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Artificial regulation of transcription applying carbohydrate–lectin interaction

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A complex of plasmid DNA–lactose conjugate with lectin is proposed as an artificial system to control transcription activity: the *in vitro* transcription of DNA in the conjugate with T7 RNA polymerase was repressed in the presence of RCA_{120}, and then the transcription ability was recovered by adding lactose or a lactose-carrying polymer to the repression system.

Artificial regulation of gene expression is an important subject for post-genome research.[1] Recently, it has been reported that gene expression was repressed with polyamides,[2] aminoglycosides,[3] and gold-nanoparticles modified with a cationic monolayer.[4] However, there has been no paper on the on–off control of gene expression with specific substances.

Recently, we reported the synthesis of several types of DNA–carbohydrate conjugates *via* diazo-coupling,[5] solid-phase synthesis,[6] and telomerization.[7] These DNA–carbohydrate conjugates can be regarded as mimics of glycosylated DNAs rarely occurring in nature[8] and also as functional biomaterials applying the characters of both DNA and carbohydrates. In this paper, we have focused on the strong affinity of DNA–carbohydrate conjugates to specific lectins. We propose that a complex between the conjugate and lectin can be regarded as a simple functional model. We demonstrate that the gene expression can be regulated *via* the carbohydrate recognition of lectin.

Fig. 1 illustrates our strategy for constructing a regulation system of DNA transcription. If the plasmid–lactose conjugate can be complexed strongly with RCA_{120}, the access of RNA polymerase to the template DNA will be hindered and the transcription of DNA will be repressed. If addition of an excess amount of lactose to the complex can relax or dissociate the binding between the plasmid and RCA_{120}, the RNA polymerase will become accessible to the DNA and the transcription of DNA will be recovered. Then, artificial on–off switching of transcription through applying the carbohydrate recognition event will be achieved.

A conjugate of pTRI-β-Actin (Mouse) plasmid DNA (Ambion Inc.) with lactose was prepared in 90% yield according to our previous report[5c,9] *via* diazo coupling with *N*-(ω-(*p*-aminobenzamido)hexanoyl)-lactosylamine,[5b] followed by centrifugal ultrafiltration using Microcon®-PCR (Millipore). The degree of substitution (DS) of the lactose derivative to nucleobases in plasmid was determined to be about 8–12% from the absorbances at 350 nm (diazo) and at 260 nm (nucleobase). The interaction between the plasmid–Lac conjugate and β-galactose-specific RCA_{120} lectin was investigated by gel-shift assay and surface plasmon resonance (SPR). The gel-shift assay showed that the conjugate was bound to RCA_{120} at a concentration above 1μM. The apparent association constant (K_a) determined from the Langmuir plot was 7.6×10^5 M^{-1} per lactose unit. The SPR time course (Fig. 2) showed that the conjugate was bound to RCA_{120}, which had been immobilized on a gold substrate *via* a self-assembled monolayer of dithiodipropionic acid, with the apparent association constant $K_a = 4.1 \times 10^5$ M^{-1} per lactose unit, and then the complex was dissociated by running a buffer. On the other hand, the native plasmid was minimally bound to the RCA_{120}-immobilized substrate, and the conjugate was minimally bound to the concanavalin A-immobilized substrate.

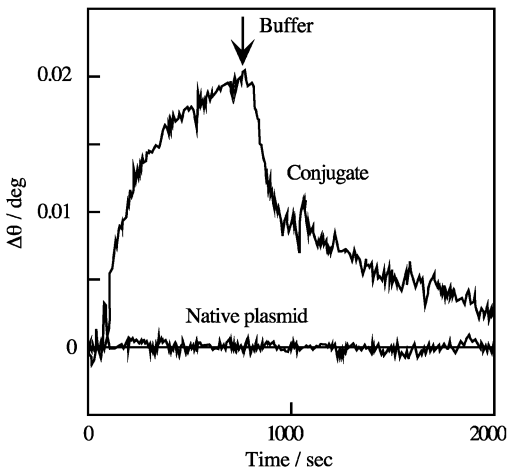

Fig. 2 Typical time courses of the angle change ($\Delta\theta$) of SPR responding to the addition of plasmid–Lac conjugate (DS = 8.2%, 220 μM base, [Lac] = 18 μM) and native plasmid (220 μM-base) to the RCA_{120}-immobilized gold surface in water at 25 °C. (SPR 670, Nippon Laser & Electronic Lab., Nagoya.)

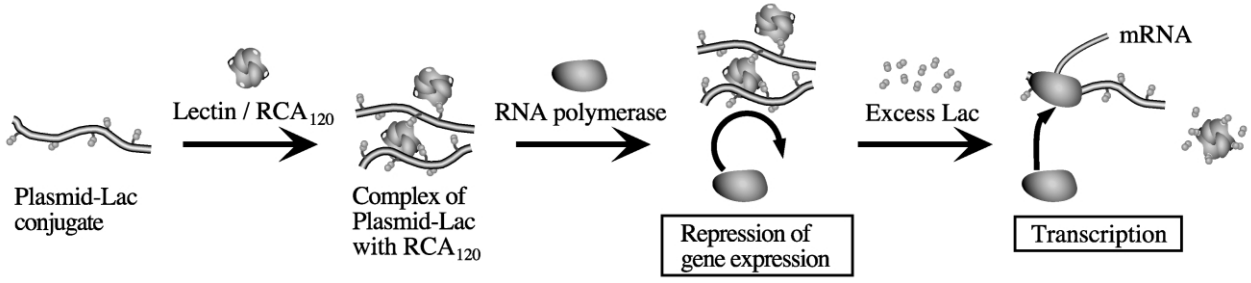

Fig. 1 Schematic illustration of artificial regulation of transcription applying the carbohydrate–lectin interaction.

