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Editing of Glutamate Receptor B Subunit Ion Channel RNAs by Four Alternatively Spliced DRADA2 Double-Stranded RNA Adenosine Deaminases

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Double-stranded (ds) RNA-specific adenosine deaminase converts adenosine residues into inosines in dsRNA and edits transcripts of certain cellular and viral genes such as glutamate receptor (GluR) subunits and hepatitis delta antigen. The first member of this type of deaminase, DRADA1, has been recently cloned based on the amino acid sequence information derived from biochemically purified proteins. Our search for DRADA1-like genes through expressed sequence tag databases led to the cloning of the second member of this class of enzyme, DRADA2, which has a high degree of sequence homology to DRADA1 yet exhibits a distinctive RNA editing site selectivity. There are four differentially spliced isoforms of human DRADA2. These different isoforms of recombinant DRADA2 proteins, including one which is a human homolog of the recently reported rat RED1, were analyzed in vitro for their GluR B subunit (GluR-B) RNA editing site selectivity. As originally reported for rat RED1, the DRADA2a and -2b isoforms edit GluR-B RNA efficiently at the so-called Q/R site, whereas DRADA1 barely edits this site. In contrast, the R/G site of GluR-B RNA was edited efficiently by the DRADA2a and -2b isoforms as well as DRADA1. Isoforms DRADA2c and -2d, which have a distinctive truncated shorter C-terminal structure, displayed weak adenosine-to-inosine conversion activity but no editing activity tested at three known sites of GluR-B RNA. The possible role of these DRADA2c and -2d isoforms in the regulatory mechanism of RNA editing is discussed.

RNA editing plays a critical role in the expression of certain gene products by changing the sequence of mRNAs, which results in synthesis of proteins not encoded in the gene sequence (44). One type of RNA editing involves the conversion of adenosine residues into inosine in transcripts of several cellular and viral genes (4). For instance, the diversity of glutamate receptor (GluR) ion channel subunits is dramatically increased by this A-to-I RNA editing (3, 9, 45). The resultant amino acid changes introduced by the editing lead to altered gating, ion conductance, ion permeability, and agonist-induced desensitization kinetics (7, 18, 22, 28, 48). At one editing site termed the Q/R site of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) GluR B subunit (GluR-B) and kainate GluR5 and GluR6 subunits, RNA editing alters the gene-encoded glutamine (Q) codon CAG to CIG, which codes for an arginine (R) (46). RNA editing occurring at the position termed the R/G site of three AMPA receptor subunits, GluR-B, -C, and -D, alters an arginine (R) codon AGA to a glycine (G) codon IGA (28). The A-to-I RNA editing requires the double-stranded RNA (dsRNA) structure formed between the exonic editing site and the downstream intron sequences (13, 15, 17, 28) and has been proposed to be carried out by dsRNA-specific adenosine deaminase activities, which were collectively designated DRADA or dsRAD in the past (32, 41, 51). The biochemical purification of the first member of this class of enzyme led to identification and cloning of DRADA1 (previously referred to as DRADA) (21, 34). The deduced primary structure of DRADA1 revealed a nuclear localization signal, three repeats of a dsRNA binding motif (DRBM), and the presence of sequences conserved in the catalytic center of other deaminases. Recombinant protein products of DRADA1 were tested recently for the site-selective editing of GluR RNAs in vitro (11, 29) and in vivo (15). The Q/R site of kainate receptor GluR6 was found to be edited to some extent by recombinant DRADA1 proteins expressed in a human embryonic kidney cell line, HEK 293 (15). In contrast, in vitro studies indicated that the Q/R site of AMPA GluR-B transcripts was not edited by recombinant DRADA1 proteins alone, although they efficiently edit an intronic "hot-spot" adenosine known to be modified also in vivo in mouse brain (11, 29). These previous studies on DRADA1 have indicated that there may be a separate DRADA1-related enzyme with a different substrate RNA specificity.

To this end, we searched for the DRADA1-related genes through human expressed sequence tag (EST) cDNA databases, which might exhibit different selectivity for the editing sites. We identified a new DRADA-like gene (DRADA2), of which expression is detected in many human tissues including brain. Characterization of DRADA2 cDNA clones isolated from human hippocampus and cerebellum libraries has revealed the presence of at least four different isoform mRNAs generated by differential splicing. Among these isoforms, DRADA2a, -2b, -2c, and -2d, the DRADA2a isoform has the highest sequence homology to the recently cloned rat RED1 (31). When tested for editing in vitro, the DRADA2a and -2b isoforms edited GluR-B RNA at the Q/R site very efficiently, as reported originally for RED1 (31). As with DRADA1, the DRADA2a and -2b isoforms edit also the intronic +60 site as well as the R/G site of GluR-B. In contrast to the DRADA2a and -2b isoforms, DRADA2c and -2d, which contain a distinct short C-terminal sequence, displayed very little A-to-I conversion and essentially no GluR RNA editing activity at all. These DRADA2 isoforms may be the RNA editing enzymes for RNAs of new target genes yet to be identified. Alternatively, they may act as regulators of the remaining two functional enzymes, DRADA2a and -2b.

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MATERIALS AND METHODS

Oligonucleotides. The following oligonucleotides used for reverse transcription (RT), PCR, primer extension assay, in vitro mutagenesis, and DNA sequencing were synthesized by DNAgency (Malvern, Pa.) and, if necessary, purified in 20% acrylamide–7 M urea gels. All DRADA2 oligonucleotides correspond to the human sequence. The nucleotide positions indicated in parentheses are based on the DRADA2b sequence. The oligonucleotides are as follows: HbUPIF, 5′-ATAAGAAT**GCGGCCGC**TATAAACATGGCTGACTACAAGG ACGACGATGACAAGGATATAGAAGATGAAGAAAAC (nucleotides [nt] 507 to 527); HbDW1, 5′-GATGGATCTTTTTT**TGATCA**TC (nt 1668 to 1688). The FLAG epitope-tag sequence is underlined. All GluR-B oligonucleotides correspond to the murine sequence. The nucleotide positions indicated in parentheses are relative to the Q/R site or the R/G site, which was assigned as position 0 (17, 28). RTBin, PCBex, EXBex, and EXBin, used for analysis of the in vitro-edited RNAs at the Q/R and +60 sites, have been described previously (11). Other GluR-B oligonucleotides used were as follows: B13UP, 5′-GTGGA CTTATATGAGGAGTGC (nt −197 to −217); B13DW, 5′-GTCCAACAGGC CTTGTTCAT (nt 896 to 915); B13Xba, 5′-GC**TCTAGA**GTACATCGAGCAGA GGA (nt −100 to −82); B13NotIm, 5′-TCGAGCGGCCGTCAGTGTGATGG (nt 340 to 362); B13ΔECS, 5′-GTTAAGAGTCTTAAAGACAGTAGGTGGGATAC TATAAC (nt 36 to 80); EXB13in, 5′-CATTGAGCATATTGTTATACTATTCC ACC (nt 5 to 33). All restriction sites within the oligonucleotides are shown in boldface type. For primer extension experiments, the oligonucleotides were labeled with [γ-^{32}P]ATP (6,000 Ci/mmol; Amersham, Arlington Heights, Ill.) and T4 polynucleotide kinase (Pharmacia, Piscataway, N.J.) as described previously (42).

Screening of database and isolation of overlapping DRADA2a, DRADA2b, and DRADA2c cDNA clones. Partial sequences displaying similarity to human DRADA1 (21, 34, 37) were identified by searching two databases of human cDNA ESTs, using the amino acid sequences of DRADA1 for the deaminase domain (amino acids [aa] 800 to 1226). A 0.6-kb insert of one of the EST cDNA clones, ES0162 (10), was used as a library screening probe. The probe was labeled with ^{32}P by random priming (42). The plaque lift was done with GeneScreen Plus Colony/Plaque Screen hybridization transfer membranes (DuPont, Boston, Mass.) as described previously (42). The membranes were incubated with a hybridization solution containing 1% sodium dodecyl sulfate (SDS), 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 10% dextran sulfate, 50% formamide, 2.5× Denhardt's solution, and 2×10^6 dpm of probe per ml at 42°C overnight, then washed with 2× SSC at room temperature for 10 min, and further washed with 2× SSC–1% SDS at 42°C for 20 min twice. Overlapping clones were isolated by hybridization screening of a randomly primed human hippocampus cDNA library (Clontech, Palo Alto, Calif.) constructed in phage λgt10 and also a human cerebellum cDNA library constructed in phage λZAP (Stratagene, La Jolla, Calif.). Inserts of cDNA clones were subjected to multiple rounds of sequencing in both directions.

Determination and analysis of the cDNA sequence. DNA sequencing was performed on an ABI377 DNA sequencer. The overlapping sequences were aligned and combined with the Fragment Assembly program (12). Alignments of different DRADA2 members were determined with the PILEUP, BESTFIT, and GAP programs, and identification of various protein sequence motifs was done with the MOTIFS program (12). A homology search of the National Center for Biotechnology Information sequence database was performed with the FASTA and BLAST programs (2).

