Symposium date: 8th September 2008

Nucleic Acids Symposium Series No. 52 **129–130**
doi:10.1093/nass/nrn066

Self-Avoiding molecular Recognition Systems (SAMRS)

Shuichi Hoshika[1*], Fei Chen[1], Nicole A. Leal[1] and Steven A. Benner[1]

[1]Foundation for Applied Molecular Evolution, 1115 NW 4th Street, Gainesville, FL 32601, USA

ABSTRACT

Reported here is a "Self-Avoiding Molecular Recognition Systems" (SAMRS), a species of DNA that can bind via simple rules to natural DNA but cannot bind to other members of the same SAMRS species. A system having these properties has been achieved with 2-aminopurine-2'-deoxyriboside (A*), 2'-deoxy-2-thiothymidine (T*), 2'-deoxyinosine (G*) and *N*4-ethyl-2'-deoxycytidine. These were designed to form more stable base pairs with natural complements than with SAMRS complements, based on the number of hydrogen bonds. Thermal melting studies were performed using duplexes containing SAMRS components. All SAMRS species, A*, T*, G* and C*, formed more stable base pairs with natural complements, T, A, C and G than with SAMRS complements, T*, A*, C* and G* respectively. This property of SAMRS would be useful for avoiding to be produced undesired products derived from intra- and intermolecular interaction between primers in multiplexed polymerase chain reactions.

INTRODUCTION

Currently, rapid genome analysis technology is needed to diagnose hereditary and/or infectious disease and personalize the treatment of patients. Since cost-effectiveness requires that many genetic sites be analyzed at the same time, many seek tools to efficiently multiplex PCR. However, as most multiplexed PCR architectures use many sets of primers in one tube, it often encounters undesired products derived from intra- and intermolecular interaction between primers.

The concept of "pseudocomplementarity" or "self-avoidance" in a molecular recognition system was introduced over a decade ago.[1,2] "Self-Avoiding Molecular Recognition Systems" (SAMRS) are those that can bind to natural DNA but not to other SAMRS species. Primers built from SAMRS would therefore not interfere with each other, permitting multiplexed PCR. This consideration prompted us to construct SAMRS which is applicable to multiplex PCR.

RESULTS AND DISCUSSION

Each SAMRS species (A*, T*, G* and C*) was designed to bind to a natural complement (T, A, C and G) more tightly than a corresponding SAMRS complement (T*, A*, C* and G*) respectively. As shown Figure 1, 2-aminopurine-2'-deoxyriboside (A*) and 2'-deoxy-2-thiothymidine (T*) can bind to T and A with two hydrogen bonds respectively. On the other hand, A* and T* can bind to each other with one hydrogen bond. 2'-Deoxyinosine

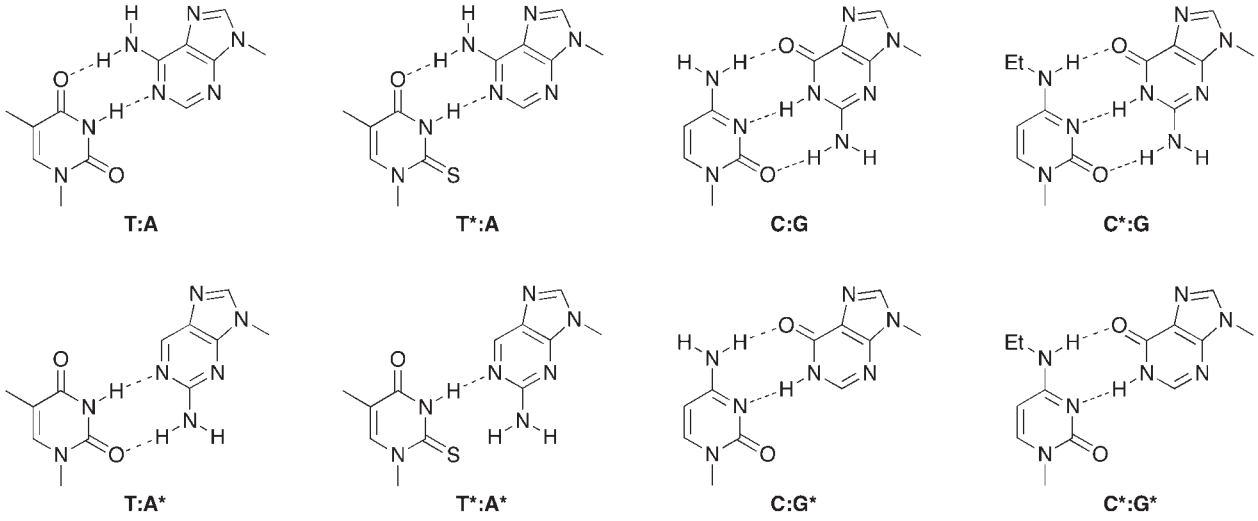

Fig. 1 Base pairing motifs of natural:natural SAMRS:natural and SAMRS:SAMRS.

