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Mechanism of Interferon Action: Functionally Distinct RNA-Binding and Catalytic Domains in the Interferon-Inducible, Double-Stranded RNA-Specific Adenosine Deaminase

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The 1,226-amino-acid sequence of the interferon-inducible double-stranded RNA-specific adenosine deaminase (dsRAD) contains three copies (R_I, R_{II}, and R_{III}) of the highly conserved subdomain R motif commonly found in double-stranded RNA-binding proteins. We have examined the effects of equivalent site-directed mutations in each of the three R-motif copies of dsRAD on RNA-binding activity and adenosine deaminase enzyme activity. Mutations of the R motifs were analyzed alone as single mutants and in combination with each other. The results suggest that the R_{III} copy is the most important of the three R motifs for dsRAD activity and that the R_{II} copy is the least important. The R_{III} mutant lacked detectable enzymatic activity and displayed greatly diminished RNA-binding activity. Site-directed mutations within the highly conserved CHAE sequence of the postulated C-terminal deaminase catalytic domain destroyed enzymatic activity but did not affect RNA-binding activity. These results indicate that the three copies of the RNA-binding R subdomain are likely functionally distinct from each other and also from the catalytic domain of dsRAD.

Double-stranded RNA-specific adenosine deaminase (dsRAD) is an interferon (IFN)-inducible dsRNA-binding protein (17, 19, 30, 31, 33, 34). dsRAD, also known as DRADA and ADAR, catalyzes the covalent modification of dsRNA but not single-stranded RNA substrates by hydrolytic C-6 deamination of adenosine to yield inosine (3, 48). Hypoxanthine, the base of the nucleoside inosine generated by the deamination, is typically recognized as guanine by the translational and transcriptional machinery (1). A-to-I modifications characteristic of dsRAD are implicated in two types of processes. First, such deaminations are found at multiple sites in viral RNAs, as exemplified by the biased hypermutations observed in negative-stranded RNA virus genomes during lytic and persistent infections (2, 4, 6, 7). Second, the deamination can be highly site specific and occur at one or a few sites in cellular mRNAs, as exemplified by the mRNAs that encode the GluR receptor channels in the brain (16).

Selective A-to-G and complementary U-to-C biased hypermutations were first observed in the genome of defective measles virus recovered from the brain of a persistently infected patient with neurodegenerative disease (8). Similar changes have subsequently been observed in the genomes of other RNA viruses (4, 6, 13). dsRAD is also the candidate enzyme responsible for the specific posttranscriptional RNA editing of transcripts in the brain that encode the AMPA and kainate glutamate-gated ion channel GluR receptor subunits (15, 16). Edited GluR mRNAs encode channel proteins that display altered Ca^{2+} permeability that affects recovery from glutamate-mediated desensitization (25). The highly selective Q-to-R and R-to-G amino acid changes in GluR subunits are dependent upon formation of dsRNA hairpin structures involving exon and intron sequences near the site of editing in the pre-mRNA substrates: exon 11 and intron 11 for the Q-to-R change (15, 52) and exon 13 and intron 13 for the R-to-G change (25). Thus, the A-to-I deaminations observed in both viral RNA genomes and cellular mRNA transcripts in the brain are dependent upon double-stranded regions within the substrate RNA.

The dsRNA-binding properties reported for purified dsRAD proteins (17, 19, 30, 31) are similar to those of some other known dsRNA-binding proteins, including the IFN-induced RNA-dependent protein kinase PKR (39), in which the prototype dsRNA-binding R motif was discovered (12, 27, 41, 46). The molecular cDNA cloning of dsRAD from human, rat, and bovine cells has recently been described (21, 32, 33). Using a screen to isolate cDNA clones of proteins regulated by IFN, we isolated from human cells a 6.5-kb cDNA encoding dsRAD (33). The 1,226-amino-acid (aa) protein sequence deduced for dsRAD from the nucleotide sequence of the human cDNA clone (21, 32, 33) possessed three copies of the dsRNA-binding R motif, a subdomain found in several known dsRNA-binding proteins (12, 27, 46). The three R-motif subdomains, designated R_I, R_{II}, and R_{III}, were positioned in the central portion of the dsRAD protein. A comparison of the three R motifs of dsRAD with each other, and with those from other mammalian proteins, showed striking conservation of sequence. The R-motif subdomain core residues established as important for dsRNA-binding activity by mutagenesis of the R_I motif of the prototype dsRNA-dependent protein kinase PKR (11, 26–28) were conserved in all three of the R subdomains found in dsRAD. The C-terminal portion of dsRAD included a 380-aa region that displayed extensive homology with the entire predicted *Caenorhabditis elegans* T20H4.4 gene product, the *Saccharomyces cerevisiae* HRA400 gene product, and the C terminus of the murine Tenr protein (33). These three proteins are of unknown function. However, the region of homology between them and dsRAD includes a subdomain sequence found in some deaminases (21, 33, 43, 51).

We report here the construction of site-directed mutants of dsRAD and their analyses for RNA-binding activity and enzymatic activity. The three RNA-binding R motifs were mutated individually at a highly conserved lysine residue of the R core, a residue previously established to be essential for dsRNA-binding activity in the case of the human PKR and vaccinia

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virus E3L proteins (9, 11, 26, 28). Our results reveal that among the three copies of the dsRNA-binding R motifs in dsRAD, R_{III} was essential for both dsRNA deaminase activity and dsRNA-binding activity whereas R_{II} was dispensable for both activities. Furthermore, site-directed mutations within the C-terminal region of the dsRAD protein caused a loss of deaminase activity but did not affect RNA-binding activity.