GluR-B plasmids. Plasmid pTA-B11 (previously referred to as pTA-B mini) contains a 754-bp fragment covering part of exon 11 and intron 11 of the mouse GluR-B gene, which is essential for the Q/R site and intronic +60 site editing (11). Plasmid pTA-B13 contains a 1,133-bp fragment of the mouse GluR-B gene, covering exon 13, intron 13, and exon 14, that is essential for the R/G site editing as described previously (28). The insert of this plasmid pTA-B13 was prepared by PCR amplification of mouse genomic DNA extracted from BALB/3T3 cells with B13UP and B13DW primers. The insert was ligated into pCRII vector (Invitrogen, San Diego, Calif.) by the TA cloning procedure.

DRADA2 expression constructs. The cDNA clone Hb2 containing the 5′ end region of DRADA2 was merged with the clones Hb8 and Hb15 at the *Bcl*I site (nt 1670) to generate two full-length DRADA2a-type and DRADA2b-type plasmids in pBluescript KSII+ vector (Stratagene), respectively. A FLAG epitope-tag sequence (18) was introduced into their N termini by PCR amplifying a 1.1-kb 5′ end fragment of Hb8, using HbUPIF and HbDW1 as a pair of primers and replacing the original 1.1-kb *Not*I/*Bcl*I (nt 1 to 1670) region of the two pBluescript clones with the PCR products. The resultant clones, termed pBS-IF-DRADA2a and pBS-IF-DRADA2b, respectively, both contain a new Kozak (25) sequence that is strongly preferred by baculovirus for protein translation initiation (35) at the new N-terminal region. The region amplified by PCR was confirmed by sequencing. Their *Mlu*I/*Xba*I fragments (nt 2421 to 3366) were further replaced by an *Mlu*I/*Xba*I fragment from Hb6 clone, which contained a short C-terminal sequence, to generate both short-COOH isoforms of DRADA2, termed pBS-IF-DRADA2c and pBS-IF-DRADA2d. The *Not*I/*Xba*I inserts (nt 1 to 3366) of these four pBluescript clones were shifted to pVL1392 vector (PharMingen, San Diego, Calif.) containing a baculovirus polyhedrin promoter, resulting in four expression constructs, pVL-F-DRADA2a, pVL-F-DRADA2b, pVL-F-DRADA2c, and pVL-F-DRADA2d. Production of recombinant baculoviruses, preparation of total cell extracts from infected Sf9 cells, and purification of recombinant proteins on an anti-FLAG antibody M2 affinity column were carried out as described previously (11, 26). The yield and purity of fractionated recombinant proteins were determined by electrophoresis on an SDS–10% polyacrylamide gel followed by silver staining. The presence of recombinant proteins was further confirmed by Western blotting analysis with an anti-FLAG antibody (Kodak) as described previously (11).

Site-directed mutagenesis. By using the Transformer Site-Directed Mutagenesis Kit (Clontech) with a selection primer (B13NotIm) and a mutagenic primer (B13ΔECS), a mutant B13Δ construct was made. In order to delete the editing site complementary sequence (ECS), present within intron 13 and essential for R/G site editing, pTA-B13 was used as a starting plasmid. A *Not*I site present in the vector was chosen as a selection site. The mutation site was confirmed directly by sequencing.

RNA synthesis. pTA-B11, linearized with the restriction enzyme *Spe*I, and pTA-B13, linearized with the restriction enzyme *Xho*I, were each transcribed at 37°C by 20 U of T7 RNA polymerase or SP6 polymerase (Promega, Madison, Wis.), for 1 h in 40 mM Tris-HCl (pH 7.5)–6 mM $MgCl_2$–2 mM spermidine–200 μg of bovine serum albumin per ml–10 mM dithiothreitol (DTT). In order to monitor the RNA synthesis and also to estimate the yield, 2 μCi of [α-^{32}P]ATP (400 Ci/mmol; Amersham) was included in the reaction mixture in addition to 500 μM (each) GTP, CTP, and UTP and 250 μM ATP. The template DNAs were eliminated by treatment with RNase-free DNase I (Boehringer Mannheim, Indianapolis, Ind.) in the presence of RNasin (Promega) as described previously (11).

In vitro RNA editing assay. Editing of a synthetic GluR-B B11 or B13 RNA was assayed in vitro with purified recombinant DRADA2 isoforms as well as DRADA1 proteins. The standard editing reaction mixture contained 20 fmol of a synthetic B11 or B13 RNA substrate, 5 ng of recombinant DRADA2 or 10 ng of DRADA1 proteins, 0.02 M HEPES (pH 7.0), 0.1 M NaCl, 10% glycerol, 5 mM EDTA, 1 mM DTT, and 250 U of RNasin (Promega) per ml. The reaction mixtures were incubated at 30°C for various times. The RNA was recovered by proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation. The final RNA pellet was dissolved in diethyl pyrocarbonate (DEPC)-treated double-distilled H_2O and stored at −70°C.

Dideoxyoligonucleotide-primer extension assay. Approximately 10 fmol of ^{32}P-labeled extension oligonucleotide EXBex (Q/R site analysis), EXBin (+60 site analysis), or EXB13in (R/G site analysis) was mixed with 5 fmol of in vitro-edited GluR B11 or B13 RNA, heated to 70°C for 10 min, and annealed at 55°C for 2 to 4 h in 1× RT buffer (Boehringer Mannheim) including 10 μM (each) dATP, dCTP, and dGTP and 250 μM dideoxy-TTP in a 9-μl volume. The primer-annealed RNA was then reverse transcribed with 1 U of avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim) at 42°C for 45 min. The reactions were stopped by the addition of 4 μl of a formamide loading buffer. Primer-extended DNAs were fractionated on a 15% polyacrylamide–8 M urea gel. The ratio of the edited and unedited GluR-B RNAs was estimated by quantifying the radioactivity of the primer-extended product with a PhosphorImager system (Molecular Dynamics, Sunnyvale, Calif.).

RT-PCR amplification and cDNA sequencing of the in vitro-edited RNAs. The in vitro-edited B11 RNA was analyzed by RNA template-specific PCR as described previously (11). The B13 RNA was analyzed similarly, except for the usage of a specific RT primer, B13DW, and the PCR primers B13Xba and B13DW. The PCR-amplified DNA fragment (1,021 bp) was digested with *Xba*I and *Hin*dIII, and a 386-bp *Xba*I/*Hin*dIII fragment purified on an agarose gel was cloned into the *Xba*I and *Hin*dIII sites of the pBluescript KSII+ vector (Stratagene). The sequence of the entire B11 or B13 region of cDNA inserts of randomly selected clones was determined with two sequencing primers, the M13 reverse for the B11 region (11) and the T7 primer for the B13 region.

Northern blotting analysis. Northern blots containing 2 μg of poly(A)$^+$ RNA from various human fetal and adult tissues (Multiple Tissue Northern Blots) were obtained from Clontech. Northern blot hybridization was carried out in buffer containing 5× SSC, 2.5× Denhardt's solution, 50% (vol/vol) formamide, 7% (wt/vol) dextran sulfate, 0.1% SDS, 1 mM EDTA, denatured salmon sperm DNA (0.1 mg/ml), and 1×10^6 dpm of probe per ml at 42°C overnight. The membranes were washed with 0.2× SSC–0.1% SDS at 50°C for 30 min twice.

S1 nuclease mapping. Total RNA (20 μg) extracted from HEK 293 and HeLa cells was analyzed by the S1 nuclease mapping procedure (5). 5′-end labeling of the probe DNA was carried out with [γ-^{32}P]ATP (6,000 Ci/mmol; Amersham) and T1 polynucleotide kinase (Pharmacia), whereas 3′-end labeling was done with [^{32}P-]dCTP (3,000 Ci/mmol; Amersham) and the Klenow fragment of *Escherichia coli* DNA polymerase I, as described previously (42). The ^{32}P-labeled DNA probe was heat denatured, hybridized in 80% formamide to total RNAs at 55°C for 10 h, digested with 80 U of S1 nuclease, and analyzed by electrophoresis on an 8 M urea–4% polyacrylamide gel.

Nucleotide sequence accession numbers. The accession numbers for the human DRADA2a, DRADA2b, and DRADA2c sequences reported in this paper are U76420, U76421, and U76422, respectively.

RESULTS

Isolation of DRADA2 cDNAs. In order to identify DRADA-related gene family members, we searched for the genes con-

taining a region highly homologous to the DRADA1 deaminase domain (21, 34, 37) through human EST databases, one available at GenBank and also one developed by Human Genome Sciences (1). Candidate cDNA clones, which contained sequences similar to that of the human DRADA1 deaminase domain (aa 800 to 1226), were obtained. The inserts of promising cDNA clones were used as probes to screen several human-brain-tissue-specific cDNA libraries to isolate additional overlapping cDNA clones. The presence of a DRBM (47) was then used as the second criterion to select a true DRADA-related gene, which resulted in the identification of DRADA2 (Fig. 1).

