Selective PCR Amplification of Functional Immunoglobulin Light Chain from Hybridoma Containing the Aberrant MOPC 21-Derived Vκ by PNA-Mediated PCR Clamping

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Cloning and assembly of immunoglobulin variable regions from hybridomas is now a widely used technology to raise single-chain Fv (scFv) with predefined specificities. Many hybridomas are prepared with fusion partners derived from the mouse myeloma MOPC-21. MOPC-21 produces a normal kappa (κ) light chain and an aberrant one with a nonfunctional VJ recombination (MOPC abVκ, Accession No. M35669) (1). The P3-X63-Ag8.653 and Sp2/O fusion cell lines have been selected from MOPC-21 for their non-expression of the normal κ chain (9). However, MOPC-21 abVκ mRNA is still present in these cells. Sets of oligonucleotides designed to amplify variable regions cannot discriminate between the functional gene derived from the fused B cell and the MOPC-21 transcript. Many groups reported its preferential amplification by reverse transcription polymerase chain reaction (RT-PCR) when attempting to clone the light chain of hybridomas derived from B cells fused to one of these fusion partners (6,10,11,13,16).

We recently tried to clone the variable regions of the rat antibody 505.1, directed against the Escherichia coli Glucosamine 6-P Synthase (4). The 505.1 hybridoma was obtained by fusion of rat splenocytes with Sp2/O cells. The 505.1 variable regions of heavy chains (VH) and light chains (VL) were first PCR-amplified as previously described (12). Fragments were subsequently assembled to generate the VH(GGGGS)3-VL insert, which was then cloned into the pSW1 vector (18). All of the clones sequenced were found to contain the aberrant Vκ derived from MOPC-21.

Several methods have been proposed to avoid the cloning of the MOPC abVκ; however, they are labor-intensive or only moderately efficient (6,10,11,13,16). Here, we present an efficient and simple method to specifically inhibit the amplification of this aberrant gene with a peptide nucleic acid (PNA) matching its CDR3. PNAs are analogs of oligodeoxynucleotides (2,7,14) that form highly stable complexes with complementary DNA but cannot function as primers for DNA polymerases (15). We designed a PNA (H2N-CGTGTAAGCTCCCTA-H; PE Biosystems, Framingham, MA, USA [formerly PerSeptive Biosystems]) to specifically block the PCR elongation of the MOPC abVκ in conditions where the functional Vκ from the hybridoma would be amplified from cDNA. Criteria for designing the PNA were its high specificity based on the analysis of the Kabat database (8) and an elevated melting temperature (Tm) (66°C) determined as recommended by the manufacturer. Six nucleotides separate the carboxy-terminus of the PNA from the 3’ end of the antisense Vκ primer, a number that could be critical for PCR clamping (15) but that was nonetheless mandatory based on the two defined criteria.

![Figure 1. Inhibition of the aberrant MOPC-21 Vκ amplification by PNA. ScFv containing the MOPC abVκ and control scFvs (7.5.4, G1.9 and YMX) cloned in pSW1 were used as templates. PCR was performed in 20 µL with Stoffel buffer (10 mM Tris-HCl, pH 8.3, 10 mM KCl), 3 mM MgCl2, 250 µM dNTPs, 0.25 µM Vκ2Back and Vκ4For, 0–25 µM PNA, 5 pg of template DNA and 1.2 U of AmpliTaq DNA polymerase, Stoffel fragment. PCR conditions were 94°C for 2 min followed by 30 cycles (94°C for 20 s, 65°C for 30 s, 50°C for 30 s and 60°C for 30 s) and a final extension step of 60°C for 2 min. The 65°C step was performed to allow preferential annealing of the PNA. The extension step was kept at 60°C to avoid melting of the PNA from the DNA matrix during elongation.](image-url)
The conditions of PNA inhibition were first optimized with a plasmid carrying the aberrant MOPC-21 Vκ (Figure 1). When Taq DNA Polymerase (AmpliTaq®; PE Biosystems, Foster City, CA, USA) was used, only a partial PCR inhibition was observed (PNA concentrations: 2.5–25 µM) (not shown). Possibly, PNA was removed during elongation, due to the strand displacement activity of the enzyme.

