METHODS

The New Technology of Hot Start Polymerase Chain Reaction

I. N. Rybalkin, N. M. Mukhamedova, T. N. Vlasik, and A. A. Chenchik

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The use of monoclonal antibodies against Taq DNA polymerase in the polymerase chain reaction is proposed. These antibodies effectively inhibit polymerase activity at temperatures <70°C, activity being restored upon DNA melting in the very first stage of amplification. As in the "hot start" reaction, the addition of the antibodies to the incubation medium markedly improves the reaction yield. The method is particularly effective for the identification of a few copies of DNA.

Key Words: *polymerase chain reaction; Taq DNA polymerase; hot start; monoclonal antibodies*

Amplification of primer dimers and nonspecific byproducts often occurs in the polymerase chain reaction (PCR). The formation of nonspecific products and their quantity depend on the primer sequence. Synthesis of nonspecific products is particularly frequent upon amplification against the background of complex (for example, genomic) DNA. The yield of specific PCR product decreases as a result of interference between the syntheses of specific and nonspecific products. The situation is often complicated by the fact that nonspecific products are close in size close to specific products.

The reason for the formation of nonspecific products is that at low temperatures oligonucleotide primers form partially complementary complexes with DNA, and DNA polymerase adds nucleotides to their 3' ends. The efficiency of thermophilic DNA polymerase is markedly reduced at temperatures below 50-55°C compared with its activity at optimal temperatures (72°C). However, even this lowered activity is sufficient to add several nucleotides to the primer, which leads to the amplification of nonspecific DNA fragments.

A hot start PCR was developed to solve the problem of nonspecific amplification [1,2]. One reagent (DNA polymerase, $MgCl_2$, primers, deoxynucleotide triphosphates, or DNA) is withheld until the reaction mixture has been heated to a high (>55°C) temperature. Then this reagent is added. The probability of partial complementary pairing of primers with DNA and with each other markedly decreases at high temperatures, and triggering of the reaction at these temperatures prevents elongation of these pairs. In another hot start PCR a wax barrier is employed to separate the reaction components [1,3]. At 55°C the wax melts, and the reagents mix with each other. The need to keep opening the sample tubes, which increases the likelihood of contamination [4], is a disadvantage of both techniques.

MATERIALS AND METHODS

We propose the use of monoclonal antibodies capable of inhibiting the activity of thermophilic polymerase at

Institute of Experimental Cardiology, Cardiology Research Center, Russian Academy of Medical Sciences, Moscow (Presented by V. N. Smirnov, Member of the Russian Academy of Medical Sciences)

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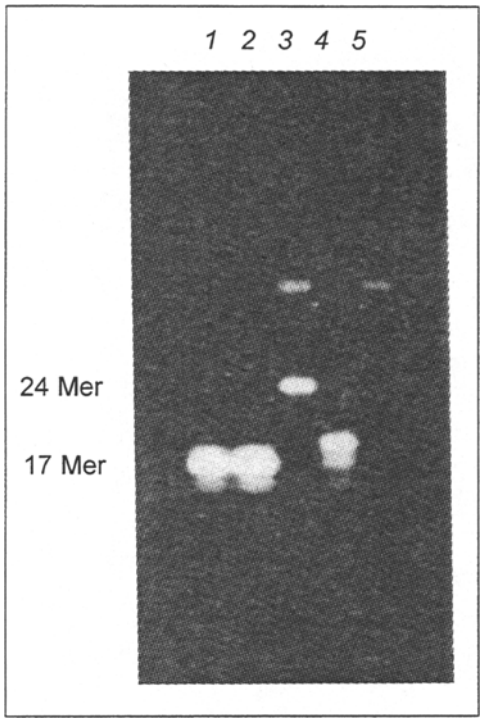

Fig. 1. Activity of Taq DNA polymerase at temperatures <72°C. *1*) initial primer; Taq DNA polymerase was added to the reaction mixture; *2*) at 95°C, *3*) at 0°C, after which the tube was placed in the amplifier heated to 95°C, *4*) at 0°C and incubated for 1 h, *5*) at 22°C and incubated for 1 h.

temperatures <65°C. When the reaction temperature is >70°C, the antibodies denature, and the enzyme regains its activity. In this case the amplification of non-

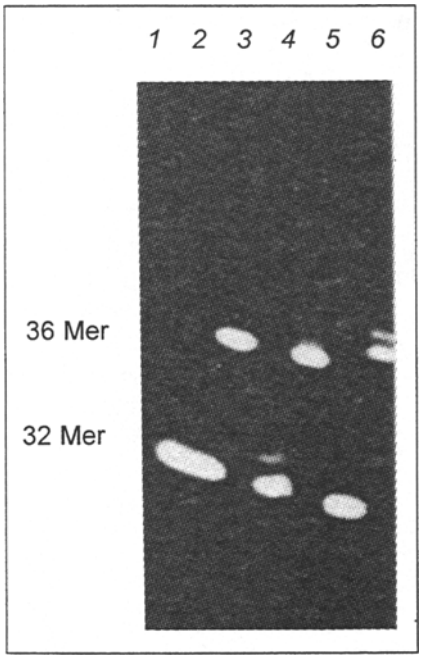

Fig. 2. Inhibition of Taq DNA polymerase by monoclonal antibodies at low temperatures: *1*) 8C1; *2*) 8F5; *3*) 9E6; *4*) 10C6; *5*) 12G1; *6*) without antibodies.

specific DNA fragments is inhibited, and the yield of specific PCR product increases.