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MATERIALS AND METHODS

Oligonucleotides. Oligonucleotides used for site-directed mutagenesis of the three dsRNA-binding R motifs (R_I, R_{II}, and R_{III}) and the postulated C-terminal catalytic (C) domain of the dsRAD were as follows, with the mutated bases underlined: R_I*(+), 5′-GCTGGAAGCGAGAAAGTGGC-3′; R_I*(−), 5′-GCCACTTTCTCGCTTCCAGC-3′; R_{II}*(+), 5′-GTGTGAGTGCTCCCAGCGAG-3′; R_{II}*(−), 5′-CCACTTTCTCGCTGGGAGCAC-3′; R_{III}*(+), 5′-CTGCGCACACAGCGAGAAGC-3′; R_{III}*(−), 5′-GCCTTGCTTCTCGCTGTGTGC-3′; HE*(+), 5′-CAATGACTGCCAGGCAGCAATAATCTC-3′; and HE*(−), 5′-GGGAGATTATTGCTGCCTGGCAGTC-3′. The symbol "+" indicates the sense primer, and the symbol "−" indicates the antisense primer. Additional oligonucleotides used for PCR and subsequent subcloning included the following: *Nde*I(+), 5′-CAACCCCTCCCATATGGC-3′ (nucleotides [nt] 1090 to 1107); *Sal*I(+), 5′-CAAGTTGGTCGACCAGTCCG-3′ (nt 2279 to 2298); *Sal*I(−), GGTCCGGACTGGTCGACCAAC-3′ (nt 2282 to 2302); and *Kpn*I(−), 5′-CAGTGCCTCTGGTACCGTCC-3′ (nt 3464 to 3483). The underlined sequences indicate the restriction sites used in subcloning; the nucleotide numbers refer to the dsRAD cDNA sequence, GenBank accession number U18121.

Oligonucleotide-directed mutagenesis of the dsRAD cDNA. The molecular cloning of dsRAD from human cells and the consensus sequence of the full-length dsRAD cDNA have been previously reported (33). Point mutants of dsRAD were prepared by a PCR-based method for site-directed mutagenesis of the dsRAD cDNA. Because the full-length dsRAD protein initiating at Met-1 is poorly expressed, and because dsRAD initiating at Met-296 is efficiently expressed and retains dsRNA-binding activity and dsRAD activity (33), the plasmid construction pcDNAI/Neo K88 (aa 296 to 1226), engineered to lack 5′-GC-rich portion of the open reading frame and initiate at Met-296, was used as the wild-type parent in our studies. For each dsRAD mutant, two rounds of PCR (37) were performed by using native *Taq* DNA polymerase and conditions specified by the manufacturer (Perkin-Elmer) to generate products possessing the desired site-specific mutations flanked by suitable restriction sites to facilitate subcloning. The methods utilized for construction of the transcription vector plasmids were essentially as described by Sambrook et al. (38). Chemicals were reagent grade, and enzymes were obtained from New England Biolabs unless otherwise specified.

R_I and R_{II} single-motif mutants. For mutations in either the R_I or R_{II} motif, primer pairs *Nde*I(+)-R_I*(−), R_I*(+)-R_{II}*(−), and R_{II}*(+)-*Sal*I(−) were used. The first-round PCR products generated by using the wild-type dsRAD cDNA as the template were *Nde*I-R_I* (628 bp), R_I*-R_{II}* (352 bp), and R_{II}*-*Sal*I (280 bp), respectively. Agarose gel-purified first-round PCR products *Nde*I-R_I* and R_I*-R_{II}*, which have 20 bp overlapping with each other, were then used as the templates with the distal primers *Nde*I(+) and R_{II}*(−) in a second round of PCR, generating the *Nde*I-R_I*-R_{II}* (960-bp) product. Similarly, first-round PCR products R_I*-R_{II}* and R_{II}*-*Sal*I, which have 27 bp of overlapping sequence, were used as a template in a second round of PCR with the distal primers R_I*(+) and *Sal*I(−) to generate the product R_I*-R_{II}*-*Sal*I (605 bp). Because there is a *Pst*I site between the R_I and R_{II} motifs, restriction digestion of *Nde*I-R_I*-R_{II}* with *Nde*I and *Pst*I, and of R_I*-R_{II}*-*Sal*I with *Pst*I and *Sal*I, resulted in two fragments, designated *Nde*I-R_I*-*Pst*I and *Pst*I-R_{II}*-*Sal*I, respectively. The corresponding fragments with the wild-type sequence, designated *Nde*I-R_I-*Pst*I and *Pst*I-R_{II}-*Sal*I, were isolated from the starting plasmid pcDNAI/Neo K88 (aa 296 to 1226). The two fragments, *Nde*I-*Pst*I and *Pst*I-*Sal*I, containing either the site-directed mutant sequence or the wild-type sequence, were then inserted by three-way ligation into the pBluescript SK^- (pBS) vector (Stratagene) containing the wild-type *Nde*I-*Sal*I fragment of the dsRAD cDNA (33). pBS vector containing the wild-type *Nde*I-*Sal*I fragment of the dsRAD cDNA was obtained from pBS vector containing the wild-type *Bam*HI-*Sal*I fragment of the dsRAD cDNA (33) by digestion with *Nde*I and *Sal*I and isolation of the corresponding pBS-dsRAD fragment. Three pBS-based constructions that contained mutations in either one or both of the R_I and R_{II} motifs resulted from the three-way ligations. The dsRAD *Bam*HI-*Sal*I cDNA fragments were isolated from each of the mutant pBS-dsRAD constructions and then subcloned into the parent plasmid, pcDNAI/Neo-AdD/M296, which had been digested with the same pair of restriction enzymes to remove the wild-type dsRAD *Bam*HI-*Sal*I cDNA fragment. Because there is an additional *Sal*I site downstream of the dsRAD coding region, the wild-type *Sal*I-*Sal*I fragment that had been deleted was subsequently inserted back into the mutant plasmids that had been cut with *Sal*I and dephosphorylated with calf intestinal alkaline phosphatase, thereby yielding the three complete dsRAD constructions containing site-directed point mutations in either one or both of the R_I and R_{II} motifs.