Northern blot analysis detected DRADA2 transcripts as a major 8.6-kb mRNA and several minor shorter-size bands, including a fetal-liver-specific 6.0-kb mRNA and a 1.9-kb mRNA (Fig. 2A, upper panels). DRADA2 mRNAs are expressed ubiquitously in all adult (data not shown) and fetal (Fig. 2A, upper panels) tissues, similar to the even distribution of DRADA1 mRNAs (Fig. 2A, lower panels). Since the coding and 5′ untranslated regions of DRADA2 are approximately 2.1 and 0.4 kb, respectively (see below), we anticipate that the major 8.6-kb mRNA contains a long 3′ untranslated region (~6 kb) with several alternative polyadenylation sites. In fact, the 3′ ends of several cDNA clones contained a polyadenylation tract preceded by a canonical polyadenylation signal sequence AATAAA (38) 16 nt upstream of the polyadenylation site (Fig. 1A). However, the total sizes of those cDNA clones are only approximately 5 kb or less in length, suggesting that there must be a major polyadenylation site 3′ to the site already identified in the isolated cDNA clones. It is currently not known how the 1.9-kb mRNA is related to the longer 8.6-kb mRNA, since it is too short to account for the DRADA2 protein coding sequence.

Four DRADA2 isoforms resulted from alternate splicing. The nucleotide sequence of 5,035 bp corresponding to the longest overlapping cDNA sequence which contained a short 5′ untranslated region (413 bp) and a 3′ untranslated region (2,396 bp), including a polyadenylation signal AATAAA (nt 5104), was determined (DRADA2b in Fig. 1A). While we were characterizing original EST cDNA clones as well as additional cDNA clones isolated from two human cDNA libraries, we noted the presence of at least three different cDNA clones that appeared to be derived from alternatively spliced DRADA2 mRNAs (Fig. 1A). These three DRADA2 isoforms, DRADA2a, -2b, and -2c, share an identical N-terminal half of the coding region where bipartite (aa 52 to 68) and classical (aa 68 to 72) nuclear localization signals (14) and the RNA binding domain are identified (Fig. 1A and C). The RNA binding domain of DRADA2 contains only two repeats of dsRNA binding motifs, which is a major structural difference from DRADA1 with three DRBMs (21, 34, 37). The deaminase domain and the C-terminal region of the DRADA2a isoform have a sequence most similar to that of DRADA1 as well as other deaminases (21, 34, 37). The DRADA2b and DRADA2c isoforms, however, contain an additional 40-aa stretch in the middle of the core deaminase region (26), which has a high degree of homology to the *Alu* cassette insert, the in-frame *Alu* element found in the protein-coding exons of many human genes (30). This *Alu* segment appears to be recognized as a part of the intron sequence and is spliced out in the mRNA for the DRADA2a isoform (Fig. 1A and B).

In addition to the presence of this extra *Alu* segment, the C-terminal structure of DRADA2c is different from that of the DRADA2a and -2b isoforms, in that it lacks the last 29 aa residues but instead contains a very short 2-aa C-terminal sequence. We found this same short C-terminal sequence of DRADA2c in the 3′ untranslated region (1,381 bp downstream of the stop codon) of several DRADA2b-type cDNA clones (Fig. 1A). Thus, the short C terminus of DRADA2c most likely arises once again from an alternative splicing event, which recognizes the long C-terminal sequence of the DRADA2a and -2b isoforms as a part of an intron, as schematically shown in Fig. 1B.

Among four cDNA clones analyzed thoroughly, two clones contained the *Alu* cassette insert (Hb6 and Hb15) and three clones contained the long C terminus (Hb8, Hb15, and Hb17), whereas one clone contained the short C-terminal structure (Hb6). There could be an additional isoform, DRADA2d, resulting from alternative splicing, which contains no *Alu* cassette insert but contains the short C-terminal structure (Fig. 1A and B), although we did not find this particular splicing pattern among the cDNA clones isolated and characterized. In order to evaluate the expression ratio of all of these DRADA2 isoforms, including the fourth hypothetical isoform DRADA2d, quantitative S1 mapping analysis for RNAs extracted from human HEK 293 and HeLa cells was carried out with specific probes, which allows us to estimate the ratio of different DRADA2 mRNA populations (Fig. 2B). We found that both HEK 293 and HeLa cells express approximately a 1:4 ratio of DRADA2 mRNAs with and without the *Alu* cassette insert. To our surprise, a large fraction of DRADA2 mRNAs expressed in both cell lines was found to contain the short C-terminal structure: the ratio of the short-to-long COOH-type DRADA2 mRNAs was 3:2 (Fig. 2B). These quantitative S1 mapping results suggest that HEK 293 and HeLa cells express DRADA2d-type mRNA, in fact, as a major type. We estimated that the DRADA2a-, -2b-, -2c-, and -2d-type mRNAs are present in a 3:1:1:5 ratio in HEK 293 and HeLa cells. Sequence comparison of DRADA2 DNA sequences in the GenBank DNA sequence database suggested that DRADA2 is a human homolog of the recently reported rat RED1 (31). However, RED1 contains a 10-aa stretch insert exactly at the place where the *Alu* cassette insert was found in human DRADA2b and DRADA2c. The C-terminal structure of rat RED1 is almost identical to the long-COOH sequence of the DRADA2a or -2b isoform (Fig. 1C). Overall, the protein structure arrangement of the DRADA2a isoform resembles most closely the rat RED1, with 97% sequence similarity.

Distinctive RNA editing site selectivity of DRADA2. All recombinant enzymes (Fig. 3A), carrying an epitope-tag peptide FLAG (18) at the N terminus, were produced in Sf9 insect cells as described previously (11, 21, 26). FLAG-epitope-tagged DRADA1 was also prepared for comparison of its enzymatic characteristics to those of DRADA2. When a long c-*myc* dsRNA (575 bp) was used as a substrate RNA for an A→I base modification assay, both DRADA2a and DRADA2b converted many adenosine residues into inosines very efficiently (Fig. 3B) as did DRADA1 (11). Both DRADA2a and DRADA2b displayed adenosine deaminase activities almost identical to those of DRADA1 (0.002 U/mol). In contrast, DRADA2c and DRADA2d with a short C-terminal sequence exhibited 5 to 10 times lower levels of the A→I deamination activity (0.0002 to 0.0004 U/mol) (Fig. 3B).

We then investigated the RNA editing site selectivity of DRADA2a in detail, especially in comparison to that of DRADA1. Two GluR-B gene transcript fragments, B11 RNA (745 nt), encompassing the Q/R site within exon 11 and part of intron 11, and B13 RNA (1133 nt), encompassing the R/G site within exon 13, intron 13, and part of "flop" exon 14, were used as in vitro RNA editing substrates (Fig. 4). The RNA editing site selectivity of the two DRADA enzymes was first tested for three major editing sites of GluR-B RNA by an in vitro RNA

