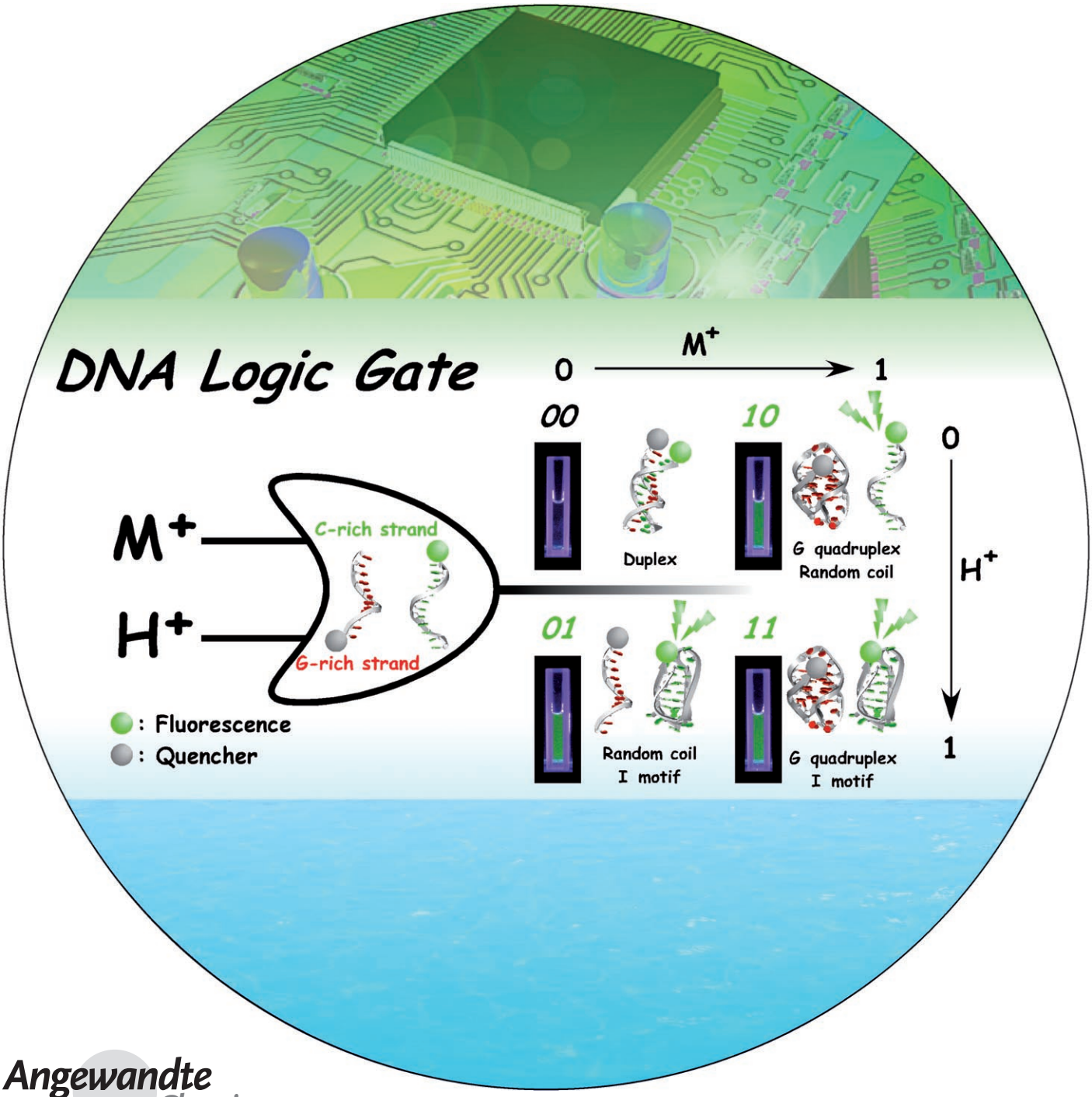

Telomeres found at the termini of linear eukaryotic chromosomes may consist of double-stranded G- and C-rich sequences and 3′-single-stranded G-rich overhangs.[1] The G- and C-rich sequences can individually fold into four-stranded helices, namely, G quadruplexes and i motifs, respectively.[2] Furthermore, most telomere DNA molecules are thought to be made up of duplexes between G- and C-rich sequences, except for regions of G-rich overhangs. Whether G quadruplexes and i motifs are formed in living cells, however, remains unclear, although there is some evidences for the formation of G quadruplexes in cells.[3] We[4] and others[5] have shown that duplex–quadruplex conversion by telomere DNA molecules, which may be useful for the construction of molecular devices, is affected by environmental factors. In fact, several studies demonstrate that telomere DNA molecules can be used to construct nanomolecular machines.[6]