R_{III} single-motif and C-domain mutants. For mutations in either the R_{III} motif or the postulated catalytic (C) domain, a PCR-based strategy similar to that used to generate the R_I- and R_{II}-motif mutants was employed. The first round of PCR, using the wild-type dsRAD cDNA template and the primer pairs *Sal*I(+)-R_{III}*(−) and R_{III}*(+)-*Kpn*I(−), yielded *Sal*I-R_{III}* (109-bp) and R_{III}*-*Kpn*I (1,121-bp) products possessing 25 bp of overlapping sequence, while primer pairs *Sal*I(+)-HE*(−) and HE*(+)-*Kpn*I(−) yielded *Sal*I-HE* (515-bp) and HE*-*Kpn*I (719-bp) products with 29 bp of overlapping sequence. These first-round PCR products were purified and used as templates in the second round of PCR with two distal primers, *Sal*I(+) and *Kpn*I(−), to generate *Sal*I-R_{III}*-*Kpn*I (1,205 bp) and *Sal*I-HE*-*Kpn*I (1,205-bp) products. Following digestion with *Sal*I and *Kpn*I, the respective mutant *Sal*I-*Kpn*I restriction fragment was ligated in a three-way reaction with the wild-type *Kpn*I-*Nsi*I fragment encoding the C-terminal region of dsRAD and pcDNAI/Neo-K88(296-1226) that had been digested with *Sal*I and *Nsi*I, yielding the mutant expression constructions containing site-directed changes in either the R_{III} motif or the C domain.

Multiple R-motif mutants. To obtain dsRAD mutants with site-directed changes in two or more of the three R motifs, the wild-type *Sal*I-R_{III}-*Sal*I fragment was replaced with the mutant *Sal*I-R_{III}*-*Sal*I fragment in each of the three plasmid constructions possessing mutations in either or both of the R_I and R_{II} motifs, thereby yielding the three R-motif mutant constructions that possess changes in R_I and R_{III}; R_{II} and R_{III}; and R_I, R_{II}, and R_{III}. Thus, all seven of the possible expression constructions possessing mutations in one or more of the three R motifs were obtained: R_I(K554E), R_{II}(K665E), and R_{III}(K776E), the three single mutants with one of the R motifs altered; R_I(K554E)R_{II}(K665E), R_I(K554E)R_{III} (K776E), and R_{II}(K665E)R_{III}(K776E), the three double mutants with two of the R motifs altered; and R_I(K554E)R_{II}(K665E)R_{III}(K776E), the triple mutant in which all three of the R motifs were altered. The site-directed mutations of all constructions were verified by sequence analysis by the dideoxy chain termination method (42), using modified T7 DNA polymerase Sequenase version 2.0 according to the manufacturer's instructions (United States Biochemical).

In vitro transcription and translation. Plasmid DNA constructions (5 μg) of the wild-type or mutant versions of dsRAD were linearized with *Xho*I and transcribed in vitro with phage T7 RNA polymerase (Promega) according to the manufacturer's instructions. After treatment with RQ-1 DNase (Promega), the reaction mixture was extracted with phenol-chloroform and then chloroform and the RNA product was recovered by ethanol precipitation. Prior to translation in vitro, the RNA message produced by in vitro transcription was heated at 75°C for 5 min and then chilled on ice for 10 min. Translation in vitro was carried out by using the nuclease-treated rabbit reticulocyte lysate (RRL) system (Promega) according to the manufacturer's recommendations. RRL protein-synthesizing reaction mixtures (50 μl) containing mRNA at a concentration of 20 μg/ml and [^{35}S]methionine at 15 mCi/ml (1,000 Ci/mmol; Amersham) were incubated for 60 min at 30°C. ^{35}S-labeled protein products were analyzed by sodium dodecyl sulfate (SDS)–10% polyacrylamide gel electrophoresis (PAGE) and autoradiography.

