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Stable minihairpin structures forming at minisatellite DNA isolated from yellow fin sea bream *Acanthopagrus latus*

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Abstract

The lengths of simple repeat sequences are generally unstable or polymorphic (highly variable with respect to the numbers of tandem repeats). Previously we have isolated a family of minisatellite DNA (GenBank accession AF422186) that appears specifically and abundantly in the genome of yellow fin sea bream *Acanthopagrus latus* but not in closely-related red sea bream *Pagrus major*, and found that the numbers of tandem arrays in the homologous loci are polymorphic. This means that the minisatellite sequence has appeared and propagated in *A. latus* genome after speciation. In order to understand what makes the minisatellite widespread within the *A. latus* genome and what causes the polymorphic nature of the number of tandem repeats, the structural features of single-stranded polynucleotides were analyzed by electrophoresis, chemical modification, circular dichroism (CD), differential scanning calorimetry (DSC) and electron microscopy. The results suggest that a portion of the repeat unit forms a stable minihairpin structure, and it can cause polymerase pausing within the minisatellite DNA.

Keywords: DNA secondary structure; Electron microscopy; Electrophoretic mobility; Fish genome; Minisatellite DNA; Repetitive DNA

1. Introduction

The occurrence of simple sequence repeats such as mini- and microsatellites are salient features of eukaryotic genomes. Moreover, the genetic instability (i.e., the high variability of the repeat number during transmission) of such sequence repeats has been a focus of attention, as several human neurodegenerative diseases are caused by expansion of certain microsatellites. In particular, the molecular mechanisms underlying the instability of disease-related triplet repeats have been intensively investigated (Mitas, 1997; Hancock and Santibanez-Koref, 1998; Sinden, 1999; Sinden et al., 2002; Lenzmeier and Freudenreich, 2003; Cleary and Pearson, 2003; Gatchel and Zoghbi, 2005; Pearson et al., 2005 for review), and their structural and functional properties have been characterized using biophysical, biochemical and genetic assays. As a result, it is now known that duplex DNA containing CTG triplet repeats shows greater flexibility than mixed sequences (Chastain and Sinden, 1998) and may conform to slipped-stranded DNA (S-DNA) (Pearson et al., 1998) and that CTG triplet repeats block DNA replication in *E. coli* (Samadashwily et al., 1997). In addition, Ohshima and Wells (1997) reported that both bacterial and eukaryotic DNA polymerases stall

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at CTG tracts during replication *in vitro* and this pausing of the polymerase promotes primer-template realignment generating a DNA hairpin structure. *In vitro* experiments also revealed that repeat expansion is induced by abasic sites (Lyons-Darden and Topal, 1999) and single nucleotide gaps (da Silva and Reha-Krantz, 2000), both of which can cause DNA polymerases to stall, and that replication pauses at various DNA sequences having the potential to form H-DNA (Samadashwily et al., 1993) and at triplet repeats (Ohshima et al., 1996). *In vivo* attenuation of replication and triplet repeat expansion has been described by Pelletier et al. (2003). They observed that CGG triplet repeats significantly attenuate replication fork progression, and that a mutation in the large subunit of the replication factor C complex (rfc1-1) increased the expansion rate for the CGG_{25} repeat approximately 50-fold in *Saccharomyces cerevisiae*. Replication pausing at GAA triplet repeats, which can form triplex DNA (Mariappan et al., 1999), sticky DNA (Sakamoto et al., 1999) and hairpin structures (Heidenfelder et al., 2003), also has been observed *in vivo*, and a model of replication blockage by (GAA)n/(TTC)n repeats leading to their expansion and contraction has been proposed (Krasilnikova and Mirkin, 2004). In that context, the instability of triplet repeats is associated with lagging-strand DNA synthesis and repair in which replication pausing, formation of alternative DNA structures at the template and nascent strands, and processing of the Okazaki fragment are involved.

Minisatellite elements, another class of simple repeat sequences, are also widespread within the genomes of eukaryotic organisms, and have been widely used as genetic markers, owing to the highly polymorphic nature of their tandem repeat number (Jeffreys et al., 1991; Turner et al., 1992; Meyer et al., 1993). Some minisatellite loci are associated with chromosome disorders (Cleary and Pearson, 2003), and the *in vivo* instability of minisatellites has been described (Larson et al., 1999; Lopes et al., 2002; Jauert et al., 2002). Variation in repeat number within minisatellite DNA may be caused by several mechanisms, including replication slippage (primer-template misalignment), genetic recombination, repair and gene conversion, but, the structural cause of this variability is not yet clearly defined.