By contrast, the AmpliTaq DNA Polymerase, Stoffel Fragment (PE Biosystems, Foster City) was more efficient than Taq DNA Polymerase, with an almost total inhibition of the MOPC ab Vκ amplification in presence of 2.5 µM of PNA. In the same conditions, amplification of 3 control Vκ was unaffected by PNA (Figure 1).

We then evaluated whether PNA inhibition could be used to clone the Vκ gene from the 505.1 hybridoma that contains the MOPC ab Vκ. We first cloned the 505.1 light chain using another approach: since the 505.1 monoclonal antibody (MAb) is a rat antibody, the amplification of the light chain was done with the Vκ2-Back primer (3) and with the primer Rat-Cκ For (5′-AGGATCCAGTTGCTACGTTCCG-3′), which specifically anneals to the rat Cκ domain and not to the mouse gene (not shown). The PCR product was re-amplified with the nested primer pair Vκ2Back/Vκ4For (3) with added restriction sites and cloned into pSW1. Sequencing revealed the presence of two variable light chains. One chain (505.1 abVκ) was nonfunctional due to the presence of two stop codons, the other chain (505.1 Vκ) was functional. Based on the 505.1 Vκ sequence, an oligonucleotide (CDRx505: 5′-TGTTAGAGGATTATCATATG-3′) specific for the CDR3 was designed for further PCR screening.

We then determined the amplification pattern of the Vκ domain with a cDNA prepared from the 505.1 hybridoma in the presence or absence of PNA. Two independent PCRs (reactions A and B) were performed with nested primers Vκ2Back/Vκ4For and with the 505.1 cDNA as template. PCR A was done in the absence of PNA and with Taq DNA Polymerase, PCR B was done in the presence of 10 µM PNA and with Stoffel fragment polymerase (see Figure 2 legend for details). PCR inserts A and B were independently ligated into the pSW1 vector. Fifteen clones from each ligation were first screened by PCR for the presence of a Vκ insert [with primers LMB3 and LMB2 (12)] and to allow specific Vκ identification using two pairs of primers (Figure 2). The CDRκ505 oligonucleotide or an oligonucleotide matching the CDR3 of MOPC ab Vκ (CDRxMOPC: 5′-CGTGTAAAGCTCCCTTCTAAT-3′), were used as antisense primers in combination with the sense primer LMB3. Almost all inserts (14/15) from PCR A cloning were typed as MOPC ab Vκ (Figure 2, Panel 2). By contrast, the MOPC ab Vκ gene was not cloned after PCR B in presence.
of PNA (Figure 2, Panel 5), while 8 out of 13 clones with an insert were shown to contain the 505.1 Vκ (Figure 2, Panel 6). Five clones out of thirteen had a Vκ insert different from 505.1 Vκ and MOPC abVκ; hence, it probably derived from 505.1 abVκ.

PNA-mediated PCR clamping was originally described to analyze point mutations (15,17). The method presented here shows the usefulness of PNA for scFv cloning from hybridomas. The only changes in usual scFv cloning protocols (12,18) involve the addition of 10 μM PNA in the RT-PCR cocktail and the use of Stoffel fragment polymerase with modified cycling conditions. This approach could be extended by designing PNAs matching the CDR3s of other known V regions whose mRNAs are transcribed by fusion partners [e.g., the rat κ-chain Y3 Ag 1.2.3 (5) and the mouse aberrant Vκ 45 T00.IL1 derived from MPC11 (19)]. It represents a versatile tool to improve the reliable cloning of the variable regions specifically produced by hybridomas.

REFERENCES

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