RNA binding assay. The RNA-binding activities of the in vitro-synthesized wild-type and mutant dsRAD protein products were assessed by RNA-Sepharose affinity chromatography (23, 28) with poly(rI) · poly(rC)-Sepharose or poly(rA)-Sepharose (Sigma) as indicated below. Briefly, RNA-Sepharose beads were prewashed with several volumes of binding buffer (20 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid], pH 7.5; 5 mM magnesium acetate; 1 mM dithiothreitol; 0.5% Nonidet P-40; 10% [vol/vol] glycerol; and NaCl as specified below) containing bovine serum albumin (BSA; 10 mg/ml); the beads were then washed three times with 5 volumes of binding buffer lacking BSA. dsRAD protein synthesized in vitro (6 μl of RRL reaction mixture) was then mixed with RNA-Sepharose beads (50 μl), either double-stranded poly(rI)·poly(rC) or single-stranded poly(rA)-Sepharose beads. After incubation at 30°C for 60 min with frequent vortexing, the Sepharose beads were washed four times with 15 volumes of binding buffer and then resuspended in SDS gel loading buffer (40 μl). After being heated for 5 min at 100°C, proteins present in the supernatant solution (30 μl) obtained by microcentrifugation were analyzed by SDS-PAGE and autoradiography. Autoradiograms were quantified by laser densitometry.

dsRAD assay. The assay conditions for measurement of dsRAD activity were essentially as described previously (31, 33). For preparation of the dsRNA substrate, ^{32}P-labeled opposing transcripts were annealed in 20 mM Tris-HCl (pH 7.9) containing 0.15 M NaCl by first being heated at 75°C for 5 min and then slowly cooled to room temperature. The standard reaction mixture (40 μl) for measurement of dsRAD activity contained 10 fmol of ^{32}P-labeled dsRNA, various amounts of in vitro-synthesized dsRAD protein as indicated, 50 mM Tris-HCl buffer (pH 7.9), 100 mM KCl, 5 mM EDTA, 10% (vol/vol) glycerol, 1 mM dithiothreitol, and 0.5 mM phenylmethylsulfonyl fluoride. After incubation at 30°C for 2 h, the dsRNA substrate was recovered from the reaction mixture by extraction with phenol-chloroform and chloroform, followed by ethanol precipitation in the presence of 0.5 μg of poly(rI) (Sigma) added as carrier RNA. Recovered RNA was washed with 70% ethanol, dried, and suspended in 10 μl of nuclease P1 buffer (30 mM potassium acetate, pH 5.3, and 10 mM $ZnSO_4$)

