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SHORT COMMUNICATION

Nucleotide sequence of the variable region of the heavy and light chains of a monoclonal IgG antibody reactive to herbicides, terbutryn and prometryn

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GenBank L 41626, L 41627

Members of the triazine family of herbicides are reliable indicators of contamination of the ground water or soil with pesticide residues. To facilitate better detection of the chemical residues using improved immunoassay procedures, several monoclonal antibodies against triazine herbicides have been developed. K1F4 is a hybridoma secreting monoclonal (IgG) antibody reactive to terbutryn and prometryn, two members of the triazine family. We have cloned the genes encoding the variable regions of the heavy and light chains of this monoclonal antibody and report the nucleotide sequence here.

KEY WORDS: Antigen binding, ground water contamination, immunoassay, kappa light chain, pesticide, triazine

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine, CSA 1912-24-9) and its many analogs together constitute the triazine family of herbicides, selective inhibitors of photosynthesis in weed species. Triazines block the photosynthetic electron transport process in weeds by preventing the binding of plastoquinone to the quinone-binding protein, D-1 (formerly known as QB protein). Some higher plants are resistant to the action of triazines because of structural variations in the site where herbicides bind in competition with plastoquinone (Fedtke and Trebst, 1987). The high degree of physiologically-based target selectivity and consequent sense of safety of triazines to non-target species including animals and humans have led to excessive use of these herbicides throughout the world, over the past four decades.

Detectable levels of triazines in contaminated surface and ground waters have thus become reliable indicators of excessive use of pesticides in general. To facilitate their detection in contaminated water samples and the study of human exposure to these chemicals by immunochemical procedures, several hybridomas secreting monoclonal antibodies have been developed against triazines, and their cross reactivity patterns against different members of the triazine family characterized (Giersch and Hock, 1990; Karu *et al.*, 1991). The murine monoclonal antibody, AM7B2.1 (Karu *et al.*, 1991), has high affinity for atrazine, the type member of the triazine family of herbicides, and K1F4 (Giersch and Hock, 1990) has high affinity for terbutryn and prometryn, two other members of the triazine family. As part of our effort to delineate the molecular genetic and structural basis of the binding properties of antibodies to small molecules (haptens) of environmental importance, we embarked on the isolation and characterization of the immunoglobulin (IgG) heavy

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and light chain variable regions (V-regions) from anti-triazine monoclonal antibodies. Recently, we have reported the cloning, sequencing and bacterial expression of the Fab region (Ward *et al.*, 1993, 1995) from AM7B2.1. Here, we report the nucleotide sequence of the V-region chain (spanning into the CH1 domain) and of the heavy light-chain of the monoclonal IgG antibody, K1F4.

Immunoglobulin (IgG) genes were isolated as cDNA copies by PCR amplification, by using primers specific for the variable regions of the heavy- and light-chains of the K1F4 monoclonal antibody (Kreissig *et al.*, 1995; Ward *et al.*, 1995). PCR-amplified DNA was first cloned into the baculovirus transfer vector pAcUW51 (PharMingen, San Diego, CA), that was altered to contain a modified mouse heavy chain leader sequence after the p10 promoter and a modified mouse light chain leader sequence after the polyhedrin promoter (Kreissig *et al.*, 1995; Ward *et al.*, 1995a), and was then sequenced after subcloning into the plasmid, pTZ18R (USB, United States Biochemical, Cleveland, OH), by using standard methods (Ausubel *et al.*, 1993; Sanger *et al.*, 1980).

The nucleotide- and deduced amino acid sequences of the variable region of the K1F4 antibody heavy chain are shown in Figure 1. The nucleotide

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FR1>>                5                  10                  15
GAG GTG AAG CTG GTG GAG TCT GGG GGA GGC TTA GTG AAC CTT GGA GGG TCC
GLU VAL LYS LEU VAL GLU SER GLY GLY GLY LEU VAL ASN LEU GLY GLY SER

          20                  25                  30   CDR1>>
CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACT TTC AGT AGC TAT TAC ATG
LEU ARG LEU SER CYS ALA ALA SER GLY PHE THR PHE SER SER TYR TYR MET

35   FR2>>           40                  45                  CDR2>>
TCT TGG GTT CGC CAG ACT CCA GAG AAG AGG CTG GAC TTG GTC GCA GCC ATA
SER TRP VAL ARG GLN THR PRO GLU LYS ARG LEU ASP LEU VAL ALA ALA ILE

52  52A 53   55                  60                  65   FR3>>
AAC CCT AAT GGT GGT ATC ACC TAC TAT CCA GAC ACT GTG AAG GGC CGT TTC
ASN PRO ASN GLY GLY ILE THR TYR TYR PRO ASP THR VAL LYS GLY ARG PHE

          70                  75                  80   82  82A 82B
ACC ATC TCC AGA GAC AAT GCC AAG AAC ACC CTG TAC CTG CAA ATG AGC GGT
THR ILE SER ARG ASP ASN ALA LYS ASN THR LEU TYR LEU GLN MET SER GLY

    82C 83   85                  90                  CDR3>>
CTG AAG TCT GAG GAC ACA GCC TTG TAT TAC TGT GCA AGA CGA GAT TAT TAC
LEU LYS SER GLU ASP THR ALA LEU TYR TYR CYS ALA ARG ARG ASP TYR TYR

    100 100A 100B 100C 100D 100E 101   FR4>>  105                 110
AGT GGT GGC TAC GGA TAT CTC GAT GTC TGG GGC GCA GGG ACC ACG GTC ACC
SER GLY GLY TYR GLY TYR LEU ASP VAL TRP GLY ALA GLY THR THR VAL THR

