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Preliminary studies on DNA retardation by MutS applied to the detection of point mutations in clinical samples

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Abstract

MutS ability to bind DNA mismatches was applied to the detection of point mutations in PCR products. MutS recognized mismatches from single up to five nucleotides and retarded the electrophoretic migration of mismatched DNA. The electrophoretic detection of insertions/deletions above three nucleotides is also possible without MutS, thanks to the DNA mobility shift caused by the presence of large insertion/deletion loops in the heteroduplex DNA. Thus, the method enables the search for a broad range of mutations: from single up to several nucleotides. The mobility shift assays were carried out in polyacrylamide gels stained with SYBR-Gold. One assay required 50–200 ng of PCR product and 1–3 μg of *Thermus thermophilus* his_6-MutS protein. The advantages of this approach are: the small amounts of DNA required for the examination, simple and fast staining, no demand for PCR product purification, no labelling and radioisotopes required. The method was tested in the detection of cancer predisposing mutations in *RET*, *hMSH2*, *hMLH1*, *BRCA1*, *BRCA2* and *NBS1* genes. The approach appears to be promising in screening for unknown point mutations.

Keywords: MutS; Retardation; Mutation detection; Cancer; DNA polymorphism

1. Introduction

Mutations are very common, which is manifested by thousands of inherited genetic diseases and a high incidence of neoplasm (caused by somatic mutations). It is estimated that more than 25% of the human

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population suffer from neoplasms and 38% are afflicted with diseases of a genetic origin (including neoplasms) [1]. However, the frequencies of individual point mutations are very low. This is why mutation detection requires efficient screening methods. It is practical to apply DNA sequencing to confirm the positive results preliminarily recognised in the screening tests. Electrophoretic screening methods like SSCP are commonly used, but none of them detects all point mutations [2]. MutS protein, which recognizes mispaired and unpaired bases, could be an alternative tool for mutation screening. The tested DNA should be mixed with the reference DNA, denatured and renatured to form heteroduplexes prior to MutS binding. MutS forms complexes with mismatched heteroduplexes. The examination of heterozygotic alleles does not require the addition of reference DNA. Whitehouse et al. [3] reported *Thermus thermophilus* MutS to identify all mismatches from single mispaired bases to three-nucleotide insertion/deletion loops (bubbles). *Salmonella typhimurium* MutS was reported to recognize mismatches and deletions or insertions up to four bases [4]. The formation of MutS–DNA complexes may be detected by DNA band shift in electrophoresis [5,6]; in filter assays [7]; using microchip devices [8,9]; atomic force microscopy [10] or thanks to DNA protection against exonuclease digestion [11,12]. Unfortunately, MutS also exhibits a weaker affinity for complementary DNA. Unlike other methods exploiting MutS binding for mutation detection, like filter binding assays, the complexes with fully complementary and mismatched DNA can be differentiated as they are observed as distinct bands [5]. Band shift assays were usually applied to investigate MutS properties, yet they were also employed to detect mutations in the cystic fibrosis gene [6]; the DNA fragments for these assays were labelled with radioactive isotopes. In our band shift assays, instead of labelling with radioactive isotopes DNA was stained in polyacrylamide gels with a sensitive fluorescent dye SYBR-Gold.

The examined samples came from patients with a family history of cancer and were selected according to mutation importance in carcinogenesis and occurrence in population. The c.5262_5263insC (also known as 5382insC) in *BRCA1* gene is reported to be one of the most common mutations in *BRCA1* gene (10.1% of all *BRCA1* mutations reported [13]). The c.657_661delACAAA mutation in *NBS1* gene (Slavic mutation) was identified in majority of Nijmegen breakage syndrome (NBS) patients [14,15]. Eng et al. [16] report that in 95% of multiple endocrine neoplasia type 2A (MEN 2A) families, the affected members carry a missense mutation of *RET* protooncogene in exon 10 at cysteine codons 609, 611, 618, 620, and in exon 11 at codon 634. Germline mutation predisposing to MEN 2B is located mainly in exon 16 at codon 918 [17] (Table 1).

2. Materials and methods

2.1. MutS protein

T. thermophilus his_6-MutS protein was isolated from overproducing *Escherichia coli* cells and purified by metal affinity chromatography followed by chromatography on ResourceQ FPLC 6 ml column (Pharmacia) [5].

2.2. DNA

The tested DNA fragments were PCR-amplified from the human genomic DNA from heterozygotic patients with a family history of cancer using the primers indicated in Table 1.

2.3. DNA retardation assay

The samples for DNA retardation were prepared in 50 μl mixtures in the retardation buffer (20 mM Tris–HCl pH 9.0; 10 mM KCl; 34 mM ammonium sulphate; 0.1% Triton X-100; 5 mM $MgSO_4$). PCR products were added directly without purification, as indicated in Fig. 1, heated to 92 °C for 10 min, then cooled to 62 °C for 20 min, and 22 °C for 5 min. His_6-tagged *Tth* MutS protein (0.5 μg/μl) was preincubated in 60 °C for 10 min in 20 mM Tris–HCl pH 9.0; 10 mM KCl; 10 mM $(NH_4)_2SO_4$; 0.1% Triton X-100; 5 mM $MgSO_4$, 2 mM ATP, then added to the DNA samples prepared as above, in the amounts indicated in the description of Fig. 1, followed by 10 min incubation at 60 °C. Twenty microlitres of each sample was subjected to electrophoresis at room temperature, in 6% polyacrylamide gel, in 89 mM Tris–borate buffer, pH 8.3 with $MgCl_2$ addition to 1 mM, at 10 V/cm for 3 h, followed by staining with SYBR-Gold