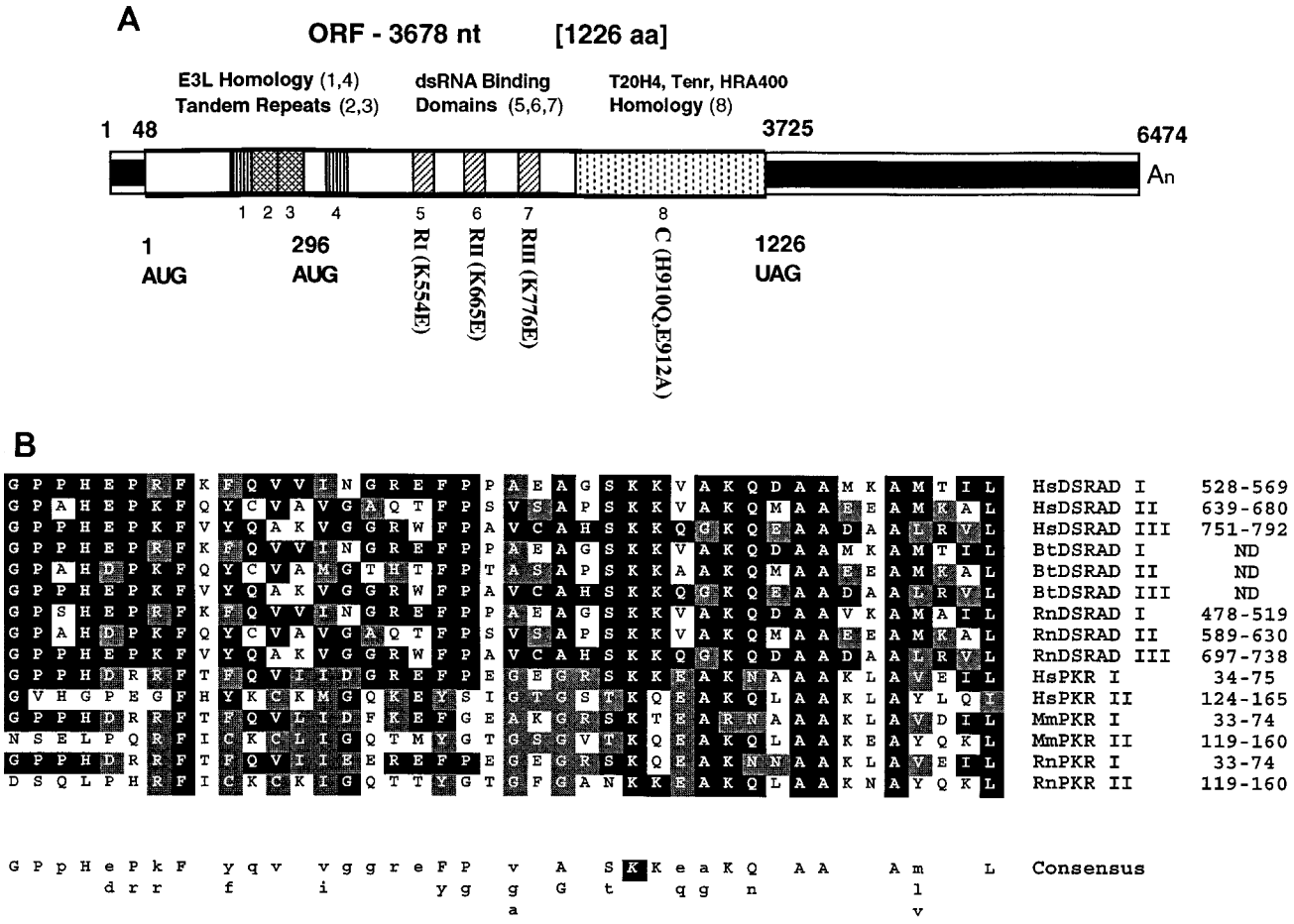

FIG. 1. Schematic representation of dsRAD cDNA and encoded RNA-binding R subdomains. (A) Schematic summary of the 6,474-nt dsRAD cDNA (GenBank accession number U18121) from human cells and deduced 1,226-aa protein. The patterned regions correspond to the following homologies: 1 and 4, the repeated region of homology with the vaccinia virus E3L protein; 2 and 3, tandem repeats; 5 to 7, the three copies of the dsRNA-binding domain; 8, the proposed deaminase catalytic domain, which has homology those of with the predicted *C. elegans* T20H4.4 gene product (accession number U00037); the spermatid nuclear RNA-binding Tner protein from mouse cells (accession number X84693); and the *S. cerevisiae* HRA400 gene product (accession number Z49149). The three R motifs and the CHAE subdomain where mutations were made are indicated below. Numbers shown above the schematic diagram are nucleotide numbers corresponding to the untranslated regions and the open reading frame, and numbers below refer to the deduced amino acid sequence, including the second AUG codon used when making the mutant constructs. (B) Comparison of the RNA-binding domain core motifs of the dsRAD (DSRAD) and the RNA-dependent protein kinase (PKR) from human (Hs), rat (Rn), mouse (Mm), and bovine (Bt) cells. dsRAD possesses three copies (R_I, R_{II}, and R_{III}) and PKR possesses two copies (R_I and R_{II}) of the dsRNA-binding motif. The position of the conserved lysine which was replaced is indicated by black shading in the consensus sequence. Identical amino acid residues are indicated by black boxes, and similar residues are indicated by grey boxes. GenBank/EMBL data bank accession numbers for dsRAD are as follows: U18121 (human) and U18942 (rat); the bovine sequence is published only (32). Those for PKR are as follows: M85294 (human), M65029 (mouse), and L29281 (rat).

before digestion with 1.5 μg of nuclease P1 (Pharmacia) for 1 h at 50°C (31, 33). IMP and AMP were resolved from each other by thin-layer chromatography on cellulose NM 300 glass plates (Macherey and Nagel) in a solvent consisting of saturated $(NH_4)_2SO_4$, 100 mM sodium acetate (pH 6.0), and isopropanol (79:19:2). Autoradiography was usually performed for 16 h at −80°C with a screen; radioactivity associated with the excised thin-layer chromatography spots was quantified by using a Beckman LS1801 liquid scintillation system.

RESULTS

Synthesis of mutant forms of dsRAD. The IFN-inducible dsRAD contains three copies of the dsRNA-binding R motif (12, 27, 46) positioned in the central region of the protein (Fig. 1A). The carboxy-terminal region of the dsRAD protein includes the postulated catalytic (C) domain of the deaminase (Fig. 1A), which displays significant homology with the *C. elegans* T20H4.4, *S. cerevisiae* HRA400, and mouse Tenr gene products (33). The R motifs of the human, bovine, and rat dsRAD proteins are highly conserved compared with each other and also with the prototype dsRNA-binding R-motif sequence of the RNA-dependent protein kinase PKR from human, mouse, and rat cells (Fig. 1B). Among the residues strictly conserved within the R motifs of dsRAD and PKR is the lysine at amino acid position 60 of PKR (Fig. 1B), a residue which has been shown to be essential for dsRNA-binding activity in the case of the PKR protein (11, 26, 28). Therefore, we used site-directed mutagenesis to alter selectively this lysine residue in each of the three R motifs of dsRAD, using mutagenic oligonucleotides in which Lys was replaced with Glu. Seven different mutant versions of the human dsRAD protein containing point substitutions in one or more of the three dsRNA-binding R motifs were constructed (Fig. 1A). Likewise, the highly conserved CHAE sequence found at amino acid positions 909 to 912 within the postulated C-terminal catalytic domain of dsRAD (21, 33) was also altered. A double mutant was constructed, in which His-910 was replaced with Gln and Glu-912 was replaced with Ala (Fig. 1A).

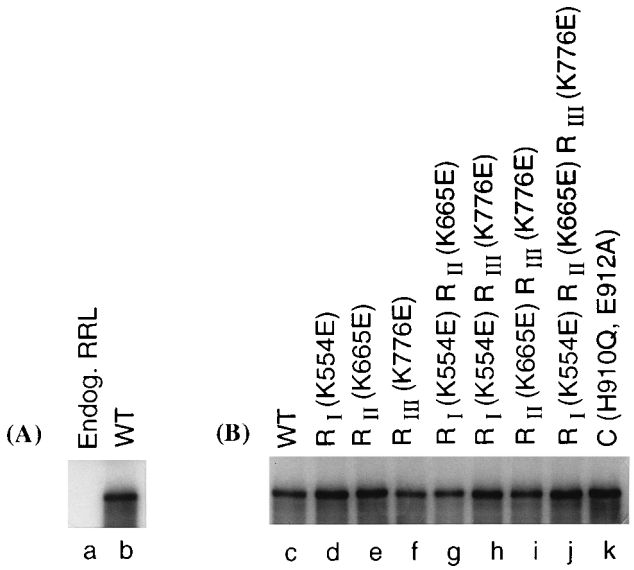

FIG. 2. Structural analysis of dsRAD-proteins synthesized in vitro. Shown is an autoradiogram of dsRAD proteins synthesized in vitro by using mRNA transcripts synthesized in vitro from wild-type and mutant dsRAD cDNA templates. (A) In vitro-translated protein products from reactions without (Endog. RRL) and with exogenously added wild-type (WT) dsRAD mRNA transcripts. (B) Protein products from RRL cell-free protein-synthesizing systems which were programmed with the exogenously added dsRAD mRNA transcripts as indicated above the lanes.

