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DNA-templated click chemistry for creation of novel DNA binding molecules

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ABSTRACT

We have developed a new methodology for producing new molecules that bind to dsDNA using DNA-templated click chemistry. The click reactions between the minor groove binding peptide and acridine intercalators were accelerated by the addition of dsDNA. Furthermore, the resulting peptide–acridine conjugate showed a slightly stronger binding to dsDNA. These results indicate that the DNA-templated click chemistry is applicable for screening new binding molecules.

DNA binding molecules with a sequence selectivity have significant potential applications in genomic studies. Recently, small molecules binding to DNA have become the focus of attention,[1] because they can target the noncanonical DNA structure, such as the quadruplex,[2] Z-DNA,[3] branched DNA,[4] etc. Such compounds are of interest as potential therapeutic agents and new tools in nucleic acid biochemistry. The recognition modes for the noncovalent binding of small molecules to DNA are intercalation, minor groove binding, coulombic attraction or a combination of them. A combination of different binding natures may produce unique binding molecule. Thus, there are several hybrid DNA ligands, for example, the bis-intercalator,[5] intercalator with a minor groove binder[6] and intercalator having DNA-cleaving molecules.[7]

In this letter, we propose a new strategy for developing novel DNA binding molecules having different binding modes by duplex DNA-templated click chemistry (Fig. 1). 'Click chemistry' is the new concept developed by Sharpless and co-workers to apply practical and reliable chemical transformations.[8] Especially, the copper (I) catalyzed 1, 2, 3-triazole formation from azides and terminal acetylenes is a powerful linking reaction with a water-tolerance.[9] Recently, the target-guided, in situ click chemistry approach has been successfully employed for discovering acetylcholinesterase inhibitors.[8a] In addition, the proximity between the two reactants has facilitated these reactions, even in the absence of the Cu(I) catalyst.[10] We have chosen the peptide and acridine derivatives as the DNA binding modules for applying the duplex DNA-templated click chemistry. Short peptides are reported to be important constituents of the DNA binding protein motif[11] and can be easily prepared by solid-phase synthesis to be applicable in combinatorial chemistry for developing DNA binding molecules.[12] Many acridine derivatives have been previously synthesized and characterized for their interaction with the dsDNA.[13] The 9-anilinoacridine-4-carboxamide derivatives have been shown to bind DNA by a threading mechanism and acridine substituents at the 4- and 9-positions are located in the major groove and minor grooves with the acridine chromophore sandwiched between the base pairs.[14] Acridine derivatives conjugated to natural[15] or unnatural[16] peptides are reported to have high affinities to dsDNA.

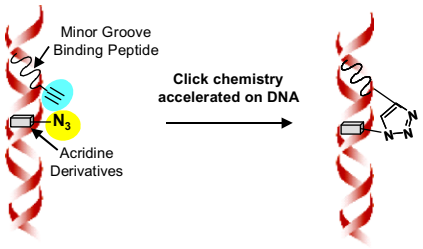

Figure 1. DNA-templated click chemistry between peptides and acridine derivatives.

We have designed acridine derivatives having an azide group at the 9-position with three different length spacers, and a pentapeptide (PRGRP), which selectively binds to the minor groove of the A, T site of the dsDNA,[17] having an acetylene group for the DNA-templated click chemistry (Fig. 2).

We expected that a dsDNA containing a binding site for the pentapeptide might facilitate the coupling reaction between the peptide and acridine having the optimal spacer for bringing the peptide and acridine into proximity. Acridine derivatives having

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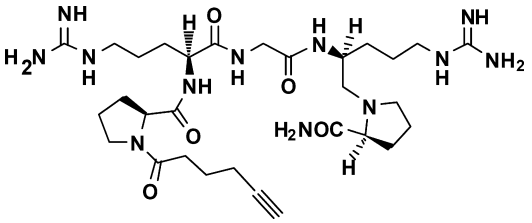
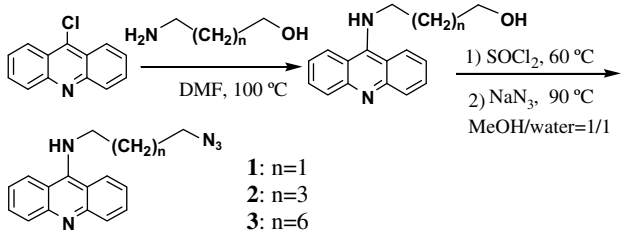


Figure 2. Structure of pentapeptide (**4**).

an azide group with different spacer lengths were obtained from 9-chloro acridine (Scheme 1). The pentapeptide (PRGRP) was synthesized using standard Fmoc peptide synthesis procedures on Rink amide resin. The acetylene group was introduced at the N-terminal position of the pentapeptide by coupling with hexynoic acid. After cleavage from the solid-support upon TFA (containing phenol, ethandithiol, and thioanisole) treatment, the peptide (**4**) was purified by reversed phase HPLC and analyzed by mass spectrometry.