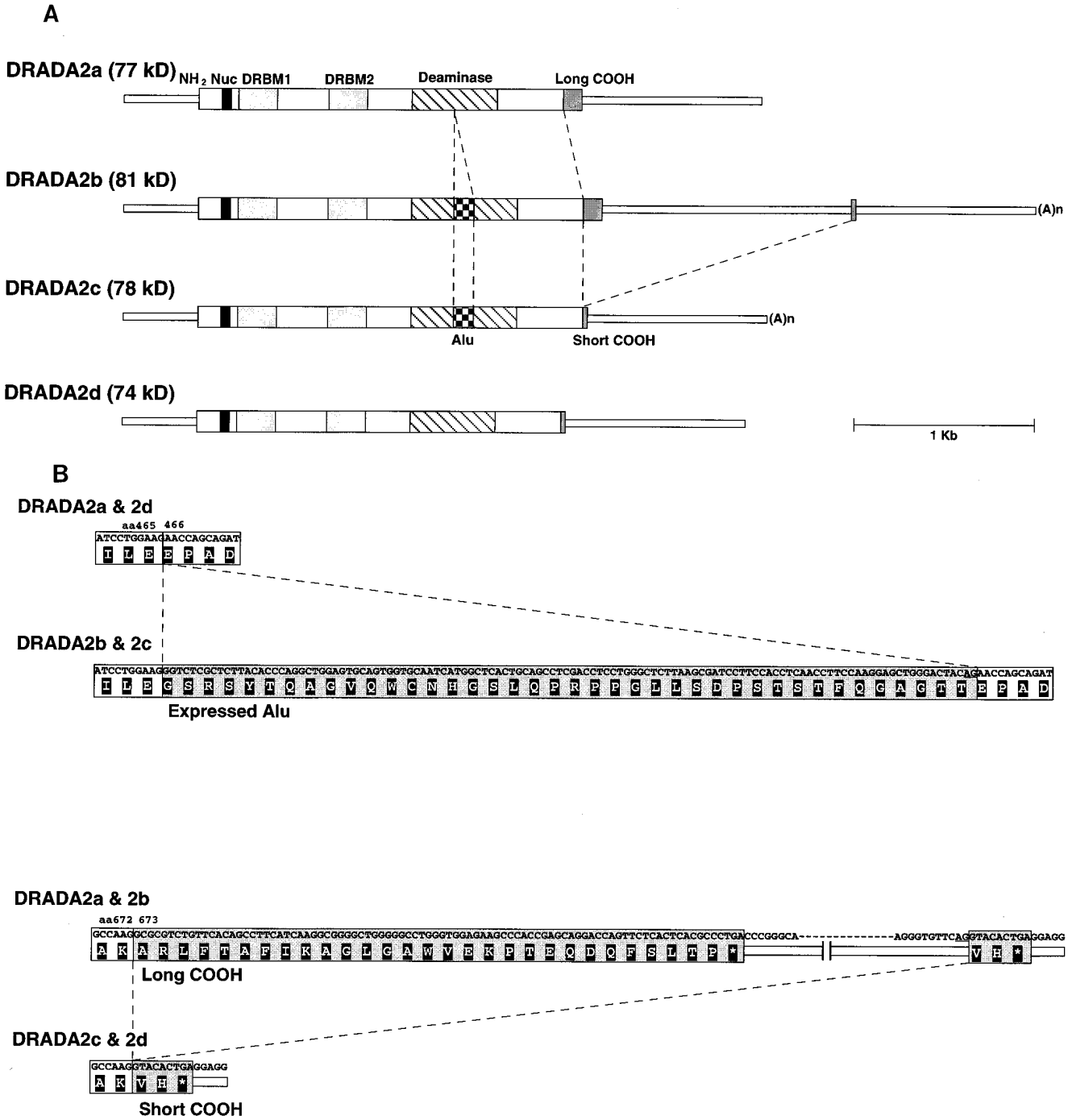

FIG. 1. Differentially spliced isoforms of DRADA2. (A) The structures of four different isoforms of DRADA2 mRNAs and proteins are schematically shown. The coding regions are indicated by thick boxes, while 5′ and 3′ untranslated regions are indicated by thin boxes. The putative nuclear localization signal (Nuc, filled box), two DRBM repeats (lightly shaded boxes), the putative deaminase domain (hatched box), the expressed *Alu* cassette insert (checkered box), and long and short C-terminal regions (dark shaded boxes) are indicated. The deduced open reading frames of DRADA2a, -2b, -2c, and -2d encode 701-, 741-, 714-, and 674-aa proteins with calculated molecular masses of 77, 81, 78, and 74 kDa, respectively. (B) The *Alu* cassette insert and alternate C-terminal regions. The nucleotide sequence as well as the amino acid sequence corresponding to the expressed *Alu* cassette insert and two alternative long and short C termini (shaded boxes) is shown. The amino acid sequence near the alternative splicing junction is also shown. Two nucleotides, AG, which are present in the 3′ end of the insert and could act as a splicing acceptor consensus sequence, are underlined. (C) Amino acid sequence alignment of the human DRADA2a isoform and rat RED1. Bipartite (aa 52 to 68) and classical (aa 69 to 72) nuclear localization signal (Nuc) sequences are indicated by thick overlining. Within the putative deaminase domain of DRADA2a, there are 13 stretches of amino acid residues (boxed) conserved also in the deaminase domain of DRADA1. Among these core deaminase residues, 4 aa previously identified to be critical for the A-to-I deamination activity of DRADA1 by site-directed mutagenesis (26) are marked by filled circles. Note that the *Alu* cassette insert (40 aa) present in DRADA2b and -2c but absent in the DRADA2a and -2d isoforms is replaced with a stretch of 10 aa residues in rat RED1 (GenBank accession number, U43534).

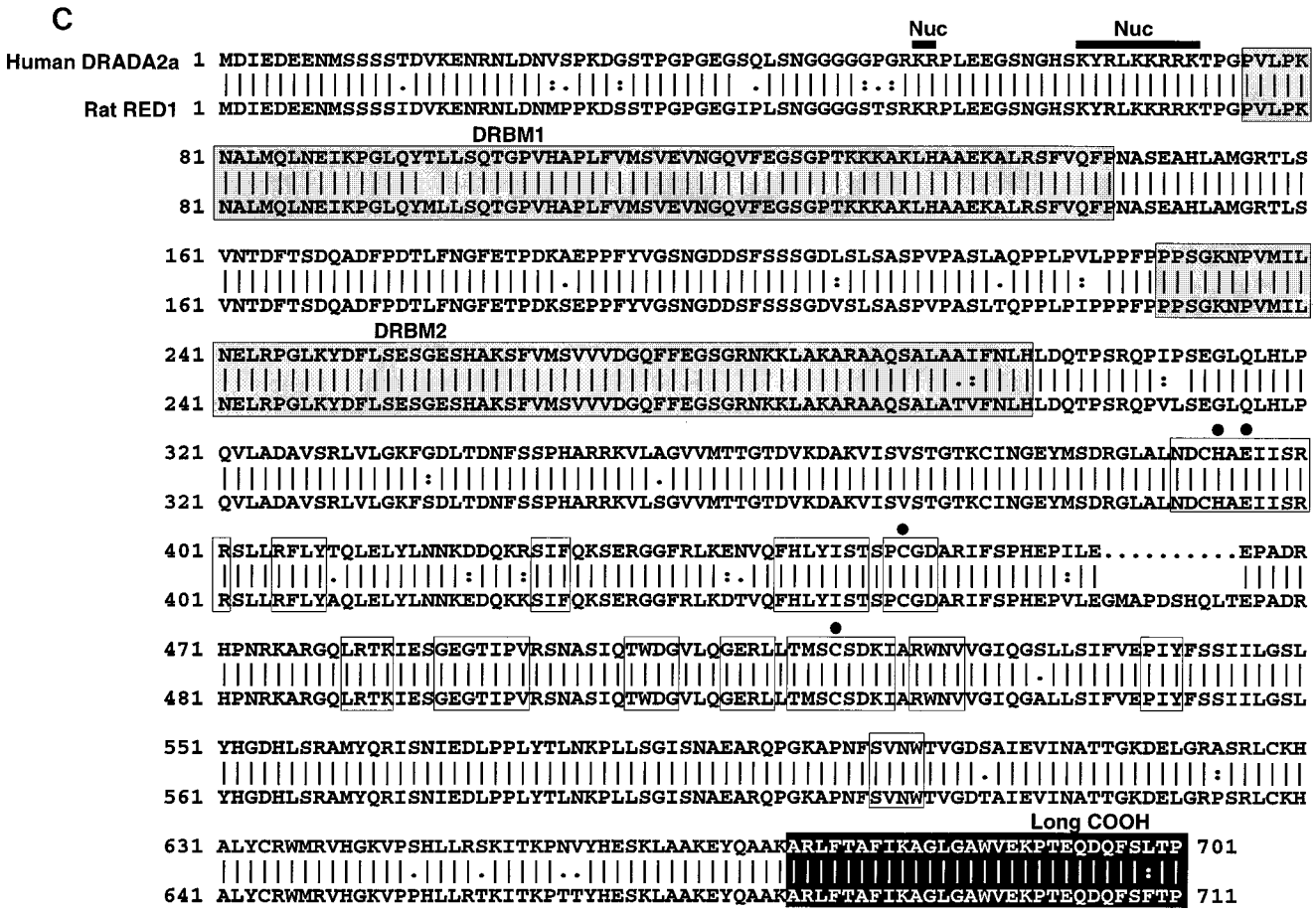

FIG. 1—*Continued.*

editing assay and subsequent dideoxynucleotide-primer extension analysis of in vitro-edited RNAs (11). We found that DRADA2a can carry out the site-selective RNA editing of GluR-B RNA at the Q/R site very efficiently, reaching 80% after only a 30-min incubation (Fig. 5A). DRADA1 can barely edit at the Q/R site by itself, as we reported previously (11), even after prolonged incubation (Fig. 5A). One more exonic (+4) and four more intronic (present in the B11 region) sites are also known to be edited in mouse and rat brains (13, 17). We carried out an additional dideoxynucleotide-primer extension experiment to monitor one of these intronic sites (+60 site) which, we and others found previously, is edited very efficiently by DRADA1 (Fig. 5A) (11, 29, 31). DRADA2a also edited this intronic site, but less efficiently. The R/G site present in the B13 region was edited by both DRADA2a and DRADA1 (Fig. 5A). Interestingly, in vitro editing at the R/G site by these two recombinant DRADA enzymes appears to be a rather slow process, progressing still at the end of the 4-h incubation period (Fig. 5A). The requirement of the dsRNA structure for the in vitro Q/R site editing by DRADA2a was confirmed by using a mutant B11M1 RNA substrate RNA (11), which contains a site-directed single base substitution of a guanosine to a cytosine at the −2 position and thus lacks a 17-bp dsRNA critical for the Q/R site editing in vivo (13, 17). We found that at the Q/R site editing of this mutated B11 RNA by DRADA2a was decreased to 30%, in contrast to 80% for the wild-type B11 RNA (data not shown). Interestingly, DRADA2a edited the mutant B11M1 RNA at the +60 intronic site as efficiently as its wild-type counterpart, suggesting that the disruption of the 17-bp dsRNA including the ECS does not affect the editing of the downstream intronic region. The requirement of the dsRNA structure for the R/G site editing by DRADA2a and DRADA1 was confirmed when we tested the mutant B13Δ RNA. The R/G site of this mutant substrate RNA, lacking the essential ECS element (28), was not edited at all by either DRADA2a or DRADA1 (data not shown).