Wild-type and mutant versions of the human dsRAD protein were produced by utilizing the RRL cell-free protein-synthesizing system programmed with dsRAD mRNA transcribed in vitro from mutant as well as wild-type dsRAD cDNA templates. These dsRAD proteins were efficiently synthesized in reaction mixtures that contained exogenously added dsRAD mRNA (Fig. 2) but were not detected in the endogenous RRL control reaction mixture lacking added mRNA (Fig. 2A). As illustrated in Fig. 3B, the seven different R-mutant dsRAD proteins and the C-mutant dsRAD protein were indistinguishable from the wild-type dsRAD protein as measured by electrophoretic mobility in SDS-PAGE, and all of the mutant dsRAD proteins were synthesized in vitro with an efficiency comparable to that of the wild-type dsRAD protein.

The R_{III} motif is essential for dsRAD activity. The wild-type and mutant versions of the dsRAD protein synthesized in vitro in the rabbit reticulocyte system (Fig. 2) were examined for deaminase activity. RRL reaction mixtures programmed with wild-type dsRAD mRNA possessed significant dsRAD enzyme activity relative to the endogenous RRL control reaction mixtures as illustrated by the autoradiogram in Fig. 3A (lanes a and b). The R_I and R_{II} single-mutant dsRAD proteins likewise displayed significant enzymatic activity (Fig. 3A, lanes c and d). By contrast, when comparable amounts of the in vitro-synthesized proteins were examined, the R_{III} single mutant, the three double mutants R_IR_{II}, R_IR_{III}, and $R_{II}R_{III}$, the $R_IR_{II}R_{III}$ triple mutant, and the C-mutant dsRAD proteins all showed very low levels of enzymatic activity (Fig. 3A, lanes e to j) relative to the wild-type and the R_I and R_{II} single-mutant dsRAD proteins (Fig. 3A, lanes b to d).

Quantitation of deaminase activity as a function of protein concentration revealed that mutation of R_{III} alone completely eliminated detectable dsRAD enzymatic activity even at the highest protein concentration examined; the single-site R_{III} mutant showed only the background level of activity observed

