O-GENE DETECTION BY ALLELE SPECIFIC AMPLIFICATION IN THE ABO BLOOD GROUP SYSTEM

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Summary We have constructed a fast and easy method for the detection of *O*-gene in the ABO blood group system. Our method included allele specific amplification using polymerase chain reaction (PCR) and subsequent analysis with polyacrylamide gel electrophoresis (PAGE) and silver staining. *O*-gene product was observed on the gel in 25 DNA samples from group O phenotype and 20 DNA samples from group A or B (genotype *AO* or *BO*) persons. No product was found in 10 DNA samples from group AB phenotype.

Key Words ABO, blood group, *O*-gene, allele specific, PCR

INTRODUCTION

The ABO blood group system is reliable and informative polymorphism for the blood banking, transfusion, personal identification, and paternity test (Race and Sanger, 1975; Salmon *et al.*, 1984). However, we could not distinguish between *AA* (or *BB*) homozygote and *AO* (or *BO*) heterozygote by serologic analysis unless we could use family pedigree data.

Yamamoto *et al.* (1990a) reported nucleotide sequences of cDNAs that coded synthetic enzymes for ABO blood group phenotypes from human gastric cancer cell lines. Determination of ABO genotype from peripheral blood leukocytes was first presented by Yamamoto *et al.* (1990b). Several variations were developed by using PCR-RFLP method (Lee and Chang, 1992), allele specific PCR (AS-PCR) method (Ugozzoli and Wallace, 1992), and PCR-denaturing gradient gel electrophoresis (PCR-DGGE) method (Johnson and Hopkinson, 1992). Yamamoto's method was shown to be accurate in these three years. However, Yamamoto's and other methods mentioned above are complex as discussed later.

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We intended to develop a simple and accurate method for the determination of *O*-gene from peripheral blood leukocytes by allele specific PCR and polyacrylamide gel electrophoresis followed by silver nitrate staining.

MATERIALS AND METHODS

Preparation of DNA. Twenty-five group O, 20 group A or B (genotype *AO* or *BO*) and 10 group AB blood samples from unrelated Japanese were collected as EDTA treated blood. Genotype *AO* or *BO* was determined from family pedigree data. Genomic DNAs from these blood samples were extracted by SDS-proteinase K-phenol method (Sambrook *et al.*, 1989) and Chelex-100 method (Hochmeister *et al.*, 1991).

Preparation of primers for PCR. Two primers for the detection of *O*-gene on PCR were synthesized by Milligene Cyclone DNA Synthesizer M7500. The synthesized primers were cleaved from the synthesizer columns with fresh 28% aqueous ammonium, dried up in Eppendorf type tubes by centrifugal vaporizer, and resolubilized in minimum amount of TE buffer containing 10 mM Tris-HCl, 0.1 mM EDTA, pH 7.5. The sequences of the primers were 5'-GAATTCGGA-TGTCCTCGTGGTA-3' for a sense primer and 5'-AAGCTTGAGCCAGCCAAG-GG-3' for an anti-sense primer.

PCR reaction. Reaction mixture contained 10 mM Tris-HCl buffer pH 9.0, 1.5 mM MgCl₂, 50 mM KCl, 0.1 mM each dNTP, 4 nmol each primer, 5–100 ng genomic DNA, and 2.5 U Taq DNA polymerase from Pharmacia Biotech, Tokyo. The amount of genomic DNAs purified by Chelex-100 method was 5 to 15 ng and that by SDS-proteinase K-phenol method was 10 to 100 ng. Before addition of Taq DNA polymerase to the reaction mixture, the mixture added one drop of mineral oil was stood on boiling water bath for 5 min. After rapidly cooling down the mixture on salted ice, polymerase was added and PCR reaction was started on a programmable multiple temperature controller (Astec PC-700, Fukuoka). Thirty cycles of PCR were carried out under the conditions, denaturation: 95°C, 40 sec; annealing: 56°C, 40 sec; extension: 72°C, 30 sec adding one sec on the extension phase in each cycle. The PCR products were stored in a freezer before use.

Detection of PCR product. Five microliters of the PCR product were mixed with 2 µl of 50% glycerol, 0.1% bromophenol blue (BPB) and 0.1% xylene cyanol FF (XC) solution. The mixtures were electrophoresed on 15% polyacrylamide gel sized 8 cm × 7 cm with TBE-buffer containing 50 mM Tris, 48.5 mM borate, and 1 mM Na₃EDTA, and under 5–7 mA constant currency at room temperature until BPB came at the front of the gel. After electrophoresis, the gel was stained with silver nitrate by the method of Budowle *et al.* (1991).