Systematic studies of the effects of multiple surrounding conditions on the structures of telomere DNA molecules are needed for the design of logic gates. Herein, we investigated the structure of two *Oxytricha* telomere DNA molecules, $d(G_4T_4)_3G_4$ (G strand) and $d(C_4A_4)_3C_4$ (C strand), under various conditions. Alone, the G strand folds into an intramolecular antiparallel G-quadruplex,[2a] whereas the C strand may fold into an i motif.[2b] Figure 1 a shows the CD spectra of a 1:1 mixture of G and C strands (total strand concentration = 50 μM) in the presence of various concentrations of Li^+ at pH 8.0. The CD spectra had a positive peak and a shoulder at 260 and 290 nm, respectively, showing that the dominant structure is a B-form duplex at all concentrations of Li^+.[4] The CD spectra at the lower concentrations of K^+ at pH 8.0 showed that the dominant structure is a B-form duplex; however, at higher concentrations of K^+, the CD intensities at 260 and 290 nm decreased and increased, respectively, indicating that the mixture mainly folds into quadruplex(es) (Figure 1 b). We further examined the structural conversion at pH 5.0. The CD spectra of the mixture in the presence of lower concentrations of Li^+ at pH 5.0 had a positive peak at 290 nm, showing that the mixture folds into quadruplex(es) (Figure 1 c). In addition, the CD intensity at 290 nm decreased with increasing Li^+ concentrations, indicating destabilization of the G quadruplex by Li^+.[7] On the other hand, the CD spectra of the mixture in the presence of K^+ at pH 5.0 had a positive peak at 290 nm (Figure 1 d), showing that quadruplexes dominate at all concentrations of K^+. Furthermore, the CD intensity at 290 nm in the presence of K^+ (200 mM) at pH 5.0 is twice that at pH 8.0 (Figure 1 b), suggesting that the i motif structure is stabilized by acidic conditions.[2b] These results are consistent with the effects of pH and monovelant cations on the structures of human telomeric DNA molecules.[5a]

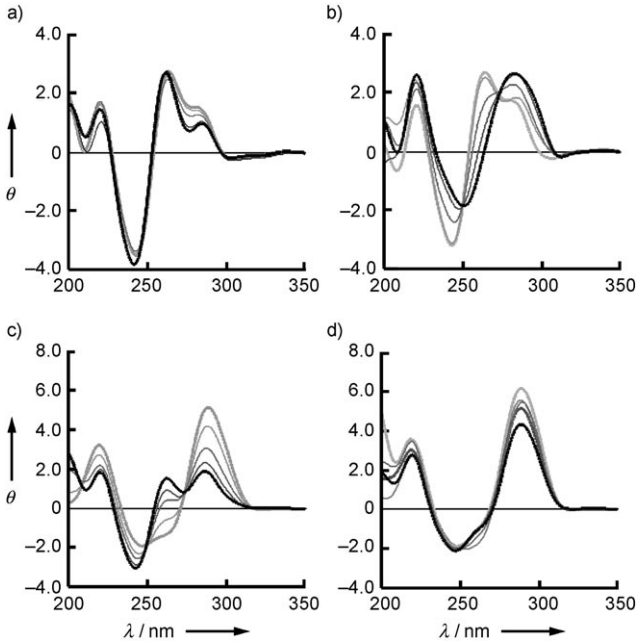

Figure 1. CD spectra (θ in 10^5 deg cm^2 dmol^{-1}) of a 1:1 mixture of $d(G_4T_4)_3G_4$ and $d(C_4A_4)_3C_4$ in the presence of various concentrations of a) Li^+ and Tris-HCl (50 mM; pH 8.0; Tris = tris(hydroxymethyl)aminomethane), b) various concentrations of K^+ and Tris-HCl (50 mM; pH 8.0), c) various concentrations of Li^+ and MES (50 mM; pH 5.0; MES = 2-(4-morpholinyl)ethane-sulfonic acid), or d) various concentrations of K^+ and MES (50 mM; pH 5.0). All measurements were carried out with a total strand concentration of 50 μM at 4 °C. The concentrations of monovalent cations (K^+ or Li^+) were 10, 20, 50, 100, and 200 mM (lines from the top to the bottom at 290 nm in panels a, c, and d and from the bottom to the top in panel b).