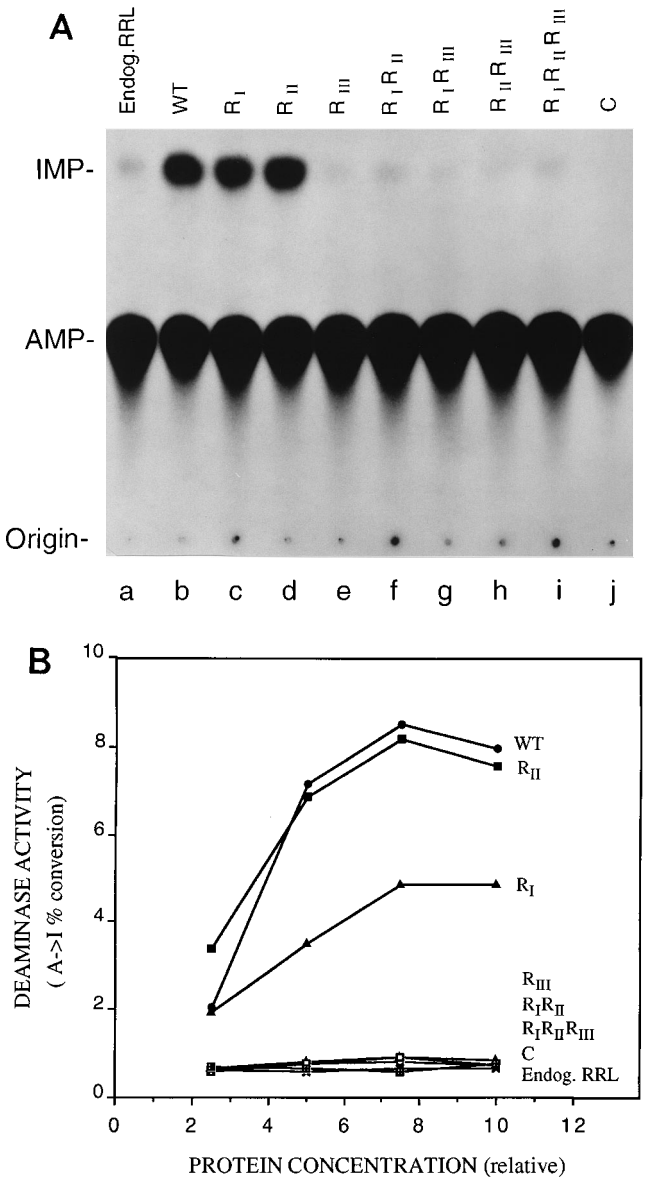

FIG. 3. Functional analysis of enzyme activity of dsRAD proteins synthesized in vitro. (A) Autoradiogram showing dsRAD enzyme activities of proteins synthesized in vitro by using mRNA transcripts synthesized in vitro from wild-type (WT) and mutant dsRAD cDNA clones. A ^{32}P-labeled dsRNA substrate was incubated with equivalent amounts of wild-type or mutant dsRAD under the standard assay conditions described in Materials and Methods. Following subsequent P1 nuclease digestion, the labeled nucleotides were analyzed by thin-layer chromatography. The positions to which the AMP and IMP standards migrated, as well as the origin, are indicated. (B) Effect of relative dsRAD protein concentration on the rate of deamination of dsRNA in the standard reaction mixture. Endog., endogenous. RRL lysate. WT, wild type; mutant in one of the RNA-binding domains, either R_I(K554E), R_{II}(K665E), or R_{III}(K776E); mutant in two of the RNA-binding domains, either R_I(K554E) R_{II}(K665E), R_I(K554E)R_{III}(K776E), or RII(K665E)RIII(K776E); mutant in all three RNA-binding domains, R_I(K554E)R_{II}(K665E)R_{III}(K776E); and mutant in the proposed catalytic domain, C(H910Q,E912A).

for the reticulocyte lysate endogenous control reaction (Fig. 3B). By contrast, the single-site R_{II} mutant possessed dsRAD activity comparable to that of the wild-type protein, indicating that the R_{II} motif was the least critical of the three R-motif copies for enzymatic activity. Mutation of the R_I motif resulted in reduction of dsRAD activity by about 50% compared with

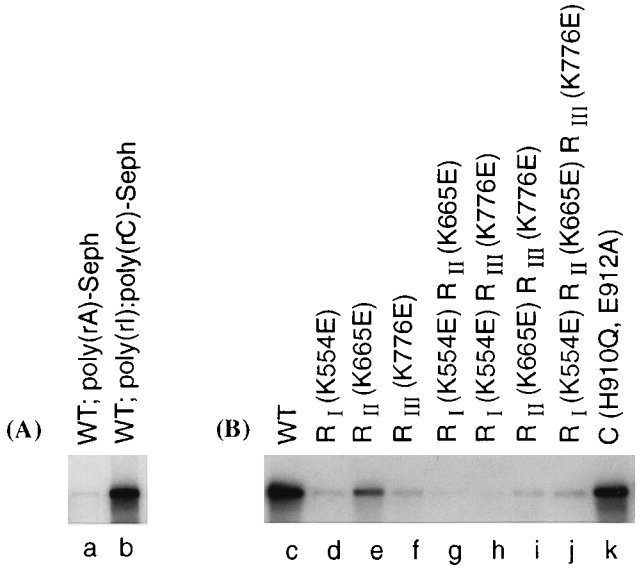

FIG. 4. Functional analysis of RNA-binding activity of the wild-type and mutant dsRAD proteins synthesized in vitro. (A) Autoradiogram of the wild-type (WT) human dsRAD protein bound to single-stranded RNA [poly(rA)-Sepharose] or dsRNA [poly(rI) · poly(rC)-Sepharose], which was analyzed by SDS-PAGE. (B) Analysis by SDS-PAGE of the wild-type and mutant dsRAD proteins bound to dsRNA [poly(rI) · poly(rC)-Sepharose). The binding assays were performed as described in Materials and Methods in the presence of 0.15 M NaCl.

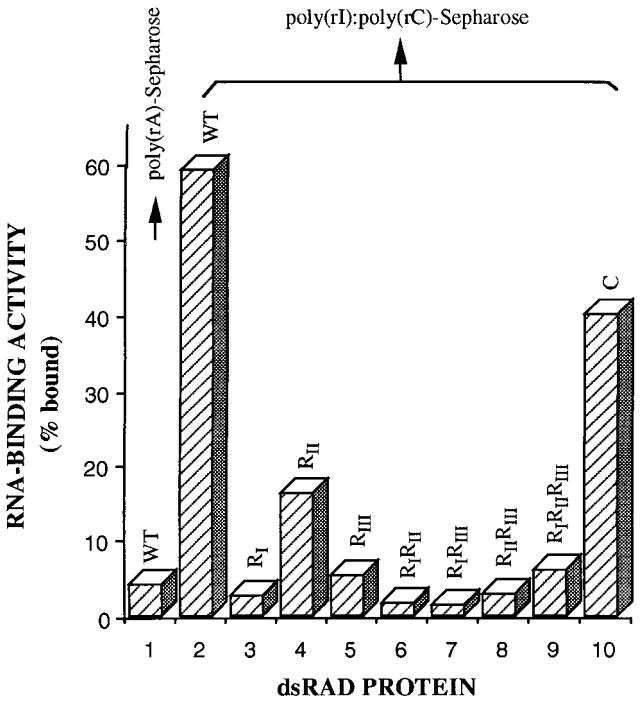

FIG. 5. Quantitation of RNA-binding activity of dsRAD proteins synthesized in vitro. The RNA-binding activities of in vitro-synthesized wild-type (WT) and mutant dsRAD proteins were quantified by laser densitometry as described in Materials and Methods. The percentage of input dsRAD protein bound to poly(rI) · poly(rC)-Sepharose beads was determined by using the standard binding assay carried out in buffer containing 0.15 M NaCl.

that of the wild-type dsRAD protein (Fig. 3B). Both of the double R mutants and the triple R mutant possessing the K-to-E substitution within the R_{III} motif lacked detectable enzyme activity (Fig. 3). The double mutant possessing the K-to-E substitution in both the R_I and R_{II} motifs also lacked detectable dsRAD activity, indicating that a wild-type R_{III} motif was not sufficient for deaminase activity (Fig. 3B). These results suggest that the three copies of the dsRNA-binding R motif present in dsRAD are functionally distinct from each other and indicate that the R_{III} motif is necessary but not sufficient whereas the R_{II} motif is neither necessary nor sufficient for deaminase activity.