Authentic samples of the triazoles were prepared from acridine derivatives (**1**–**3**) and the peptide (**4**) in the presence of 100 mM sodium ascorbate and 100 mM copper sulfate in a DMF at room temperature. After purification by HPLC, the structure was determined by MS and ^{1}H NMR as the isomerically pure anti-triazole (Scheme 2).

When acridine derivatives (**2**) and peptide (**4**) incubated at 50 °C without the Cu (I) catalyst 6 days, an equimolar mixture of two separable peaks, each having the same molecular weight, were observed by HPLC. Thus, the equimolar mixture of the 1, 4-triazole and 1, 5-triazole regioisomers was obtained as separable peaks under these conditions. These results have suggested that the regioisomer assignment for the triazoles would be accomplished by comparing the HPLC traces.



Scheme 1. Synthesis of the acridine derivatives. Synthesis is described in Supporting information.

The cycloaddition between each acridine (0.1 mM) and peptide (1 mM) was performed at 30 °C in 2 mM Tris–HCl buffer (pH = 7.0) containing 2 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 100 mM NaCl, 0.25 mM $CuSO_4$, and 0.25 mM sodium ascorbate. The reaction was followed by an HPLC analysis. Under these conditions, without dsDNA, only a 2–3% conversion to **5**–**7** was observed after 24 h. Figure 3 illustrates the HPLC change in the reaction between the acridine derivatives (**1**) and peptide (**4**) in the presence of dsDNA ($^{5'}$CGCGAATTCGCG$^{3'}$; AATT12, 0.05 mM), which have the peptide binding sequence. Remarkably, the addition of dsDNA to the reaction mixture promoted substantial acceleration of the cycloaddition. In this reaction, only a single peak appeared as a product and this new peak was proven to be identical to the authentic 1, 4-triazole regioisomer.

The acceleration due to the addition of AATT12 was also observed in the reaction using acridine derivatives (**2**–**3**). The conver-

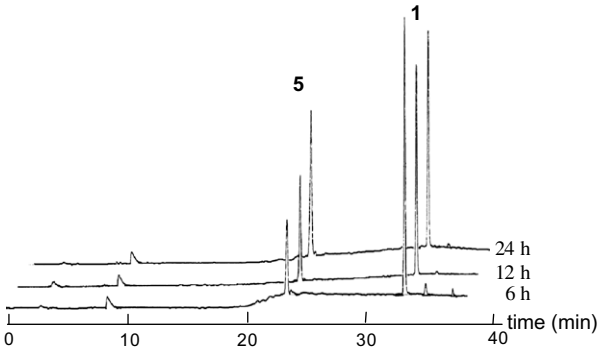
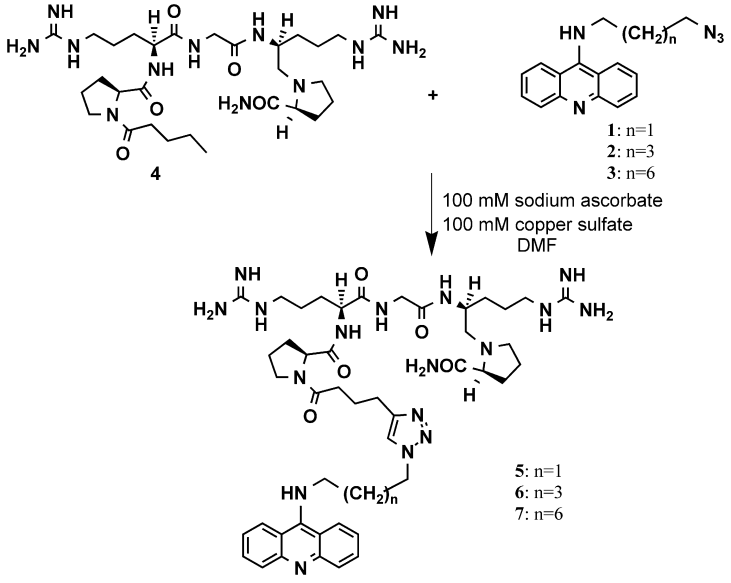


Figure 3. HPLC traces of the reaction between 1 and 4 in the presence of AATT12 ($^{5'}$CGCGAATTCGCG$^{3'}$). HPLC conditions: column: Nacalai tesque Cosmoil 5C18ARII 4.6 × 250 mm; solvent A 0.1% TFA water, solvent B 0.1% TFA MeCN, B, linear gradient from 0% to 30% over 30 min, from 30% to 100% in another 30 min; monitored at 254 nm.