RESULTS

Taq DNA polymerase is active at temperatures lower than 72°C. The Taq DNA polymerase used in this study was a cloned protein expressed by *E. coli*. According to SDS electrophoresis, the enzyme was 80% pure. The activity of Taq DNA polymerase at various temperatures was measured by elongation of M13 (17 Mer) sequence primer (labeled with ^{32}P at the 5' end) melted with a single-stranded DNA of bacteriophage lambda. The reaction mixture contained dATP, dGTP, and dCTP. Buffer conditions were standard for PCR. Elongation of the primer from 17 to 24 bases indicated the presence of polymerase activity. Discrete bands corresponded to the position of dTTP omitted from the reaction mixture. The reaction was terminated by the addition of saturated urea solution with stain. The samples were analyzed by electrophoresis with subsequent radioautography. During heating of oligonucleotide melted with single-stranded DNA in the presence of triphosphates (Fig. 1, track *3*) from 0 to 95°C, the polymerase elongated the primer and thus stabilized the primer-template complex. The heating rate was the maximal possible for a Perkin-Elmer amplifier. The polymerase activity was noticeable at the temperature range of 0-22°C (Fig. 1, tracks *4* and *5*) and was sufficient for the stabilization of partially complementary complexes. Thus, the polymerase was active in the whole range of temperatures at which mixtures for PCR are prepared.

The antibodies inhibit the activity of Taq DNA polymerase. The ability of antibodies produced by various hybridoma clones to inhibit Taq DNA polymerase was tested by elongation of M13 (32 Mer) sequence primer (labeled with ^{32}P at the 5' end) melted with a single-stranded DNA of bacteriophage lambda. The experimental conditions were similar to those described above. Taq DNA polymerase premixed with the antibodies was added to the reaction mixture. Incubation was carried out for 1 h at 41°C. The reaction mixture contained dATP, dGTP, and dCTP. Elongation of the primer from 32 to 36 bases indicated the presence of polymerase activity.

We obtained three hybridoma clones (8C1, 9E6, and 12G1) producing antibodies that inhibited Taq DNA polymerase. Antibodies 8F5 and 10C6 displayed no inhibitory activity (Fig. 2).

Antibodies 8C1 increase PCR sensitivity and decrease nonspecific amplification. The ability of the antibodies to improve PCR yields was demonstrated in a system consisting of successive dilutions of phage lambda DNA (template) and two primers. The

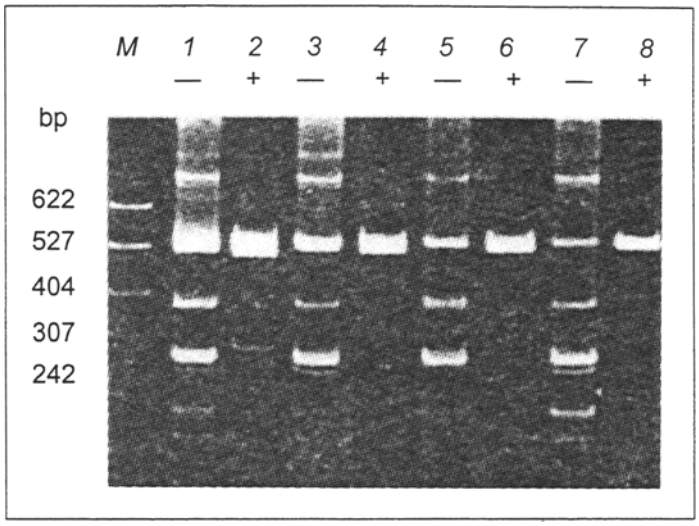

Fig. 3. Amplification of a small number of specific DNA copies in the presence of a complex genomic DNA: *1*, *2*) 1000 copies; *3*, *4*) 200 copies; *5*, *6*) 40 copies; *7*, *8*) 8 copies. *M* - pBR322/ MspI DNA markers. With (+) and without (-) antibodies. Ethidium bromide staining.

primers were chosen to provide amplification of the phage genome 530 bp long. Amplification was carried out in the presence of human genomic DNA providing the synthesis of nonspecific products. The DNA (100 ng) was mixed with the given number of phage lambda DNA copies; 1 μM phage lambda DNA primers (24 Mer), 200 μM each dNTP, and 0.25 unit Taq DNA polymerase with (+) and without (-) 8C1 antibodies (40 cycles, first step 94°C for 2 min) were then added. The amplification products were analyzed by electrophoresis in 12% polyacrylamide gel with ethidium bromide staining. Figure 3 displays the positive effect of the antibodies on the PCR yield. Large amounts of nonspecific products were synthesized in the absence of the antibodies. The nonspecific synthesis was almost completely inhibited by the antibodies when they were added to the reaction mixture prior to Taq DNA polymerase, and the yield of specific product markedly increased (particularly for a low number of copies, Fig. 3, tracks *7* and *8*). Thus, the use of monoclonal antibodies that inhibit the activity of Taq DNA polymerase is a reliable and simple technique of hot start PCR. The antibodies reduce nonspecific synthesis and increase the yield of specific product.

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