RESULTS

O-gene product from all DNAs of the group O phenotype was stained on polyacrylamide gel at the same electrophoretic migration place as that of xylene cyanol FF (Fig. 1). No *O*-gene product was found on the gel from group AB phenotype DNAs. Heterozygote *AO* or *BO* DNAs also showed *O*-gene product the same as the group O DNAs (Fig. 2). *O*-gene product amplified from group O phenotype DNAs purified by Chelex-100 method was less in amount than those purified by SDS-proteinase K-phenol method (data not shown).

DISCUSSION

We developed a new simple method for the detection of *O*-gene by allele specific amplification. Our sense primer contained number 243 to 258 base sequence of *O*-gene cDNA reported by Yamamoto *et al.* (1990a) at the 3' end. The anti-sense primer contained number 260 to 273 base sequence common to *A*, *B*, and *O*-gene cDNAs at the 3' end. Our method uses a simple reaction mixture using a buffer system attached by Taq DNA polymerase manufacture and can detect the reaction product by polyacrylamide gel electrophoresis following non-isotopic silver staining. Our method requires only 4 hr to obtain results starting from

Fig. 1. Photograph showing the allele-specific amplification product of *O*-gene. Lanes 1-10, group O; lanes 11-14, group AB. Arrow shows the position where *O*-gene product and xylene cyanole FF appear.

Fig. 2. Photograph showing the AS-PCR product of *O*-gene from heterozygote *AO* or *BO*, and homozygote *OODNA*. Lanes 1-3, 8, 12, 13, *AO* genotype; lanes 4-6, 10, 11, *BO* genotype; lanes 7, 9, 14, *OO* genotype.

blood collection. When Chelex-100 method was carried out for preparation of genomic DNA, we can detect *O*-gene. However, the detected band from the DNA was weaker than that from the genomic DNA prepared by SDS-proteinase K-phenol method. This may be due to low concentration of the DNA and/or contamination of inhibitors of polymerase such as a heme-complex.

Ugozzoli and Wallace (1992) reported an allele specific PCR method. Their primers were overlapped at numbers 237–258 bases for a sense primer and 257–276 bases for an anti-sense primer from *O*-gene cDNA and they used a complex reaction mixture and a radioisotope for detection of the PCR product.

Yamamoto *et al.* (1990b) and Lee and Chang (1992) reported PCR-RFLP method. This method was used most popularly at this time. However, it needs two step enzyme reactions, namely, Taq DNA polymerase reaction and restriction enzyme digestion. These two step enzyme reactions make the PCR-RFLP a complicated method. Sometimes the second enzyme fails to digest the PCR product, and the method has a possibility of leading to a wrong conclusion.

PCR-denaturing gradient gel electrophoresis (PCR-DGGE) method (Johnson and Hopkinson, 1992) is a simple and good procedure for the detection of wide varieties of nucleotide base changes. However, preparation of the denaturing gradient gel is complicated and it takes 19 hr for electrophoresis. Because of these reasons, PCR-DGGE method will hardly become popular.

The allele specific (AS)-PCR method we developed has advantages such as simplicity to detect a single base change, a requirement of small amount of DNA, and an easiness for detection of PCR products. However, this method also has a disadvantage that we cannot always deny a base change when we find no PCR product. Generally no AS-PCR product meant (1) no base change (mutation) at the detection point on the target DNA, (2) denatured or fragmented DNA, (3) a denatured polymerase or contamination of polymerase inhibitors, or (4) an equipment trouble.

Our method can be applied to small fragmented DNA because we amplified only 43 base pairs. Our genomic DNAs used in this study seemed to have enough base length for the AS-PCR, since the DNAs had at least 130 base pairs that were detected from X-chromosome specific sequence according to the method of Witt and Erickson (1989) (data not shown). In addition, no discrepancy was found between serologic analysis data to ABO blood group and the AS-PCR results in this study. These show that this method can clear problems mentioned above as far as we use adequate controls. Our method may be useful to not only paternity tests but also to criminal investigations. The application data will be published elsewhere in a forensic journal.

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