We systematically tested various conditions, which may alter the efficiency and site selectivity of the two deaminases for in vitro RNA editing. Among the conditions tested, we found that the salt (NaCl) concentration affects the in vitro RNA editing efficacy at certain sites to some extent (Fig. 5B). In general, the efficiency of RNA editing at the R/G site by DRADA2a and DRADA1 as well as that at the Q/R site by DRADA2a was unchanged in the presence of up to 0.15 M NaCl, but it decreased in the presence of 0.2 M and higher concentrations of NaCl. In contrast, the efficiency of RNA editing at the +60 site by DRADA1 was essentially unaffected even in the presence of high salt, whereas this intronic site was edited more efficiently by DRADA2a if the editing assay was carried out in the presence of a relatively low salt concentration. For instance, a threefold increase of the +60 site editing by DRADA2a was observed by decreasing the NaCl concentration from 0.15 to 0.05 M (Fig. 5B). The overall RNA editing site selectivity displayed by DRADA2a was essentially as described for rat RED1 recently (31), despite the presence of an extra 10-aa

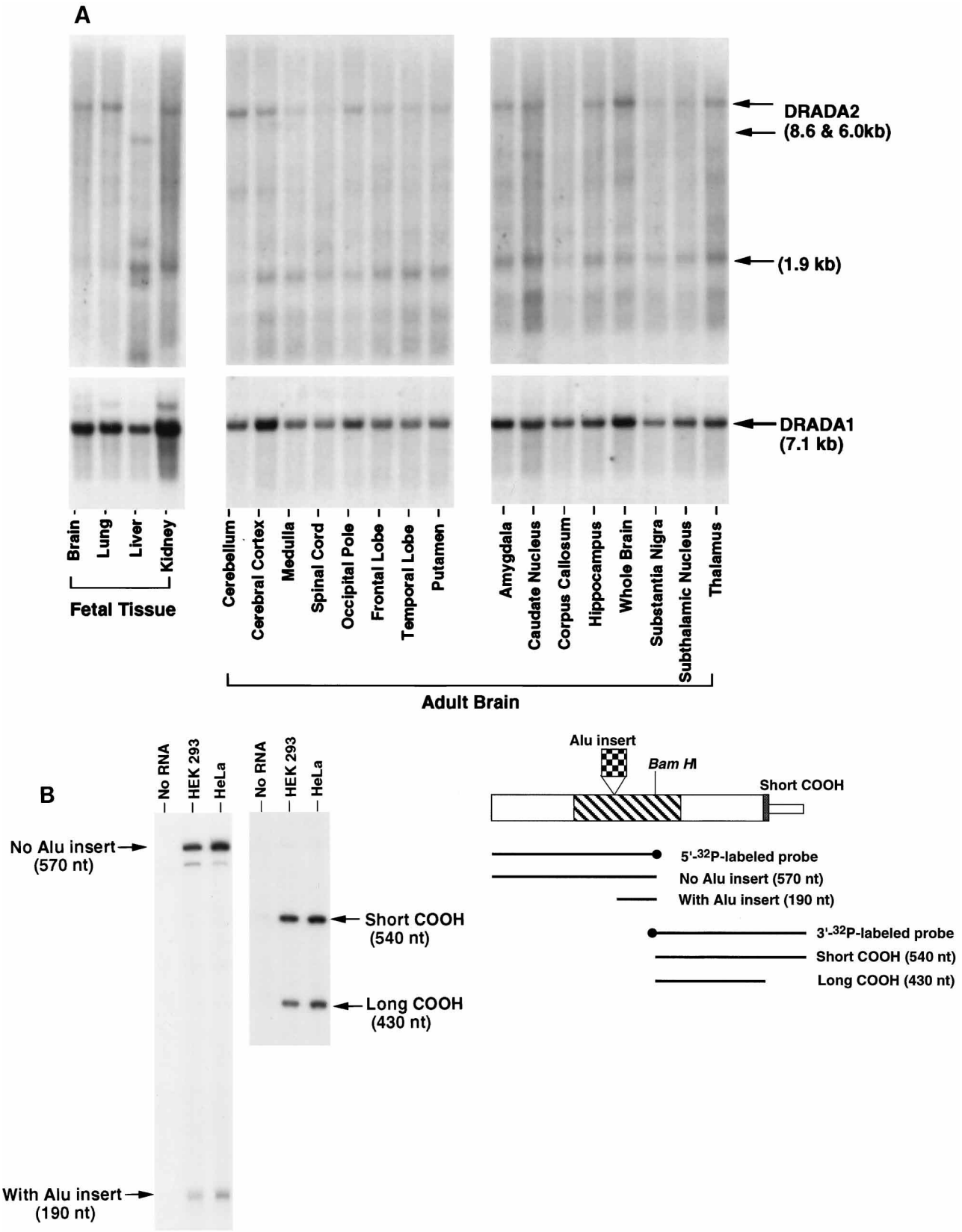

FIG. 2. Expression of DRADA2 mRNAs. (A) Northern blot analysis for DRADA2 mRNAs. Northern blots of poly(A)$^+$ RNA extracted from human fetal tissues and various parts of human adult brain tissues were hybridized with a 0.5-kb *Eco*RI/*Bam*HI fragment of the human DRADA2a cDNA, covering a part of the deaminase domain. The same Northern blots were rehybridized with a 1.2-kb *Sal*I/*Kpn*I fragment of the human DRADA1 cDNA, covering also the deaminase domain. The variation of the amount of poly(A)$^+$ RNA loaded in different lanes, checked by rehybridization of the blots with a glyceraldehyde-3-phosphate dehydrogenase cDNA

insert in this rodent homolog. It should be pointed out, however, that both intronic +60 and R/G sites can be edited efficiently by DRADA2a and DRADA1 as well if higher concentrations of enzymes are applied in the presence of the optimum concentration of salt. Thus, the relative strength of the editing site selectivity for these two sites between the two deaminases shown in Fig. 5A is somewhat arbitrary. The exception was the highly efficient Q/R site editing displayed by DRADA2a. DRADA1 was incapable of editing at this site even at very high enzyme concentrations and over a varying range of salt concentrations (Fig. 5).

Accuracy of RNA editing in vitro by DRADA2a. To monitor the accuracy of the in vitro editing of GluR-B RNAs by DRADA2a, cDNA was synthesized from the B11 and B13 RNAs edited in vitro by DRADA2a (5 ng), amplified by PCR, and cloned into the pBluescript KSII+ vector as described previously (11). Eighteen independent colonies derived from the B11 region and 16 independent colonies derived from the B13 region were selected and analyzed directly by DNA sequencing of the entire B11 region and the 5′ half of the B13 region. The sequence analysis for the B11 region confirmed the primer extension data showing the high efficiency of RNA editing by DRADA2a at the Q/R site (72%, 13 of 18 clones) and less frequent editing at the intronic +60 site (17%, 3 of 18 clones). The cDNA sequence analysis also revealed other RNA editing sites selected by DRADA2a, such as the exonic +4 site (61%, 11 of 18 clones) and the intronic sites +262 (22%, 4 of 18 clones), +263 (67%, 12 of 18 clones), and +264 (67%, 12 of 18 clones), which are known to be edited in vivo in brain (11, 13, 17, 41).

Similar analysis for B11 RNA edited in vitro by DRADA1 has revealed previously a partly overlapping but yet very different site selection pattern of this first member of the dsRNA adenosine deaminase: editing at the exonic +4 (18%) and intronic +60 (79%) and +262 (4%) sites but no editing at the Q/R, +263, and +264 sites (11). We also noted that several intronic sites not edited in brain were selected by DRADA2a. Although these nonspecific intronic site selections occurred with relatively low frequency at most sites (+79, +92, and +196 sites; 17%), one site (+269) was edited as efficiently as the Q/R site (72%, 13 of 18 clones) by DRADA2a in this in vitro system. Interestingly, we noted previously that this nonspecific intronic site was edited in vitro by nuclear extracts made from HeLa cells and also by DRADA1 (11). A similar RT-PCR and cDNA sequencing analysis for the B13 region was conducted with RNAs edited in vitro by DRADA2a (5 ng) and also by DRADA1 (20 ng). We confirmed by sequence analysis that the R/G site is edited by both DRADA2a (60%, 9 of 15 clones) and DRADA1 (64%, 9 of 14 clones). In the B13 region also, certain nonspecific sites were found to be edited by both enzymes: the +19 and +41 sites (6%) by DRADA2a and the +19, +39, and +41 sites (7, 36, and 14%, respectively) by DRADA1. The results suggest that both deaminases are prone to select in vitro certain nonspecific adenosines, which seem to be protected in vivo by a currently unknown mechanism.

Functional difference among four DRADA2 isoform enzymes. The RNA editing activity and site selectivity of four different DRADA2 isoforms were next examined and compared to each other. We found that the RNA editing site selectivity of DRADA2b is almost identical to that of DRADA2a for the three editing sites (Q/R, +60, and R/G) examined (Fig. 6), suggesting that the presence of an *Alu* cassette insert in this isoform enzyme does not alter its RNA editing function, at least in the in vitro RNA editing assay system used in the present study. In contrast, DRADA2c and DRADA2d isoform enzymes containing the short-COOH structure displayed essentially no RNA editing activity at any of the three sites tested (Fig. 6). Since the A-to-I conversion activity of these two short-COOH isoforms is much reduced in comparison to that of the other two long-COOH isoforms (Fig. 3B), we repeated the RNA editing analysis with the higher concentrations of DRADA2c and DRADA2d, which confirmed their lack of activity (data not shown).