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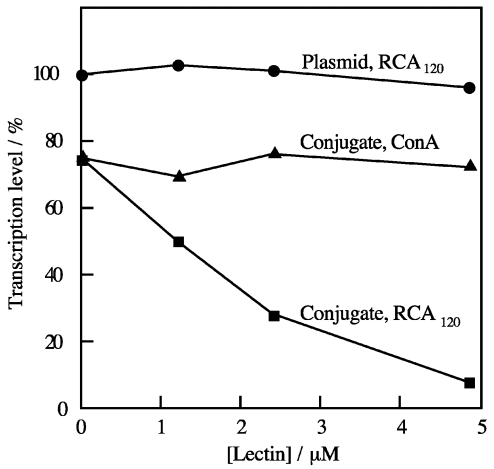

Fig. 3 Effect of concentration of RCA_{120} on *in vitro* transcription of plasmid–Lac conjugate (DS = 12%). [plasmid] = 1.8 mM base, at 25 °C.

Transcription of plasmids was carried out with a MAXIscript® transcription kit containing T7 RNA polymerase and NTPs at 25 °C for 2 h in the absence or presence of lectin. Production of mRNA was monitored on 8 M urea-denatured 15% polyacrylamide gel electrophoresis (Atto, PAGEL®) with a densitometer (Atto densitograph software library lane & spot analyzer ver. 6). The amount of transcription was normalized against that of the native plasmid as 100%. Fig. 3 compares the effect of lectin on the transcription amount of the conjugate and the native plasmid. It is important to note that the transcription could be performed even with the conjugate, although the amount of transcription was decreased to 76% of that of the native plasmid.[10] This is an example demonstrating that T7 RNA polymerase can bypass some modified DNA templates during transcription elongation.[11] When RCA_{120} was added to the conjugate, the amount of transcription was decreased with the increase of RCA_{120} concentration, reaching almost 0 % at 5 μM of RCA_{120}. On the other hand, the transcription of the native plasmid was little affected even with 5 μM of RCA_{120}, and, likewise, the transcription of the conjugate was also little affected even with 5 μM of non-specific Con A. These results suggest that the repressive effect of RCA_{120} on the transcription of the conjugate was caused by the specific interaction of RCA_{120} with the lactose residues along the DNA strand.

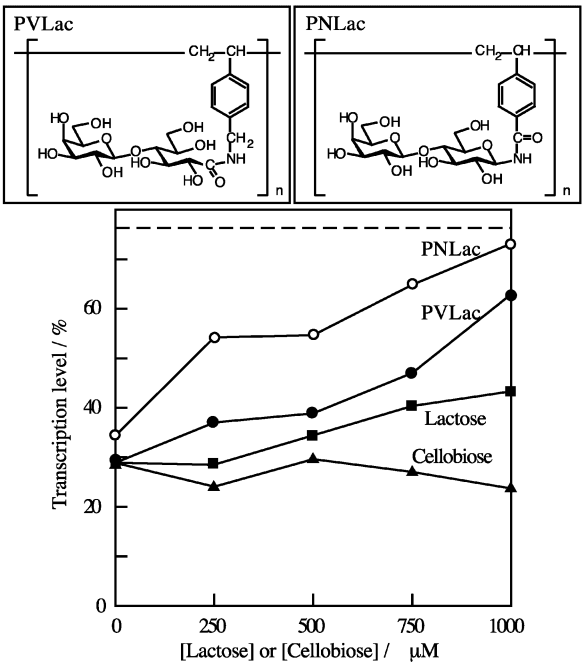

Fig. 4 Effect of lactose derivatives on *in vitro* transcription of plasmid–Lac conjugate (DS = 12%) repressed with RCA_{120}. [plasmid] = 1.8 mM base ([Lac] = 220 μM), [RCA_{120}] = 2.4 μM, at 25 °C. The broken line indicates the transcription level (76%) of plasmid–Lac conjugate in the absence of RCA_{120}.

The effect of lactose and two different lactose-carrying polystyrenes (PVLac[12] and PNLac[13]) as recovering signals was investigated to switch on the transcription. The plasmid–Lac conjugate (790 μM base) was incubated with RCA_{120} (2.4 μM, 2 h) at 25 °C for 10 min and then the recovering signal (0 to 1 mM) was added and incubated at 25 °C for 10 min. As shown in Fig. 4, the repressed transcription was recovered to 43% with excess lactose (1 mM). Addition of PVLac and PNLac achieved more effective recovery (62% for PVLac and 71% for PNLac) of transcription, probably owing to stronger lectin-binding. Since cellobiose was minimally affected, it is reasonable to assume that the specific binding of excess lactose to the lectin brought about relaxation of the conjugate-RCA_{120} complex, which resulted in the recovery of transcription.

In conclusion, we have demonstrated the first successful on–off regulation of gene expression with an artificial recognition system using a combination of a plasmid–carbohydrate conjugate with lectin.

Notes and references

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