Table 1 T_m values (°C) of duplexes containing natural:natural, SAMRS:natural and SAMRS:SAMRS base pair.

X:Y	T	T*	A	A*	C	C*	G	G*
A	55.5	56.8	43.7	46.5	45.1	45.3	46.7	49.8
A*	54.5	**52.0**	46.8	45.5	48.1	46.8	45.8	46.8
T	46.3	48.0	54.0	52.5	44.6	41.6	48.4	46.3
T*	47.0	50.0	54.0	**50.3**	40.9	40.3	44.6	45.1
G	49.5	47.0	47.0	45.1	58.8	55.7	47.0	46.0
G*	48.8	47.0	50.5	45.1	54.1	**52.0**	46.0	46.3
C	44.0	40.6	42.8	47.1	43.8	42.0	59.0	52.6
C*	41.8	40.0	43.0	45.0	41.8	40.8	55.8	**51.5**

Sequence. 5'-ACCAAGCXATCAAGT-3' and 3'-TGGTTCGYTAGTTCA-5'.

Boxes with bold outline hold T_m values of duplexes containing complementary X:Y pair.

T_m values of duplexes containing complementaly SAMRS:natural base pair are underlined.

T_m values of duplexes containing complementaly SAMRS:SAMRS base pair are in boldface.

Conditions. 20 mM Na cacodylate (pH 7.0), 100 mM NaCl, 3.0 μM duplex.

(G*) can bind to C with two hydrogen bonds and *N*4-ethyl-2'-deoxycytidine can bind to G with two hydrogen bonds (and weak one hydrogen bond).[3] On the other hand, G* and C* can bind to each other with one hydrogen bond (and weak one hydrogen bond).

"Self-avoidance" properties of A*, T*, G* and C* were tested by T_m measurement of 15 mer duplex containing each one SAMRS residue in either strand or both strands (Table 1). As the result, all SAMRS species preferred the natural complements to corresponding SAMRS complements. The duplexes containing base pair consisting of A* and T or T* and A showed higher T_m values than the duplexes containing base pair consisting of A* and T* (X:Y = A*:T; 54.5 °C and A:T*; 56.8 °C vs. A*:T*; 52.0 °C and T:A*; 52.5 °C and T*:A; 54.0 °C vs. T*:A*; 50.3 °C). Similarly the duplexes containing base pair consisting of G* and C or C* and G showed higher T_m values than the duplexes containing base pair consisting of G* and C* (X:Y = G*:C; 54.1 °C and G:C*; 55.7 °C vs. G*:C*; 52.0 °C and C:G*; 52.6 °C and C*:G; 55.8 °C vs. C*:G*; 51.5 °C). In addition, all duplexes containing complementary SAMRS:natural base pairs showed the highest T_m compared with the duplexes containing any other mismatched base pairs. These results suggest that SAMRS can not only avoid to form intra- and intermolecular self-structure but also discriminate the natural complements from the natural and the SAMRS mismatches.

CONCLUSION

We reported a "Self-Avoiding Molecular Recognition Systems" (SAMRS) using 2-aminopurine-2'-deoxyriboside (A*), 2'-deoxy-2-thiothymidine (T*), 2'-deoxyinosine (G*) and *N*4-ethyl-2'-deoxycytidine (C*). From thermal melting studies, we found that all SAMRS species preferred the natural complements to the SAMRS complements and showed good discrimination to the natural and the SAMRS mismatches. These results suggest that SAMRS would empower multiplexed PCR by avoiding any undesired primer-primer interactions.

Further applications of SAMRS to enzymology and PCR are now in progress.

REFERENCES

1. Kutyavin, I. V., Rhinehart, R. L., Lukhtanov, E. A., Gorn, V. V., Meyer, R. B. Jr. and Gamper, H. B. Jr. (1996) *Biochemistry*, **35**, 11170-11176.
2. Gamper, H. B. Jr., Arar, K., Gewirtz, A. and Hou, Y. M. (2006) *Biochemistry*, **45**, 6978-6986.
3. Nguyen, H. K., Bonfils, E., Auffray, P., Costaglioli, P., Schmitt, P., Asseline, U., Durand, M., Maurizot, J. C., Dupret, D. and Thuong, N. T. (1998) *Nucleic Acids Res.*, **26**, 4249-4258.

*Corresponding author. E-mail: shoshika@ffame.org