In an attempt to obtain some insights into the basis of significantly different enzymatic activities found with different DRADA2 isoforms, we next investigated binding of the DRADA2 isoforms and also DRADA1 to B11 and B13 RNAs by a nitrocellulose filter binding assay (20, 26). The K_ds, obtained by Scatchard plot analysis of the RNA binding assay data (data not shown), were essentially identical for different DRADA2 isoform enzymes and DRADA1 (0.3 nM for B11 RNA and 0.06 nM for B13 RNA). The results indicate that the dramatically different RNA editing site selectivities observed with different DRADA enzymes is not simply due to their altered affinity to the RNA substrates harboring potential editing sites. It should be pointed out, however, that the filter binding assay used may not be sensitive enough to detect minor differences in the affinities of each deaminase to different regions and RNA duplex structures present within B11 or B13 RNA.

DISCUSSION

Identification of the second dsRNA adenosine deaminase gene DRADA2. In this study, we have identified DRADA2, the second member of the dsRNA adenosine deaminase gene family, by searching through the human EST databases (1) for the genes with a high degree of sequence similarity (overall 56% similarity) to the first member of this class of enzyme, DRADA1 (21, 34, 37). A different approach, a combination of low-stringency screening of a rat brain cDNA library using the DRADA1 deaminase domain DNA sequence information and expression cloning of candidate cDNAs in HEK 293 cells, has also led to identification of RED1, which appears to be a rat homolog of DRADA2 (31). The complete characterization of four overlapping DRADA2 cDNA clones subsequently isolated from two human-brain-tissue-specific libraries has revealed the presence of four differentially spliced DRADA2 mRNAs and their coding protein products. One of these isoforms, DRADA2a, has the highest degree of sequence simi-

control probe, was negligible (data not shown). (B) Analysis of differentially spliced DRADA2 isoform mRNAs. The levels of DRADA2 isoform mRNAs were examined by quantitative S1 mapping analysis. The specific probes used and the expected S1 nuclease-resistant DNA products are indicated in the diagram. The DRADA2a-specific cDNA plasmid probe, cleaved at a *Bam*HI site located within the deaminase domain and 5′ ^{32}P end labeled, or the DRADA2c-specific cDNA plasmid probe, cleaved at the same *Bam*HI site and 3′ ^{32}P end labeled, was hybridized in 80% formamide to 20 μg of total RNA from HEK 293 and HeLa cells at 55°C, digested with S1 nuclease, and analyzed by electrophoresis on a 7 M urea–4% polyacrylamide gel. Identical experiments with 5′-end-labeled DRADA2b- and 3′-end-labeled DRADA2a-type cDNA plasmid probes were carried out to confirm the levels of DRADA2 mRNAs with and without the *Alu* cassette insert and with the long or short C-terminal sequence (data not shown). The linear relationship of S1 nuclease-resistant DNA bands representing DRADA2 mRNAs with and without the *Alu* cassette insert and with the short or long C terminus was confirmed by control experiments using varying amounts of HeLa total RNAs.

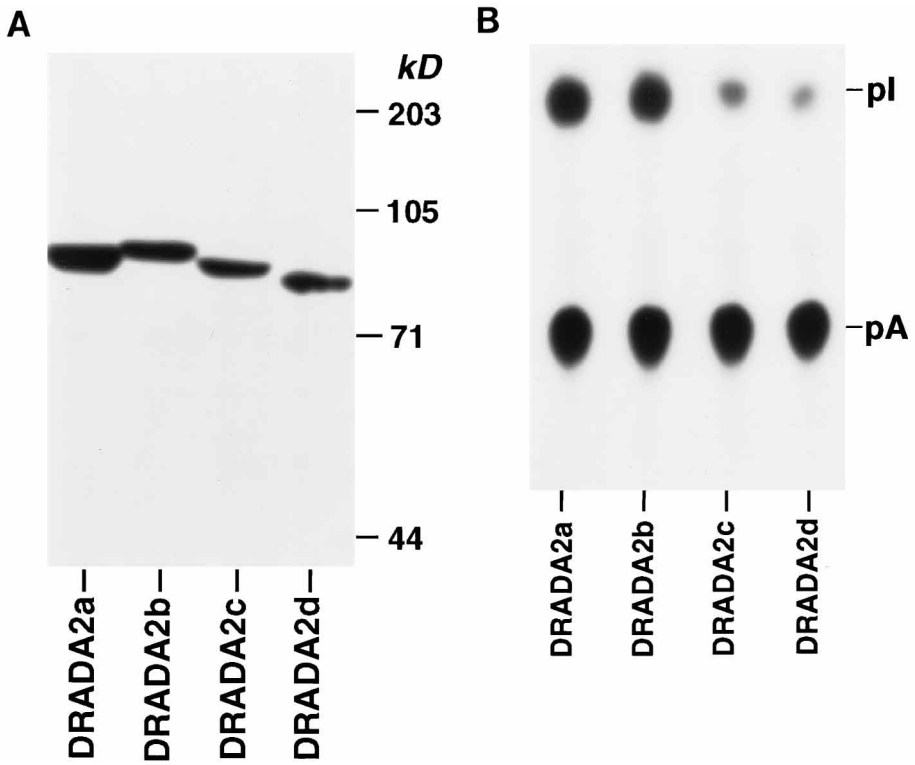

FIG. 3. Purification of recombinant DRADA2a proteins and their A→I conversion activity. (A) Expression of recombinant DRADA2 isoform proteins from pVL-F-DRADA2a, pVL-F-DRADA2b, pVL-F-DRADA2c, and pVL-F-DRADA2d under the control of the baculovirus polyhedrin promoter. All recombinant DRADA2 proteins contained an epitope-tag peptide FLAG at their NH_2 terminus. Recombinant FLAG-DRADA2 fusion proteins (10 ng) were fractionated by SDS–10% polyacrylamide gel electrophoresis and analyzed by immunoblotting with anti-FLAG M2 monoclonal antibody (Kodak). Molecular mass standards used were prestained myosin heavy chain, phosphorylase B, bovine serum albumin, and ovalbumin (BRL-Gibco, Gaithersburg, Md.). Note that all DRADA2 isoform proteins migrate as bands approximately 10 kDa larger than their molecular masses estimated from their protein sequences. This is very similar to the apparent molecular weight of DRADA1, which migrates on an SDS-polyacrylamide gel slower than its calculated molecular mass, 136 kDa, possibly due to its posttranslational modification (21). (B) The A→I conversion activity of four different recombinant DRADA2 isoform enzymes (5 ng each) was determined by a base modification assay using 10 fmol of ^{32}P-ATP-labeled c-*myc* dsRNA (20). Following incubation for 1 h at 37°C, the reaction products were deproteinized, digested with P1 nuclease, and analyzed by one-dimensional thin-layer chromatography (20). From the average of several independent assays, the A→I conversion activity of DRADA2 isoforms with short C termini (-2c and -2d) is estimated to be approximately 5 to 10 times less than that of isoforms with long C termini (-2a and -2b).

larity (97%) to rat RED1 (31), which contains an extra 10 aa residues in the middle of the deaminase domain. Two other DRADA2 splicing variants, DRADA2b and DRADA2c, contain an *Alu* cassette of a 40-aa insert at the identical location. We predict that the rat RED1 gene expresses also a shorter completely spliced variant without the 10-aa residues. In addition to this alternate splicing for the *Alu* cassette, another alternate splicing takes place in the C-terminal region of DRADA2, which results in the creation of two different C termini, one long (29-aa) and another short (2-aa) sequence. These two C-terminal structures seem to have a significant impact on the RNA editing activity despite their very minor differences in terms of the overall DRADA2 polypeptide sequence (see below). The long, but not the short, C-terminal sequence seems to be present in DRADA1, although as a much less conserved form. We could not identify so far any significant functional motif or feature that may distinguish this COOH end region.

