Binding of 5-bromouracil-containing S/MAR DNA to the Nuclear Matrix

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Abstract

Substitution of thymine with 5-bromouracil in DNA is known to change interaction between DNA and proteins, thereby inducing various biological phenomena. We hypothesize that A/T-rich scaffold/nuclear matrix attachment region (S/MAR) sequences are involved in the effects of 5-bromodeoxyuridine. We examined an interaction between DNA containing an intronic S/MAR sequence of the immunoglobulin heavy chain gene and nuclear halos prepared from HeLa cells. Upon substitution with 5-bromouracil, the S/MAR DNA bound more tightly to the nuclear halos. The multi-functional nuclear matrix protein YY1 was also found to bind more strongly to 5-bromouracil-substituted DNA containing its recognition motif. These results are consistent with the above hypothesis.

Key words: BrdU; 5-bromouracil; S/MAR sequence; nuclear matrix; IgH gene; YY1

We have shown that 5-bromodeoxyuridine (BrdU) clearly induces a senescence-like phenomenon in mammalian cells regardless of cell type or species.1 In this system, the senescence-associated genes and some tissue-specific genes are immediately induced or suppressed by BrdU.1–6 We have obtained a few clues to the molecular basis for the action of BrdU. In an earlier study, AT-binding ligands such as distamycin A and AT-hook proteins such as HMG-I markedly potentiated the effects of BrdU.7 Further, since distamycin A and AT-hook proteins are able to bind to specific A/T-rich sequences and displace histone H1 and other DNA-binding proteins, it is possible that BrdU targets such A/T-rich sequences. We thought that such A/T-rich sequences might be scaffold/nuclear matrix attachment region (S/MAR) sequences because A/T-rich sequences are clustered in S/MAR sequences in mammalian cells.8–10 In addition, S/MAR sequences have been shown to dramatically affect their neighboring promoter activities, and this effect is manifested when reporter constructs are integrated into the host genome.11–13 For example, the S/MAR binding protein SATB1 modulates the expression of a set of tissue-specific genes.14 Furthermore, we have found that S/MARs are linked to the senescence-associated genes affected by BrdU (unpublished data). With these lines of circumstantial evidence, we hypothesize that A/T-rich S/MAR sequences are involved in the biological phenomena induced by BrdU. Substitution of thymine with 5-bromouracil is thought to alter chromatin structure by changing the interaction between DNA and the nuclear matrix. In this regard, filter binding assays carried out by Lin and Riggs have shown that some structural and regulatory proteins bind more tightly to DNA substituted with 5-bromouracil.15–17

As a first step to verify the above hypothesis, we examined the interaction between S/MAR DNA and the nuclear matrix. The S/MAR DNA sequence we used was a concatemer of oligonucleotide (5'-TCTTTAATTTCTAATATATTTAGAA-3') containing an intronic S/MAR sequence derived from the immunoglobulin heavy chain (IgH) gene enhancer,18 because it is one of the best characterized S/MARs. As nuclear halos are often used for the nuclear matrix, we prepared the halos from HeLa cell nuclei by the method described by C. Mielke et al.19 with slight modification. The resulting halos were digested with restriction endonucleases to remove endogenous DNA to empty S/MAR binding sites. The S/MAR DNA was end-labeled with T4 polynucleotide kinase and [γ-32P]ATP and mixed with the halos. After incubation in the dark at 37°C overnight in the presence of an excess amount of carrier DNA, the mixture was centrifuged to yield pellet and supernatant fractions. DNA was purified from each fraction and electrophoresed on a polyacrylamide gel.

We examined the binding of the IgH S/MAR DNA to
Figure 1. Binding of IgH S/MAR DNA to nuclear halos. IgH S/MAR DNA was prepared as described. An oligonucleotide containing the intronic S/MAR sequence was self-ligated to yield a heptamer and cloned into the EcoRV site of the plasmid pBluescriptSK(−). This plasmid was used as a template to prepare S/MAR DNA containing thymine (T) or 5-bromouracil (B) by PCR with T3 and T7 primers and dTTP or 5-BrdUTP as a substrate. A PCR product of pBluescriptSK(−) (vector DNA) was used as non-S/MAR DNA. Radiolabeled 357-bp probe DNA (IgH S/MAR) and 165-bp vector DNA (vector) with equivalent radioactivity (typically 3 × 10⁶ cpm) were mixed, incubated with nuclear halos (7 × 10⁶ cell equivalents) prepared from HeLa cells cultured in the absence (T) or presence (B) of 50 µM BrdU for 7 days, and centrifuged to yield pellet and supernatant fractions. DNA was purified from each sample and resolved in 100 µl TE buffer. A, DNA samples amounting to 1000 cpm were loaded on each well, and run on 5% polyacrylamide gel to prepare an autoradiogram. B, Samples of equal amounts of DNA were run on the gel as described above. I, input DNA; P, pellet fraction; S, supernatant fraction.