Characterization of the structures of the mixture by gel electrophoresis was found to be difficult as the equilibrium of the mixture between the duplex and the quadruplexes is shifted towards the duplex (data not shown). This may be due to changes in the pH of the buffer solution and/or other surrounding conditions during electrophoresis, although the mechanism of the structural changes of the mixture induced by the multiple surrounding conditions is not fully confirmed. Thus, we further characterized the structural conversion by isothermal titration calorimetry (ITC) in the presence of M^+ (Li^+ or K^+; 20 mM) at pH 8.0 or 5.0. Heat changes caused by the interaction between G and C strands were observed only

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in the presence of Li^+ at pH 8.0 (see the Supporting Information), demonstrating the formation of duplexes, although the ionic concentrations are slightly different from these for CD measurements. On the basis of these results, we conclude that the structural conversions of the G and C strands can be controlled by both M^+ and H^+ (dominant structures of the G and C strands are shown in Figure 2). We

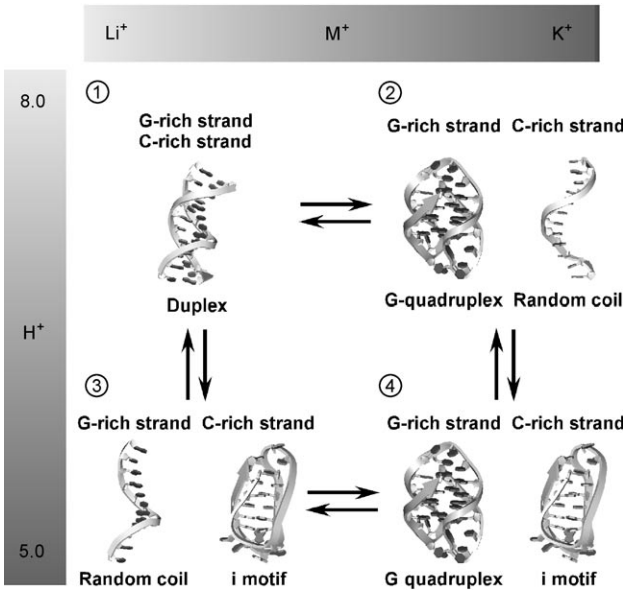

Figure 2. Schematic illustration of the structural conversion of the 1:1 mixture of G and C strands among duplex, quadruplex, and random-coil forms as controlled by M^+ (Li^+ or K^+) and H^+. 1) a duplex; 2) a G quadruplex and a random coil; 3) a random coil and an i motif; and 4) a G quadruplex and an i motif.

chose this ionic concentration (20 mM) to induce the random-coil state of the G strand in the presence of Li^+ at pH 5.0, which is required for functions of several logic gates as we will discuss later. Moreover, CD measurements confirmed that continuous structural conversions among the four states are induced by annealing the DNA structures after the conditions were changed (see the Supporting Information); however, we found that the conversions from the structural state 1 to 3 and 3 to 4 are very slow or do not occur (see the Supporting Information). These results suggest that the annealing process is required for the complete conversion of the mixture.

Based on the conversion of the telomere DNA molecules among these four states, we conceived a new concept for a DNA logic gate that responds to two chemical input signals (M^+ and H^+) and generates a change in fluorescence intensity as the output signal. As a proof of concept for the logic-gate function, we first built an OR logic gate by using a mixture that responds to two input signals, namely K^+ (20 mM; i_1) and acidic conditions (pH 5.0; i_2). Rhodamine Green, whose emission is independent of the pH,[6c] and 4-(4-dimethyl aminophenylazo)benzoic acid (Dabcyl) were attached to the strands as the fluorescent probe and quencher, respectively (see Figure 3a and b for the truth table and the positions of the fluorophores). Figure 3c shows the fluorescence spectra of the mixture (total strand concentration = 1 μM) excited at