Efficient editing of GluR-B RNA in vitro at the Q/R site by DRADA2a and DRADA2b. The two DRADA2 isoforms with the long C terminus edited at virtually all GluR-B editing sites, including the two exonic Q/R and R/G sites and the so-called intronic hot spots, the +60, +262, +263, and +264 sites, present within intron 11. Thus, as reported originally for rat RED1 by Melcher et al. (31), human DRADA2a and -2b do indeed mark their editing site pattern in vitro on GluR-B RNA, which is very similar to that known to take place in vivo in brain (11, 13, 17, 41). Although a part of this editing pattern can be achieved (R/G site and intronic +60 and 262 sites) also by DRADA1, it did not edit certain intronic sites (+263 and +264) and, most importantly, the Q/R site (11, 29). Interestingly, however, we found previously that supplementation of nuclear extract proteins made from HEK 293 cells to DRADA1 resulted in modest (up to 30%) editing of GluR-B RNA at the Q/R site in vitro, which appeared to be mediated by DRADA1. HEK 293 cells have been considered to be deficient in the expression of the enzyme(s) responsible for GluR RNA editing (11, 29, 31, 32), in contrast to many other editing-competent cell lines such as HeLa (11, 41, 51). We proposed that DRADA1 incapable of editing at the Q/R site of GluR-B may become competent to recognize this exonic site only in the presence of an additional factor present in HEK 293 cells. With the discovery of DRADA2, or RED1, a new member of the DRADA gene family capable of editing the Q/R site very efficiently by itself, results from these previous studies on DRADA1 could have an alternative explanation also (11). Interestingly, our S1 mapping analysis suggests that all of the DRADA2 isoforms, including the GluR-B RNA editing-competent DRADA2a and DRADA2b, are present in HEK 293 cells as well as in HeLa cells. It may be that endogenous DRADA2 deaminases present in HEK 293 cells are sequestered by some mechanism and may be released only in the

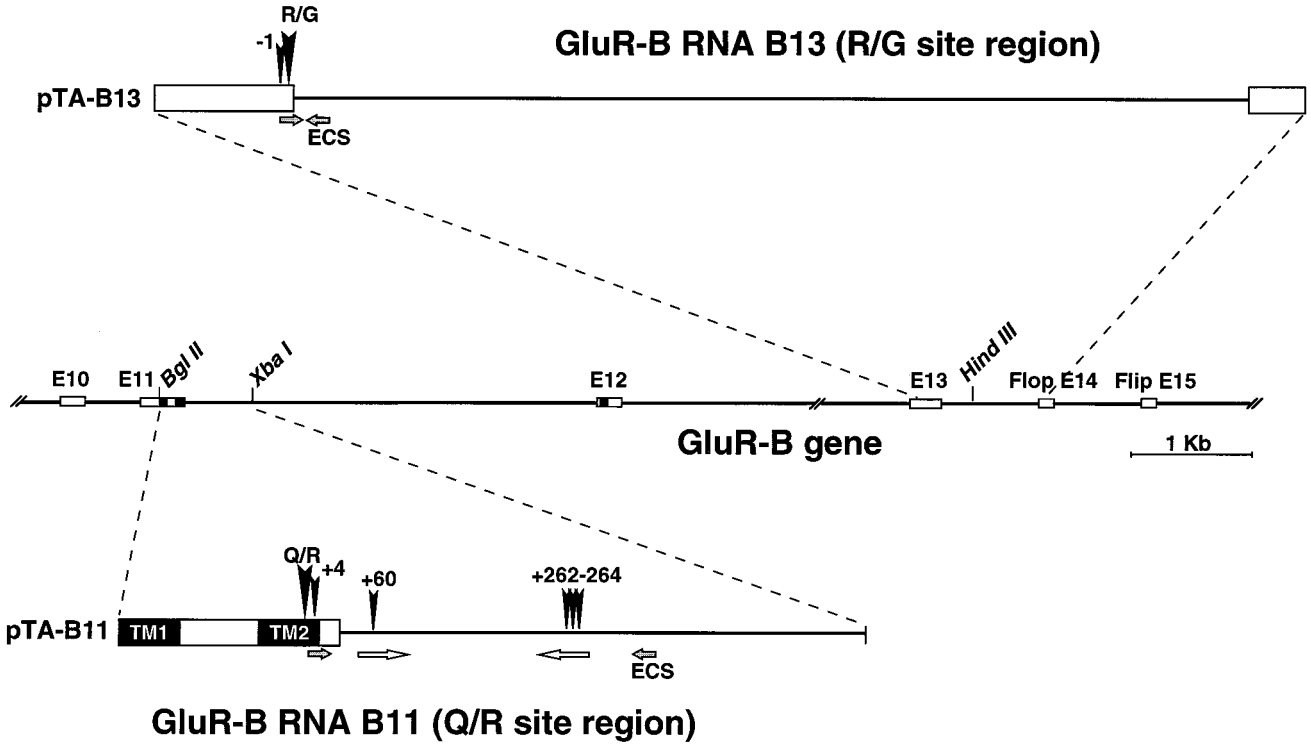

FIG. 4. Two regions of GluR-B gene harboring RNA editing sites. A part of the murine AMPA GluR-B gene, encompassing exons 10 to 15 (23), and its two regions (expanded), B11 and B13, are shown schematically. All exons are indicated by open boxes, and putative transmembrane domains are indicated by filled boxes. Two inverted repeats including the ECS essential for the Q/R site editing within the B11 region (13, 17) and also for the R/G site editing within the B13 region (28) are indicated by shaded arrows. Longer inverted repeats also required for the Q/R site editing and present in the B11 region are indicated by open arrows. All editing sites known to take place in vivo in brain, Q/R and +4 exonic sites and +60, +262, +263, and +264 intronic sites located in the B11 region and R/G and −1 exonic sites located in the B13 region, are indicated by filled arrowheads. The two segments of the GluR-B gene transcript, B11 and B13 RNAs, were prepared by in vitro transcription using two GluR-B mini gene constructs (pTA-B11 and pTA-B13) and used as substrates for the in vitro RNA editing assay.

presence of excess recombinant DRADA1 protein, resulting in the editing of the Q/R site by the activated DRADA2 (11). An apparent suppression of the RNA editing mechanism, which has also been noted recently in human embryonic NT2 cells, is progressively activated during their terminal differentiation into postmitotic neurons (27). Thus, the levels as well as activities of DRADA2 might be subject to a cell type-specific or differentiation-dependent regulation. In regard to the possible regulation of DRADA activities, it should be pointed out that the extent of RNA editing of certain GluR subunits changes significantly during brain development (6, 28). In addition, the sizable variation in the levels of RNA editing of different GluR subunits has been detected in different regions of brain or in different neuronal cells (33, 36, 39, 40). In view of coexpression of multiple DRADA gene family members and splicing variants with distinct and yet overlapping editing site selectivity in brain, these previous observations might now be explainable at least in part. The differential editing noted previously might result from possible differential regulation of these multiple DRADA genes and their expressions in various ratios in different regions of brain and/or during development.

The highly discriminating editing site selection at the Q/R site displayed by DRADA2a (or -2b) and DRADA1 indicates a significant difference in their substrate RNA interaction mechanisms. Certain features in the RNA binding domains are clearly different between DRADA2 and DRADA1, where the molecular basis of their editing site selectivity may reside. First, DRADA2 lacks a part of the N-terminal region of DRADA1, just upstream of its RNA binding domain, where DRADA1 contains two repeats of a Z-DNA binding motif (16). Thus, the RNA binding domain of DRADA2 may be more open for interacting with dsRNA substrates, whereas the access of DRADA1 to certain dsRNA substrates might be restricted because of its long N-terminal extension next to the RNA binding domain. Second, there are only two repeats of DRBMs in the RNA binding domain of DRADA2, in contrast to three repeats present in DRADA1. In addition, the space between the DRBMs of DRADA2 (83 aa residues) is twice as long as that of DRADA1 (40 or 41 aa residues). Our previous mutagenic studies on the three DRBMs of DRADA1 have suggested that spacer length between DRBMs may be important to give structural flexibility for the neighboring DRBMs (26). Finally, the sequence of DRADA2 DRBM is quite divergent from that of DRADA1 and more similar to that of other dsRNA binding protein members such as nuclear factor NF90 (19) and microtubule-associated protein SPNR (43). The understanding of the importance of these differences of DRADA2 and DRADA1 for the editing site-selective mechanism requires structural studies of these different DRADAs complexed with the Q/R site region of GluR-B RNA.

Biological function of different DRADA2 isoforms. It is currently not clear why certain alternatively spliced DRADA2 isoforms are expressed in cells. Surprisingly, the presence of an in-frame *Alu* cassette in the middle of the putative deaminase domain of DRADA2b seems to have essentially no effect on its A-to-I conversion activity or GluR-B RNA editing site selectivity. Although *Alu*-related sequences are found within the protein-coding regions of more than a dozen independent

