Programmable DNA translation system using cross-linked DNA mediators†

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Received (in Cambridge, UK) 4th March 2005, Accepted 26th April 2005
First published as an Advance Article on the web 24th May 2005
DOI: 10.1039/b503247d

The information of template DNA strands was converted into the specific sequences in a programmable way by following the mediation of cross-linked DNAs.

The information transfer and processing on the basis of the molecular recognition and assembly is one of most important issues for construction of the programmed supramolecular system.[1,2] Molecular assembly realizing this system needs processing machinery where information of input molecules is transferred through non-covalent interaction and finally the transferred information is expressed by output molecules. These systems have been partially realized using small molecules,[1,2] however, these require multiple molecular components based on the reliable molecular recognition and assembly. One of the suitable candidates for construction of the programmable system is an oligodeoxyribonucleotide (DNA), because large varieties of recognition molecules can be prepared by just changing the sequences. DNA computation[3,4] and programmed chemical synthesis[5,6] using DNA as a template are the good examples of the precise and reliable recognition of DNA molecules.

In work reported here, we intended to create the molecular translation system using multiple cross-linked DNAs as mediators. The translation system we planned to create consists of three parts; (1) one template DNA strand (input) which directs the sequential molecular assembly, (2) mediator molecules which non-covalently connect template DNA and the short DNA fragments, and (3) short DNA fragments which are connected to be a translated product (output) by ligation. For this purpose, we employed a bismaleimide cross-linked oligonucleotide (XL-DNA) having two different sequences of DNA strands (Fig. 1). Because 10 bases correspond to one helical turn in the duplex, 10 mer DNA strands are used as one unit for alignment of the orientations of cross-linkers and ligation sites. In the XL-DNA, one DNA strand is complementary to a 10 mer DNA fragment, and the other recognizes a 10 mer DNA block of a template DNA strand (Scheme 1). We used 40 mer template strands containing three parts of 10 mer recognition sequences (**1t**, **2t**, and **3t**) for assembling the components such as XL-DNAs and 10 mer DNA fragments. We finally connected the assembled 10 mer fragments by ligation and identified the translated products.

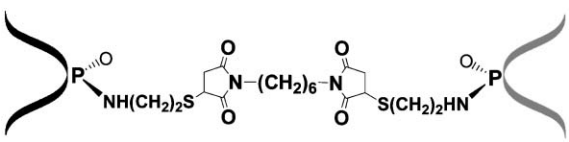
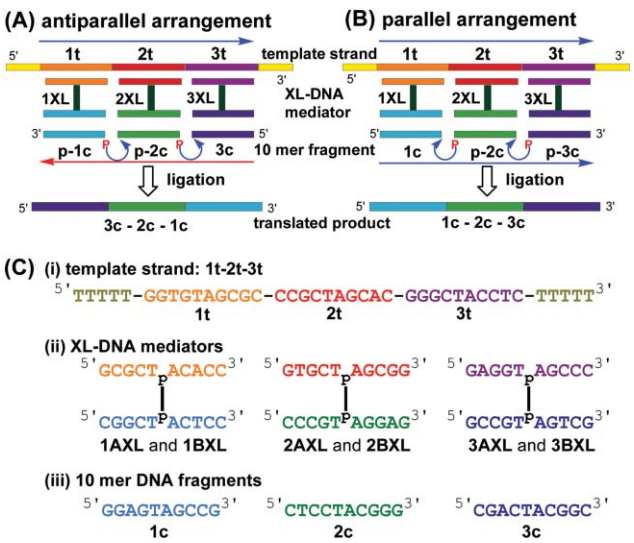


Fig. 1 The cross-linked DNA (XL-DNA) mediator in which two different DNA strands are connected by a bismaleimide linker.

Synthesis of the XL-DNA was carried out according to the previously reported method with some modification (Scheme S1†).[7–10] Because a linker is introduced to a phosphorus atom, two adjacent diastereomer peaks appeared on a reversed-phase HPLC, and the faster and slower eluted diastereomers on a reversed-phase HPLC are denoted as **a**- and **b**-diastereomers, respectively (Fig. S1†).[7–9] In the case of XL-DNAs, two different sequences of DNA strands having the same diastereochemistry were connected, for example, **1AXL** was prepared from **1a** and **1'a**, and **1BXL** was from **1b** and **1'b** (Fig. S1 and S2†). The sequences of template strands, three XL-DNAs, and three 10 mer DNA fragments are shown in Scheme 1C.