          113 CH1>>                                             127
GTC TCC TCA GCC AAA ACG ACA CCC CCA TCT GTC TAT CCA CTG GCC CCT GGA
VAL SER SER ALA LYS THR THR PRO PRO SER VAL TYR PRO LEU ALA PRO GLY
                                                              144
TCT GCT GCC CAA ACT AAC TCC ATG GTG ACC CTG GGA TGC CTG GTC AAG GGC
SER ALA ALA GLN THR ASN SER MET VAL THR LEU GLY CYS LEU VAL LYS GLY
                                                  158
TAT TTC CCT GAG CCA GTG ACA GTG ACC TGG AAC TCT GGA TCC
TYR PHE PRO GLU PRO VAL THR VAL THR TRP ASN SER GLY SER
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Figure 1 Nucleotide- and deduced amino acid sequences of the V-region of the heavy chain of the monoclonal antibody, K1F4. The cDNA clones were isolated from K1F4 by PCR amplification procedures (Ausubel *et al.*, 1995) and sequences determined completely on both strands by using standard dideoxynucleotide termination reactions (Sanger *et al.*, 1980) containing 7-deaza dGTP, using [^{35}S]dATP as the label. The sequencing reactions were analyzed on 6% polyacrylamide wedge gels containing 8M urea. The primers synthesized for PCR amplification and additional internal sequencing primers were used in sequencing reactions. The comparative analysis of the cDNA sequences and deduced amino acid sequences was performed using the HIBIO MENU (HIBIO DNASIS and PROSIS) of the Hitachi Software Engineering America Ltd (Brisbane, CA). The complementarity determining regions 1, 2 and 3 (CDR1, CDR2, CDR3) are underlined, and the framework regions (FR1-FR4) and the constant CH1 region are as indicated. The mature amino acid sequence is numbered above the sequence by following the convention of Kabat *et al.* (1991).

sequence analysis revealed that the K1F4 heavy chain is a member of the IgG1 subgroup (Kabat *et al.*, 1991). Comparisons with known antibody amino acid sequences revealed significant homology between the heavy chains of the terbutryn-prometryn antibody K1F4 (this study), the triazine antibody AM7B2.1 (Ward *et al.*, 1993) and the dioxin antibodies DD1 and DD3 (Recinos III *et al.*, 1994); nevertheless, K1F4 and AM7B2.1 heavy chain sequences (Ward *et al.*, 1993) differed at several positions in the complementarity determining regions (CDRs) 1, 2, and 3, as reported elsewhere (Kreissig *et al.*, 1995).

The nucleotide- and deduced amino acid sequences of the kappa (κ) light chain of the antibody, K1F4, are shown in Figure 2. The K1F4 (IgG1, κ) light chain shows structural features typical of a light chain, e.g., the conserved cysteine residue at position 23 and CDR3 of 9 amino acids (Kabat *et al.*, 1991).

Knowledge of the structure-function relationship between DNA sequence and the antigen-binding site will help us to identify specific residues that determine specificity and cross-reactivity patterns of antibodies. Once identified, these residues can be changed to design antibodies with desired, previously unavailable specificities, that would have been impossible to produce by using conventional methods offered by polyclonal or monoclonal antibody technologies. Specially designed antibodies will be very useful in various applications such as enzyme immunoassays and biosensors (Choudary *et al.*, 1995). A judicious combination of the converging technologies, including the manipulation of antibody genes to achieve new properties, production of functional antibodies in various heterologous hosts, affinity purification using engineered antibodies and the development of increasingly sensitive immunoassay or biosensor procedures will certainly advance the field of small molecule analysis and have a significant impact on our efforts to apply this burgeoning technology in efficient detection and possible remediation of environmental contaminants (Choudary *et al.*, 1995; Kreissig *et al.*, 1995).

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FR1>>                5                  10                  15
GAC ATC CAG ATG ACC CAG CCT CCT GCT TCC TTA GGT GTA TCT CTG GGG CAG
ASP ILE GLN MET THR GLN PRO PRO ALA SER LEU GLY VAL SER LEU GLY GLN

          20                 CDR1>>    27  27A 27C 27D 27E 28      30
AGG GCC ACC ATC TCA TGC AGG GCC AGC AAA AGT GTC AGT ACA TCT GGC TAT
ARG ALA THR ILE SER CYS ARG ALA SER LYS SER VAL SER THR SER GLY TYR

                FR2>>                40                  45
AGT TAT AGT CAC TGC TAC CAA CAG AAA CCA GGA CAG CCA CCC CAA GTC CTC
SER TYR SER HIS TRP TYR GLN GLN LYS PRO GLY GLN PRO PRO GLN VAL LEU

          CDR2>>          55        FR3>>       60
ATC TAT CTT GCA TCC AAC CTA GAA TCT GGG GTC CCT GCC AGG TTC AGT GGC
ILE TYR LEU ALA SER ASN LEU GLU SER GLY VAL PRO ALA ARG PHE SER GLY

65                  70                  75                  80
AGT GGG TCT GGG ACA GAC TTC ACC CTC AAC ATC CAT CCT GTG GAG GAG GAG
SER GLY SER GLY THR ASP PHE THR LEU ASN ILE HIS PRO VAL GLU GLU GLU

          85                 CDR3>>                95          FR4>>
GAT GCT GCA ATC TAT TAC TGT CAG CAC AGT AGG GAA CTT CCT CTC ACG TTC
ASP ALA ALA ILE TYR TYR CYS GLN HIS SER ARG GLU LEU PRO LEU THR PHE

     100                 105       107
GGT GCT GGG ACC AAG CTG GAG CGT AAA
GLY ALA GLY THR LYS LEU GLU LEU LYS
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Figure 2 Nucleotide- and deduced amino acid sequences of the V-region of the light chain of the antibody, K1F4. Experimental dures and other details are as described in Figure 1 legend.

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