We have isolated a novel minisatellite, AL79, from the yellow fin sea bream *Acanthopagrus latus* (Elmesiry et al., 2005). It is comprised of a tandem array of a 30-bp unit that includes an 18-bp pyrimidine/purine-biased stretch and a 12-bp A/T-rich stretch, as following: 5′-TCCTCCTCTCCTCCTCCTGAGGATATAAAG-3′. The pyrimidine/purine-biased sequences can be arranged in imperfect mirror symmetry with neighboring 30-bp units and thus it may have the potential to form intramolecular triplexes, while the occurrence of TpG and TpA dinucleotide steps may increase DNA duplex flexibility (bendability). Multiple copies of AL79 minisatellite are present within the *A. latus* genome, but it is not present in the genome of *Pagrus major*, which belongs to same family, Sparidae. This means that AL79 first appeared in *A. latus* after speciation and propagated within the genome on a relatively short evolutionary time scale. To better understand the molecular processes involved in the generation and propagation of minisatellite elements within genomes, we examined *in vitro* replication catalyzed by *Taq* DNA polymerase to evaluate the effect of template strand structure, and characterized several structural properties of the AL79 minisatellite DNA. We found that repeat expansion and contraction occurs during PCR amplification, that a small hairpin structure may be formed within a repeat unit, and that the mechanical properties of single-stranded fragments of AL79 differ from their respective complementary strands. While several protein factors are working *in vivo* to maintain the stability of, or to destabilize mini- and microsatellite loci during mitosis and meiosis (Lopes et al., 2002; Jauert et al., 2002; Pelletier et al., 2003; Liu et al., 2004; Panigrahi et al., 2005), the structural features of the AL79 sequence itself may be one of the determinants causing variation of tandem repeats within genomes.

2. Materials and methods

2.1. DNA and chemicals

A pUC19 derivative containing the AL79 minisatellite sequence (pAL79) was obtained previously (Elmesiry et al., 2005). The nucleotide sequence of AL79 appears in the GenBank/EMBL/DDBJ international databases under the accession number AF422186. Oligodeoxyribonucleotides used for PCR and analysis of circular dichroism (CD) spectra were purchased from Takara-Bio (Kusatsu, Japan) and Kurabo (Osaka, Japan). Oligodeoxyribonucleotides used for differential scanning calorimetry were purchased from Hokkaido System Science (Sapporo, Japan). The sequences of the oligonucleotides used in this study are listed in Table 1. Dimethylsulfate (DMS) and diethylpyrocarbonate (DEPC) were purchased from Wako Pure Chemicals (Osaka, Japan) and Nakalai Tesque (Kyoto, Japan), respectively. *Taq* DNA polymerases were from Takara-Bio (Kusatsu, Japan) and TOYOBO (Osaka, Japan). Agarose was from Nakalai Tesque (Kyoto, Japan). Acrylamide and bisacrylamide were from Wako Pure Chemicals (Osaka, Japan).

2.2. PCR amplification and purification of AL79 DNA

AL79 was amplified by PCR using pAL79 DNA as a template. The standard reaction mixture (100 μL) contained

Table 1
Sequences of the DNA primers used in this study

Primer name	Sequence
M13F	5′-CGCCAGGGTTTTCCCAGTCACGAC-3′
eM13F[a]	5′-CGCCAGGGTTTTCCCAGTCACGAC-3′
M13R	5′-GAGCGGATAACAATTTCACACAGG-3′
eM13R[a]	5′-GAGCGGATAACAATTTCACACAGG-3′
79FA1	5′-TAATTCACCTCAAAATGATGAACACA-3′
e79FA1[a]	5′-TAATTCACCTCAAAATGATGAACACA-3′
79RA1	5′-TGTACTGGGAAAATCATATCTATACT-3′
e79RA1[a]	5′-TGTACTGGGAAAATCATATCTATACT-3′
79F1	5′-TCCTCCTCTCCTCCTCCTGAGGATATAAAG-3′
79F2	5′-CCTCCTCCTGAGGATATAAAGTCCTCCTCT-3′
79F3	5′-GAGGATATAAAGTCCTCCTCTCCTCCTCCT-3′
79R1	5′-CTTTATATCCTCAGGAGGAGGAGAGGAGGA-3′
79R2	5′-AGAGGAGGACTTTATATCCTCAGGAGGAGG-3′
79R3	5′-AGGAGGAGGAGAGGAGGACTTTATATCCTC-3′

[a] These oligomers are labeled with rhodamine at their 5′ termini.