At first, we examined the DNA-templated translation with two XL-DNAs and two 10 mer DNA fragments (Scheme 1 and S2†). In this system, antiparallel and parallel arrangements can be formed.[8] Using DNA ligation with different 5′-phosphate 10 mer



Scheme 1 The DNA translation systems with three XL-DNAs **1XL**, **2XL**, and **3XL**. (A) Antiparallel arrangement with **p-1c**, **p-2c**, and **3c** strands, and ligated product **3c-2c-1c**.‡ (B) Parallel arrangement with **1c**, **p-2c**, and **p-3c** strands, and ligated product **1c-2c-3c**. (C) The sequences of template strand **1t-2t-3t**, XL-DNAs **1XL**, **2XL**, **3XL**, and 10 mer DNA fragments **1c**, **2c**, **3c**.§

† Electronic supplementary information (ESI) available: synthesis of XL-DNAs, HPLC profiles and PAGE images of DNA ligation. See http://www.rsc.org/suppdata/cc/b5/b503247d/

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DNA fragments, the efficiencies of the formation of two arrangements can be investigated by analysis of ligated 20 mer products. In the experiments, the formation of the antiparallel arrangement was examined by ligation with **p-1c** and **2c** fragments (product **2c-1c**), and the formation of the parallel one was also examined using **1c** and **p-2c** (product **1c-2c**) (Scheme S2†).

The DNA strands were assembled by mixing same equivalent of a template DNA, XL-DNAs, and 10 mer DNA fragments, and annealed by gradually cooling from 85 °C to 15 °C.[11] The ligation reaction with DNA ligase was carried out at 16 °C for 16 h, and the ligation products were analyzed on HPLC. After the reaction, new peaks appeared in both cases of the antiparallel and parallel arrangements (Fig. S3†), and the production of 20 mer strands **2c-1c** (antiparallel) and **1c-2c** (parallel) was confirmed by comparing with the retention times of the authentic samples of these 20 mer DNA strands. Product yields are summarized in Table 1. By analysis of the ligation efficiencies, the diastereochemistry of XL-DNAs affected the ligation reaction, and the DNA ligation with XL-DNAs having A-diastereomers was preferable (Table 1). Because the 5′-phosphates on the 10 mer DNA strand should direct towards the counterpart duplex (template strand) for ligation and the bulky DNA ligase should occupy the narrow space between the two duplexes,[12] the chain orientations of the cross-linkers from different diastereomers would affect the efficiencies of the ligation reactions differently. However, the relatively small difference in the yields of the products between these diastereomers would be attributable to the flexibility of the cross-linkers. Negative controls of the reactions were also performed. Without XL-DNAs, no detectable peak appeared. In the case of the reaction without template DNA, 2.7% of the ligated product appeared. These indicate that this system is template strand dependent, and the selective formation of the ligation products is controlled and mediated by the XL-DNAs.

To expand the system, we employed three XL-DNAs and three 10 mer DNA fragments. After ligation under the same conditions as described above, the products were analyzed by denaturing polyacrylamide gel electrophoresis (PAGE) (Fig. 2).[11] In the case of the reaction without template DNA (lane 3), no specific band corresponding to the 30 mer DNA was observed, and non-specific bands having the length over 30 mer appeared. On the other hand, in the presence of template DNA, 20 mer (30% yield) and 30 mer (37% yield) DNA strands were selectively produced (lane 1). These results indicate that the template DNA directs the following ligation reaction with three XL-DNAs. In addition, the XL-DNA can control the production of the final products. In the case of the reaction using two XL-DNAs **1AXL** and **2AXL**, most of the reactions stopped at the position of the 20 mer DNA product (lane 2). These results indicate that the XL-DNAs mediate the sequence selective reactions and control the formation of final translated products.

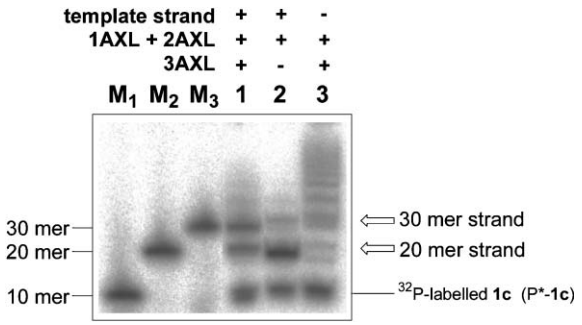

Fig. 2 Denaturing PAGE analysis of the products from the ligation reaction with three XL-DNAs as shown in Scheme 1. The arrangement is antiparallel, and template **1t-2t-3t**, 10 mer DNA fragments **p*-1c**, **p-2c**, and **3c** were used. **p*** represents a 5′-^{32}P-labelled phosphate group. M_1, M_2, and M_3 denote markers of 10, 20, and 30 mer DNA strands, respectively.