Scheme 2. Synthesis of authentic sample for adducts between the peptide and acridine derivatives.

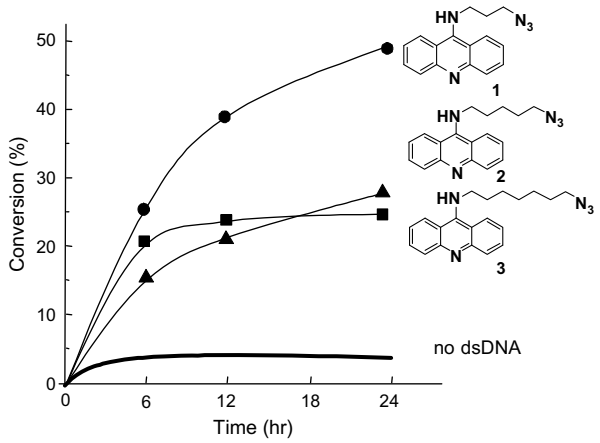


Figure 4. Graph showing the product (**5**–**7**) formation as a function of time in the reaction between the peptide (**4**) (1 mM) and acridine derivatives (0.1 mM) (1) (closed circle), (2) (closed triangle), and (3) (closed square) in the presence of AATT12 (0.05 mM). The thick line corresponds to the reaction with **4** and **1**–**3** in the absence of AATT12.

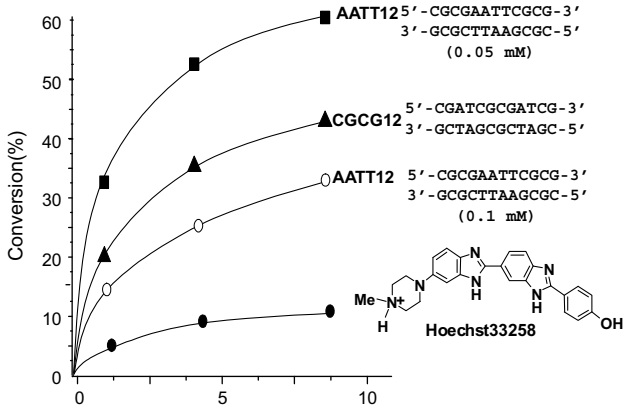


Figure 5. Graph showing the products (**5**) formation as a function of time in the reaction between the peptide (**4**) and acridine derivatives (**1**) in the presence of dsDNA. Closed square: AATT12 (0.05 mM), closed triangle: CGCG12 (0.05 mM), open circle: AATT12 (0.05 mM) in the presence of Hoechst 33258 (0.1 mM). Reaction conditions were same in Figure 4.

sion yields from (**1**–**3**) to (**5**–**7**) were plotted in Figure 4 and were in the order of **1**, **2**, **3** as shown in this figure. These results have suggested that the spacer length of the acridine derivatives would have an apparent effect on the reaction between the acridine derivatives and peptide in the presence of AATT12.

The influence of the amount of dsDNA on the DNA-templated click reaction was investigated. It showed that the rate increased upon reducing the DNA concentration from 1.0 to 0.5 equiv for the acridine derivatives (Fig. 5).

A higher ratio of the acridine derivatives to the dsDNA leads to multiple bindings and increases the probability that the acridine derivatives bind in close proximity to the peptide on the dsDNA.

The effects of the dsDNA sequence on DNA-templated click reaction have been studied (Fig. 5). These reactions carried out below the T_m of both template DNA (AATT12: 54.3 °C, CGCG12: 58.5 °C). There was slight difference in the reaction rate between AATT12 having a binding motif to the pentapeptide (PRGRP) and CGCG12 without a binding motif. In these experiments, we used a high concentration of the pentapeptide (1 mM), because the binding affinity of peptide to dsDNA relatively weak (association constants in the millimolar range). It has been reported that the electrostatic attraction by the arginine side chain of the pentapeptide might nonspecifically contribute to the binding affinity at high peptide:DNA ratios.[17] We have considered that the acceleration of the cycloaddition between the pentapeptide and acridine on the dsDNA would be attributed to the proximity effect by the electrostatic interaction mainly between the dsDNA and pentapeptide. In the presence of Hoechst 33258, which is a representative minor groove binder with a high specificity to a sequence of AATT, the reaction was efficiently inhibited. These results suggested that the reaction between the peptide and acridine derivatives would be accelerated by binding the two components to dsDNA.