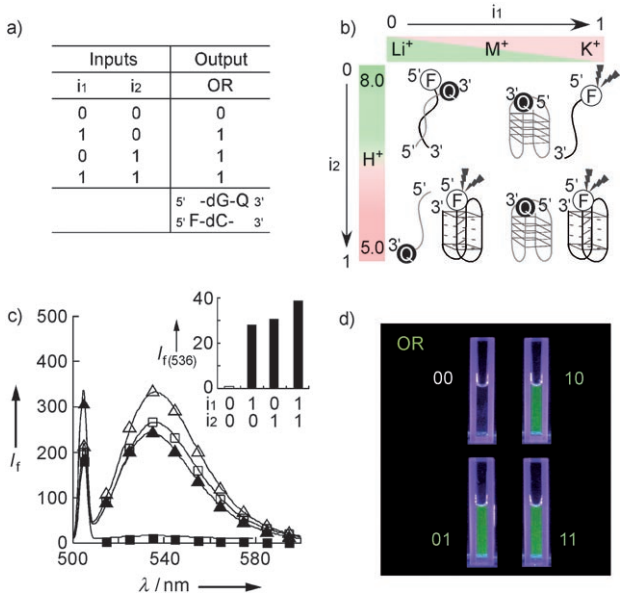

Figure 3. a) The truth table and design of the OR logic gate. b) Schematic illustration of the OR function of the 1:1 mixture of G and C strands and their response to M^+ and H^+. Gray and black lines indicate the G- and C-rich strands, respectively. c) Fluorescence spectra of the OR logic gate in the presence of Li^+ (20 mM; pH 8.0) ■, K^+ (20 mM; pH 8.0) ▲, Li^+ (20 mM; pH 5.0) □, or K^+ (20 mM; pH 5.0) △. All measurements were carried out at a total strand concentration of 1 μM and at 4 °C. Inset: normalized fluorescence intensities at 536 nm for the OR logic gate at 4 °C and ($i_1 = 0$, $i_2 = 0$), ($i_1 = 1$, $i_2 = 0$), ($i_1 = 0$, $i_2 = 1$), or ($i_1 = 1$, $i_2 = 1$). d) Fluorescence images of the OR logic gate. All measurements were carried out at a total strand concentration of 1 μM and at room temperature. F = Rhodamine Green, Q = Dabcyl. I_f = fluorescence intensity, $I_{f(536)}$ = fluorescence intensity at 536 nm.

504 nm in the presence of Li^+ (20 mM) at pH 8.0 ($i_1 = 0$, $i_2 = 0$), K^+ (20 mM) at pH 8.0 ($i_1 = 1$, $i_2 = 0$), Li^+ (20 mM) at pH 5.0 ($i_1 = 0$, $i_2 = 1$), and K^+ (20 mM) at pH 5.0 ($i_1 = 1$, $i_2 = 1$). The inset in Figure 3c shows the normalized fluorescence intensities at 536 nm of the mixture under the four listed conditions. The fluorescence intensities at the ($i_1 = 1$, $i_2 = 0$), ($i_1 = 0$, $i_2 = 1$), and ($i_1 = 1$, $i_2 = 1$) conditions were, respectively, 28-, 31-, and 39-fold higher than that of the ($i_1 = 0$, $i_2 = 0$) condition, confirming OR gate behavior, wherein the fluorescence signals can be recognized even by the naked eye (Figure 3d). In addition, these results are consistent with those from CD and ITC measurements, although the DNA strand concentrations used in CD, ITC, and fluorescence measurements are different. Moreover, we found that the structural conversion can be utilized to construct XOR (exclusive OR) and NOTIF logic gates, which generate fluorescence signals under either two or one conditions, respectively (see the Supporting Information). Notably, not only the logic gates developed here, but other logic gates as well can be created based on the structural conversion and incorporation of fluorophores in different combinations and at different positions (see the Supporting Information for molecular design of these logic gates).

In the current studies, we developed simple and robust DNA logic gates. Compared with DNA logic gates previously developed,[8] ours have a few potential disadvantages.

Because the kinetics of folding and unfolding by G-quadruplex structures are very slow, conversion requires annealing (see the Supporting Information). Regardless, our system provides a simpler and faster readout of the output signals than systems employing gel electrophoresis. In addition, the kinetics of folding and unfolding can be controlled in our system by modulating the DNA sequences.[9] Thus, further optimization of the logic gates may be possible. On the other hand, only one type of logic gate can be made for each vessel because the solution conditions (input signals) affect all of the logic gates in the vessel and because we did not utilize sequence-specific hybridization as an input signal. The control of the structural conversion, however, can be extended to any telomere DNA. A previous report showed that the polymorphic structures of telomere DNAs can be controlled by their sequences and by surrounding factors such as divalent metal ions and molecular crowding as well as monovalent cations and pH.[4,5] Furthermore, a single base difference in the telomere sequences was reported to have a drastic effect on their structures.[4c] Therefore, further optimization should make it possible to individually control the behavior of multiple logic gates in a single vessel. These results demonstrate that it should be possible to employ the structural polymorphism of telomere DNA molecules in a broad spectrum of nanobiotechnological applications, such as logic gates under cell-mimicking conditions in which both the pH and concentration of monovalent cations control various biological events.

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