Next, the sequence dependence of the system using three XL-DNAs was examined. We used six combinations of the three 10 mer DNA blocks (**1t**, **2t**, and **3t**) on the template strands (Scheme 1). A ^{32}P-labelled phosphate was introduced to the specific 10 mer fragment for detection of the translated 30 mer strand in both antiparallel and parallel arrangements. As shown in Scheme 1, when ^{32}P-**1c**, p-**2c**, and **3c** strands are employed, the resulting ligated 30 mer product should be **3c-2c-1c**, while the parallel arrangement in this case produces a 20 mer ligated strand. After ligation of the three 10 mer DNA fragments using this system, 30 mer ligated appeared strands in both antiparallel and parallel arrangements. (Fig. S4†). The efficiencies of the ligation reactions are summarized in Table 2. The yields depended on the sequences and arrangements, and low yield ligation was found using the templates **1t-2t-3t** and **1t-3t-2t** in the parallel

Table 1 Efficiencies of the ligation reaction with two 10 mer DNA fragments in the translation system with two XL-DNAs using a template strand **1t-2t-3t**[a]

Arrangement	XL-DNAs	10 mer fragments	Product	Yield (%)
Antiparallel	**1AXL**, **2AXL**	**p-1c**, **2c**	**2c-1c**	50
Parallel	**1AXL**, **2AXL**	**1c**, **p-2c**	**1c-2c**	49
Antiparallel	**1BXL**, **2BXL**	**p-1c**, **2c**	**2c-1c**	27
Parallel	**1BXL**, **2BXL**	**1c**, **p-2c**	**1c-2c**	30

[a] A and B denote the diastereomers of phosphoramidates.

Table 2 Efficiencies of the ligation reaction with three 10 mer DNA fragments in the translation system with three XL-DNAs using six template strands[a]

Template	10 mer fragments	Ligation product in antiparallel arrangement	Yield (%)	10 mer fragments	Ligation product in parallel arrangement	Yield (%)
1t-2t-3t	p*-1c, p-2c, 3c	**3c-1c-1c**	32	1c, p-2c, p*-3c	**1c-2c-3c**	15
1t-3t-2t	p*-1c, p-3c, 2c	**2c-3c-1c**	37	1c, p-3c, p*-2c	**1c-3c-2c**	15
2t-1t-3t	p*-2c, p-1c, 3c	**3c-1c-2c**	33	2c, p-1c, p*-3c	**2c-1c-3c**	43
2t-3t-1t	p*-2c, p-3c, 1c	**1c-3c-2c**	44	2c, p-3c, p*-1c	**2c-3c-1c**	56
3t-1t-2t	p*-3c, p-1c, 2c	**2c-1c-3c**	39	3c, p-1c, p*-2c	**3c-1c-2c**	51
3t-2t-1t	p*-3c, p-2c, 1c	**1c-2c-3c**	27	3c, p-2c, p*-1c	**3c-2c-1c**	47

[a] Asterisk represents a ^{32}P-labelled phosphate group.

arrangement. Although the relationship between the sequence and ligation efficiency is still unclear from this experiment, the XL-DNAs mediate the assembly of the template and DNA fragments and the selective production of the ligated strands by following the information of template DNA strands.

In these experiments, we have created the DNA translation system mediated by XL-DNAs, which can transfer molecular information of a template strand into the ligation products. Using this method, various exchanges of the molecular information would be possible by just changing the sequences of XL-DNA mediator strands.

This work has been partly supported by a Grant-in-Aid for Scientific Research (Project 17105005, Priority Area (417), 21st Century COE Research, and others) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japanese Government.

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Notes and references

‡ P in (A) and (B) represents a 5′-phosphate group. The arrows represent the orientations of the 5′ to 3′ direction of the template DNA and 10 mer DNA fragments.

§ Six combinations of the template strands are possible by exchanging the sequences of **1t**, **2t**, and **3t**. In the case of the translation system with two XL-DNAs, template **1t-2t-3t**, XL-DNAs **1XL**, **2XL**, and 10 mer fragments **1c**, **2c** were used.

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11 Phosphorylation of 10 mer DNA strands was carried out at 37 °C for 2 h in a solution (60 μL) containing 10 mer DNA (1.0 nmol), 50 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM DTT, 1 mM ATP, and T4 polynucleotide kinase (10 units). After heating at 65 °C for 20 min, the phosphorylated product was purified by HPLC. A sample solution (20 μL) containing 2 μM template DNA, 2 μM cross-linked DNAs, 2 μM 10 mer DNA strand, 2 μM 10 mer phosphorylated DNA strands, 66 mM Tris-HCl buffer (pH 7.6), 6.6 mM $MgCl_2$, 0.1 M NaCl, 10 mM DTT, and 1 mM ATP was heated at 85 °C for 5 min, then gradually cooled down to 15 °C (1.0 °C min^{-1}) by a thermal cycler. T4 DNA ligase (8 units) was added to the mixtures, and the ligation reaction was carried out at 16 °C for 16 h (Fig. S5, see ESI). After the reaction, the samples were frozen by liquid nitrogen until analysis by HPLC. For PAGE analysis, the same volume of formamide was added to the sample, and the mixture was heated at 85 °C, then analyzed by 20% denaturing PAGE.
12 Crystal structure of T7 ligase is available in the following literature in which DNA binding site of T7 ligase is proposed. H. S. Subramanya, A. J. Doherty, S. R. Ashford and D. B. Wigley, *Cell*, 1996, **17**, 607.