nuclear halos under various conditions. Upon substitution of thymine with 5-bromouracil, the proportion of the S/MAR DNA in the pellet fractions (P fractions) markedly increased (Fig. 1A, lanes 5 and 7 and Fig. 1B, lane 5). We also compared nuclear halos prepared from cells cultured with or without BrdU. The nuclear halos of the BrdU-treated cells only slightly decreased the binding with the DNA probe, probably due to competition with 5-bromouracil-substituted genomic DNA attached to the nuclear halos rather than to an alteration of the composition of the nuclear matrix (Fig. 1A lane 1 vs. 3 and lane 5 vs. 7). Control DNA (a vector sequence) did not bind to the nuclear halos under any conditions. When mouse L929 cells were used as the source of nuclear halos, similar results were obtained (data not shown). These results suggest that the substitution of thymine with 5-bromouracil in IgH S/MAR DNA per se is involved in the increase in its binding affinity to the nuclear matrix.

Then, we examined an interaction between YY1 and an oligonucleotide containing its A/T-rich recognition sequence by electromobility shift assay (EMSA). YY1 is a multi-functional zinc finger transcription factor located in the nuclear matrix. Substitution of thymine in the oligonucleotide with 5-bromouracil increased its binding affinity to YY1 in a protein extract by about 2.5-fold (Fig. 2, lanes 1 vs. 5). We also confirmed that YY1 preferentially bound to the 5-bromouracil-containing probe (Fig. 2, lane 9–16). The addition of BrdU to HeLa cells did not affect the above binding (not shown). In the EMSA competition experiments, we estimated the apparent equilibrium dissociation constant ($K_d$) in terms of the
Figure 2. Binding of YY1 to DNA containing its binding motif. Protein extracts were prepared from HeLa cells and EMSA were performed according to the method by T. C. Lee et al.29,30 Double-stranded oligonucleotide 5′-GACGCCATTTTAAGTCCTAACGA-3′ containing a YY1 binding motif31 was inserted into the EcoRV site of pBluescriptSK(−). This plasmid was used as a template to prepare probe DNA and competitor DNA containing thymine (T) or 5-bromouracil (B) by PCR with T3 and T7 primers and dTTP or 5-BrdUTP. Two nanograms (final concentration 1.09 nM) of the end-labeled probe DNA was mixed with 3 µg of the protein extract and the competitor DNA as indicated in a 15-µl reaction volume. NC lacks YY1 binding motif and was used as a negative control. Competitors containing thymine (T) or 5-bromouracil (B) were added in molar excess of 10-fold (×10) and 20-fold (×20).

effective molar concentration of the binding sites (i.e., the molar concentration of probe DNA). Under the same condition as shown in Fig. 2, the thymine-containing DNA probe was competed with 0.5-, 1-, 2-, and 5-fold molar excess of competitors containing thymine or 5-bromouracil. The YY1-specific shifted bands and free DNA bands were quantified using a phosphoimager (BAS 2000II, FUJI film). We estimated that YY1 bound to the probe DNA with $K_d = 2.58 \pm 0.70$ nM (mean value ± S.D.) and $K_d = 0.47 \pm 0.04$ nM, respectively, for competitor DNA containing thymine and 5-bromouracil.

We showed that IgH S/MAR DNA increased the binding capacity of the DNA to the nuclear matrix upon substitution with 5-bromouracil. Although the chemical nature of the stabilizing effect by BrdU is still unknown, the presence of a bromine atom at the 5 position of uracil in double-stranded DNA somehow provides an altered environment favored by the S/MAR-binding proteins like YY1. Such an alteration could occur in at least two general non-exclusive ways. First, the increased affinity between S/MAR proteins and the 5-bromouracil-substituted DNA may occur because bromine is more electronegative than a methyl residue, and this may stabilized electrostatic interactions. Alternatively, bromine substitution might induce a change in the topology of DNA, e.g., the degree of bending along the axis or stacking of A/T-bases. These altered forms might bind more strongly to the S/MAR proteins. In fact, we have already demonstrated that substitution of thymine with 5-bromouracil in a rat S/MAR sequence reduced its degree of bending (T. Suzuki et al., submitted). Furthermore, there are some reports suggesting that BrdU alters the chromatin structure. For example, BrdU decondenses A/T-rich constitutive heterochromatin and Giemsa-dark bands in mitotic chromosomes, especially in human chromosome 1, 9, 15, 16 and Y.21,22 Thus, it seems plausible that DNA conformation and electrochemical properties, in addition to DNA sequence, may be important determinants for the increased binding of S/MAR proteins to 5-bromouracil-containing DNA. Recently, we have also shown that only 5-halogenated thymidine analogues commonly induce the senescence-like phenomenon.23 Further work with the other halogenated analogues (chlorine and iodine derivatives) will give useful information regarding the relative importance of the electro-negative environment and the change in DNA topology.

The nuclear matrix is thought to execute many important functions within the nucleus.24 It plays a critical role in higher-order organization of chromatin structure by tethering actively transcribed genes via S/MARs.25–28 Any alteration in the interaction between S/MARs and
the nuclear matrix will no doubt lead to altered expression of neighboring genes. The results presented in this report support the above hypothesis that BrdU might induce or suppress a particular type of genes.

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