The equilibrium binding constants of the acridine derivatives (**1**) and acridine conjugated peptide (**5**) were evaluated by fluorescence titrations. Figure 6 shows the change in the fluorescence spectra of the acridine derivative (**1**) with the increasing concen-

Table 1
Equilibrium binding constants of acridine derivatives (**1**), the acridine conjugated peptide (**5**), and peptide (**4**) toward dsDNA (AATT12 and CGCG12) obtained by fluorescence titrations[a]

Compound	K_s, 10^5 M^{-1}	
	AATT12	CGCG12
1	1.6	2.0
5	7.3	3.6
4	~0.01[b]	—[c]

[a] Values were obtained by a nonlinear least squares data analysis.
[b] Refer to 17.
[c] Not determined.

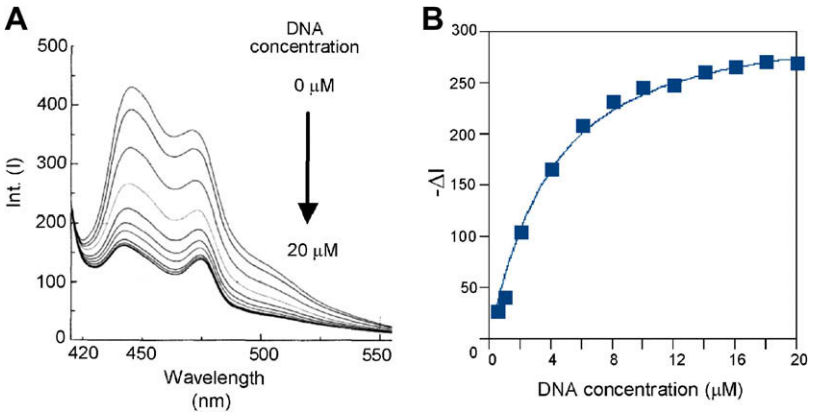


Figure 6. (A) Change in fluorescence spectra of **1** (2 μm) in 2 mM Tris–HCL buffer (pH = 7.0) containing 2 mM KCL, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 100 mM NaCl with increasing concentrations of AATT12. Excitation wavelength, 410 nm. (B) Changes in fluorescence intensity at 450 nm are plotted versus the DNA concentration.

Table 2
ΔT_m values for dsDNA (AATT12 or CGCG12) by addition of acridine derivatives (**1**), the acridine conjugated peptide (**5**), and peptide (**4**)

Compound	T_m (ΔT_m) °C	
	AATT12	CGCG12
1	57.4 (3.1)	60.5 (2.0)
5	62.4 (8.1)	62.4 (3.9)
4	54.3 (0.0)	58.5 (0.0)

2 μM of each DNA duplex was used in 2 mM Tris–HCl buffer (pH = 7.0) containing 2 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, and 100 mM NaCl. 10 μM of each ligand was used.

trations of dsDNA in 2 mM Tris–HCl buffer (pH = 7.0) containing 2 mM KCl, 2 mM $MgCl_2$ 1 mM $CaCl_2$, and 100 mM NaCl.

The addition of dsDNA (AATT12) to a solution of **1** resulted in decreased absorption of the acridinium chromophore. The binding constants were obtained by analysis of the titration curve (Table 1). The binding affinity of the acridine conjugated peptide (**5**) to AATT12, which has a binding motif to the peptide, is slightly higher than that of the acridine derivatives (**1**). The low sequence selectivity and small enhancement of binding constant of the acridine conjugated peptide (**5**) would be attributed to the lack of sequence selectivity and low affinity to the duplex DNA of peptide (**4**).

Table 2 summarizes the effects of **1**, **4**, and **5** on the melting temperature of AATT12 and CGCG12. The melting temperature (T_m) of dsDNA was not changed by addition of the peptide (**4**). The addition of the acridine derivative (**1**) increased the T_m by 2–3 °C relative to the individual DNA duplexes. On the other hand, the significant stabilization of AATT12 (ΔT_m = 8 °C) was observed by the addition of the acridine conjugated peptide (**5**). Such stabilization did not occur with the addition of **5** to CGCG12 without the binding motif to the peptide. This high stabilization of dsDNA by **5** might be attributed by the cooperative binding of intercalator and minor groove binder.

In conclusion, we have demonstrated the DNA-templated click chemistry between the minor groove binding peptide and acridine derivatives. The resulting peptide–acridine conjugate showed a slightly stronger binding to dsDNA. These results have suggested that the click chemistry on dsDNA can be useful for developing novel binding molecules having different binding modes. The search for the novel dsDNA binding molecules is currently ongoing based on the combinatorial technology with click chemistry between the peptide library and the acridine derivatives.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.08.074.

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