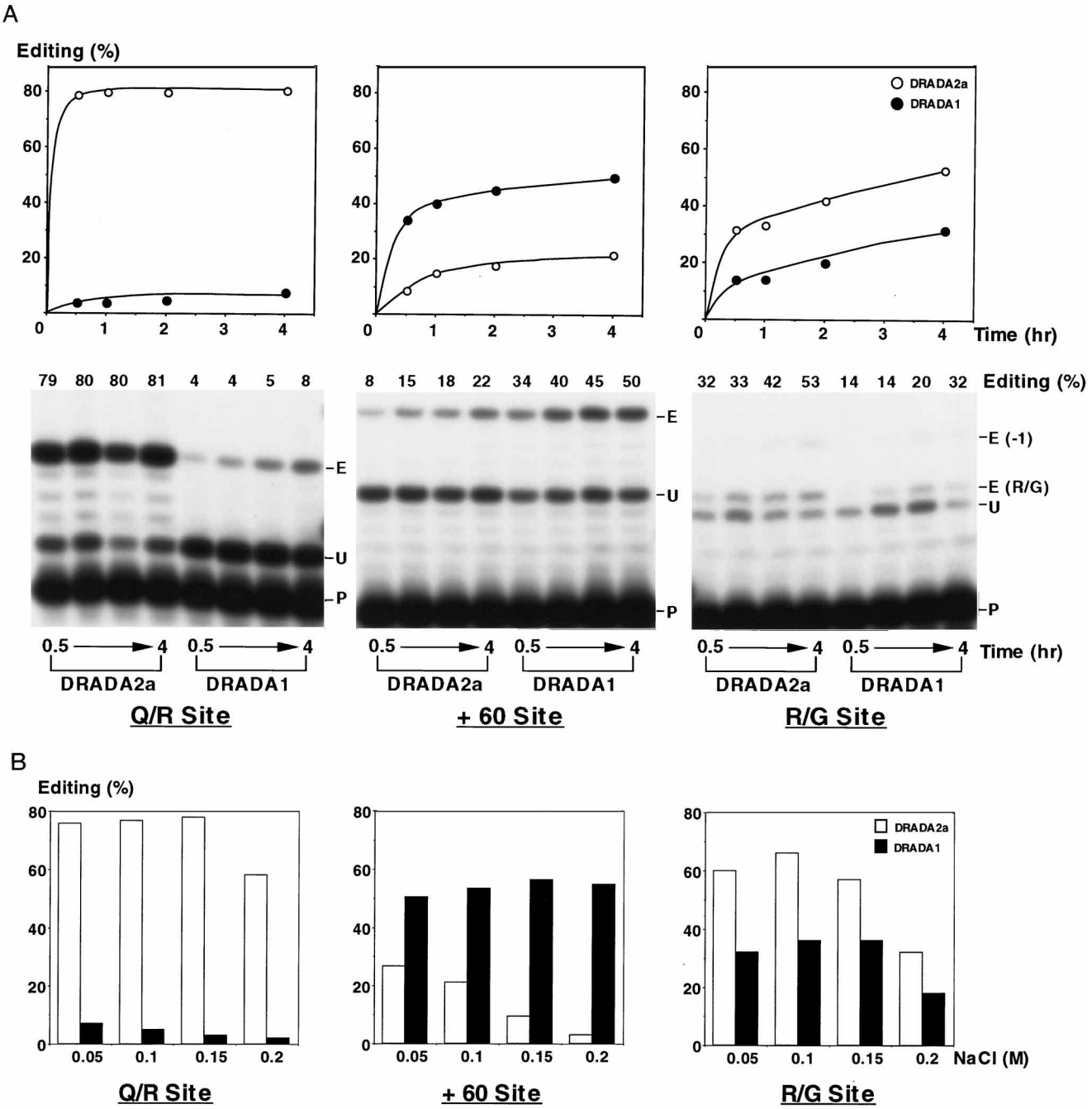

FIG. 5. RNA editing site selectivity of recombinant DRADA2 and DRADA1. Editing site selectivity of purified recombinant DRADA2a proteins was examined in vitro at the Q/R, intronic +60 and R/G sites of GluR-B RNAs. For comparison, purified recombinant DRADA1 proteins were tested simultaneously. (A) Time course analysis of site-specific editing of GluR-B RNAs. B11 RNA encompassing the Q/R and intronic sites and B13 RNA encompassing the R/G site were used as substrate RNAs for an in vitro RNA editing assay. The 100-μl reaction mixture contained 5 ng of DRADA2a or 10 ng of DRADA1 recombinant proteins and 20 fmol of B11 or B13 RNA in an editing assay buffer containing 0.02 M HEPES (pH 7.0), 0.1 M NaCl, 10% glycerol, 5 mM EDTA, 1 mM DTT, and 250 U of RNasin per ml. Following incubation for various times at 30°C, the reaction product was deproteinized and ethanol precipitated. The RNAs were examined by RT-primer extension analysis with ^{32}P-labeled oligonucleotide primers specific for the Q/R (EXBex), +60 (EXBin), or R/G (EXB13in) site. The RNA editing efficiency (in percents) was estimated by quantitating the ratio of the primer (P) extended band specific for edited (E) and unedited (U) RNA with a PhosphorImager System (Molecular Dynamics). For R/G site editing analysis, two extended bands can be detected: one representing RNA edited only at the R/G site (R/G) and another edited at both the R/G and −1 sites (−1). (B) The effects of salt on differential editing of GluR-B RNA by DRADA2a. The in vitro RNA editing was carried out as described for the time course experiments, but with various NaCl concentrations. The RNA editing reaction mixture was incubated at 30°C for 4 h.

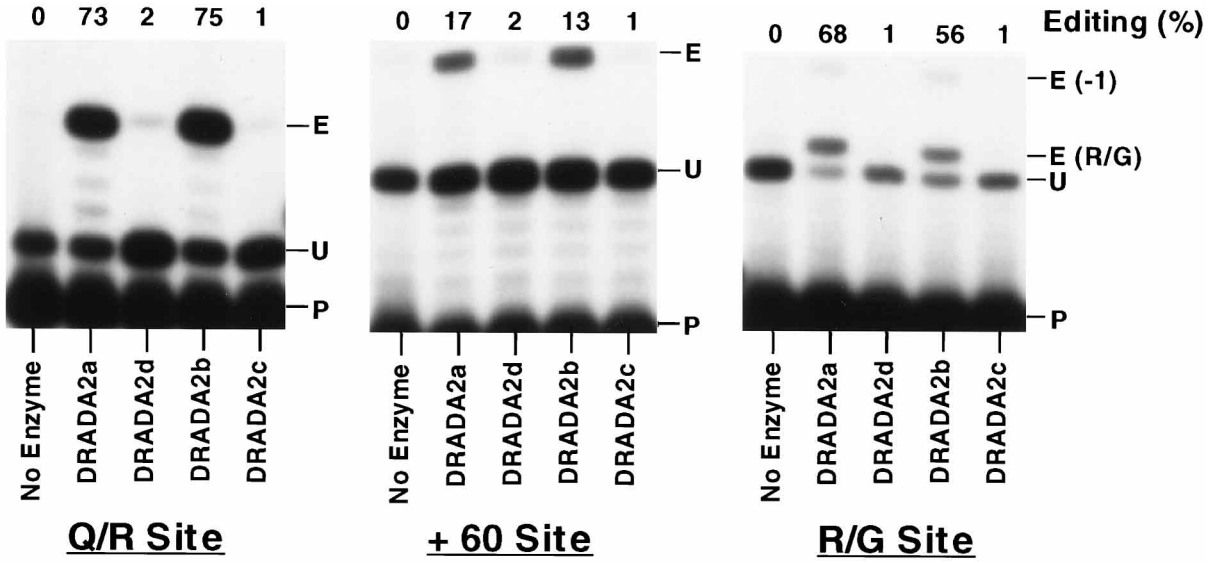

FIG. 6. Differential RNA editing by four DRADA2 isoform enzymes. Four different DRADA2 isoform proteins (5 ng each) were tested for their editing activity and/or site selectivity. The standard RNA editing reaction was carried out in the presence of 0.1 M NaCl at 30°C for 4 h. Abbreviations are as defined for Fig. 5A.

genes, resulting from the splicing of intronic *Alu* elements, the biological significance of these *Alu* cassettes is unknown except for a few cases (30). The presence of an *Alu* cassette in decay accelerating factor (DAF), a membrane-bound glycoprotein that binds activated complements, has been predicted to create a different hydrophilic C-terminal region in the peptide, which in turn leads to synthesis of a secreted form of DAF variant (8). Interestingly, the *Alu* cassette inserted in the DRADA2b and -2c isoforms has the highest homology to that of DAF. It is possible that the subtly different hydrophilicities of DRADA2b and -2c affect the localization of these minor-form splicing variants within the nucleus and, thus, their interaction with target substrate RNAs such as GluR.

More enigmatic is the expression of the two isoforms, DRADA2c and DRADA2d, carrying the truncated C terminus, which do not edit any of the Q/R, intronic +60, and R/G sites. Incompetence of these DRADA2 variants in GluR-B RNA editing may explain why this type of rat RED1 isoform was not noted previously, since RED1 was identified by expression cloning, screening for capability of GluR-B RNA editing at the Q/R site (31). These DRADA2 isoforms do display at least the A-to-I conversion activity when they are tested with a synthetic long dsRNA substrate, though it is 5- to 10-fold reduced in comparison to that of the other two isoforms. They can also bind to the substrate GluR-B RNAs harboring potential editing sites such as B11 and B13 regions. Thus, these DRADA2 splicing variant forms could potentially act as competitive inhibitors of the other two editing-competent enzymes, especially in view of the fact that the RNA editing has to take place within the limited time window between transcription and splicing (17). Alternatively, these DRADA2 forms with their altered C-terminal structure may be specialized to edit transcripts of currently unidentified target genes. As anticipated from their ubiquitous expression in a wide range of tissues, both DRADA2 and DRADA1 enzymes are likely to be also involved in editing of RNAs other than GluR and hepatitis delta virus transcripts (3, 4, 9, 45).

One of the human EST cDNA clones, 21ES0162, used as a specific probe to identify the DRADA2 gene, was originally isolated from a human fetal brain library specifically selected for human chromosome 21, and its subchromosome location has been mapped to the q22.2 region (10). In this region, there have been several important genes involved in hereditary disease, such as Down syndrome (24), whose molecular bases are not yet identified. It is possible that the pathogenesis of some of these human central nervous system diseases could involve inappropriate RNA editing of certain gene transcripts such as GluR normally processed by DRADA2 and DRADA1. The DRADA1 gene has been mapped to chromosome 1, region q21, recently (49, 50). The availability of the human DRADA gene probes and sequence information will certainly make it possible to test such candidate diseases.

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