His-910 and Glu-912 are important for dsRAD activity. Sequence alignment of the proposed C-terminal catalytic domain of dsRAD with those of other known deaminases (21), as well as with the highly conserved sequences of the predicted *C. elegans* T20H4.4 gene product, the mouse spermatid nuclear RNA-binding Tenr protein, and the *S. cerevisiae* HRA400 gene product (33), revealed several highly conserved residues. Among them, His-910 and Glu-912 within the highly conserved CHAE sequence have been postulated to be involved in the zinc chelation and proton transfer functions, respectively, of the deaminase (21, 44). To test this possibility, we replaced His-910 with Gln and Glu-912 with Ala. As shown in the Fig. 3A autoradiogram and quantified in Fig. 3B, the in vitro-synthesized C(H910Q,E912A) double-mutant protein lacked deaminase activity. This finding is consistent with the notion that one or both of the residues His-910 and Glu-912 play a role in the catalytic mechanism of dsRAD.

Both the R_I and R_{III} motifs are important for dsRNA-binding activity. To investigate whether the loss of dsRAD activity following site-directed point mutation within the R motifs correlated with the dsRNA-binding activity of the mutant dsRAD proteins, we examined the dsRNA-binding activities of the mutant proteins. RNA-binding activity was measured by poly(rI) · poly(rC)-Sepharose affinity chromatography. Wild-type dsRAD protein synthesized in vitro efficiently bound poly(rI) · poly(rC) dsRNA but bound poorly to poly(rA) single-stranded RNA (Fig. 4A). Similar results were recently reported for the native dsRAD from human U cells (33). Of the seven dsRAD proteins possessing site-directed mutations in one or more of the three R motifs, the single-site R_{II} mutant retained the greatest amount of RNA-binding activity. By contrast, the R_I and R_{III} single-site mutants, as well as all of the double R-motif mutants and the triple R-motif mutant, exhibited greatly diminished dsRNA-binding activity relative to that of the wild-type protein (Fig. 4B and 5). The R_{II} mutant protein, which retained significant RNA-binding activity (Fig. 4B and 5), also retained maximal deaminase activity relative to the other mutant dsRAD proteins (Fig. 3). Somewhat surprisingly, the R_I mutant showed a relatively low level of dsRNA-binding activity even though the level of enzymatic activity of the R_I mutant, while lower than those of the wild type and the R_{II} mutant, was clearly higher than those of the various multiple-site R mutants (Fig. 3B). As expected, the mutations in the C domain did not detectably affect dsRNA-binding activity; the dsRNA-binding activity of the C(H910Q,E912A) mutant was comparable to that of the wild-type dsRAD protein (Fig. 4B and 5). When examined at various concentrations of NaCl, the dsRNA-binding activity of the R_{II} mutant appeared to be more sensitive to increased concentrations of salt than that of the wild-type dsRAD protein (data not shown), indicating that while the R_{II} motif was not required of RNA-binding activity, the motif still contributed to the dsRNA-binding activity. Finally, the triple R-motif mutant showed binding to single-stranded RNA similar to that of the wild-type dsRAD protein (data not shown), implying that this activity was not related to the three R motifs.

DISCUSSION

The availability of a functional cDNA clone for the IFN-inducible dsRAD (21, 32–34) has permitted a structure-function analysis of the domains of the enzyme responsible for RNA binding and for catalysis. We constructed equivalent site-directed mutations in each of the three R-motif copies of dsRAD and then examined the effects of the mutations on RNA-binding activity and adenosine deaminase activity. Site-directed mutations were also generated in the postulated catalytic C-terminal domain of dsRAD and characterized (21, 33, 44). Two important points emerge from the results of our mutational analysis reported herein. First, the RNA-binding and catalytic domains of the dsRAD protein are indeed functionally distinct from each other. Second, the relative importance of each individual copy of the three R-motif subdomains of dsRAD differs appreciably as measured by the dsRNA-binding activity and deaminase enzyme activity displayed by the mutant proteins relative to those of wild-type dsRAD protein.

Among the amino acid residues strictly conserved between the R motifs of dsRAD and PKR is a lysine residue (Fig. 1B) which is essential for the dsRNA-binding activity of PKR, an enzyme that possesses two copies of R (11, 26, 28), and also for that of E3L, a vaccinia virus protein that possesses only a single copy of R (9). As an approach to examining the functional importance of each of the three copies of the R motif found in dsRAD, we constructed a family of seven dsRAD point mutants in which the lysine was replaced with glutamic acid in either one, two, or all three of the R copies in dsRAD. The results that we obtained for the seven different R-motif mutants of dsRAD possessing the identical K-to-E amino acid substitution in one or more of the R motifs clearly established that the three repeated R motifs, R_I, R_{II}, and R_{III}, are not functionally equivalent, either for dsRNA-binding activity or for mediating dsRAD activity. These results are similar to those obtained for PKR, in which the R motif is repeated twice (10, 12, 27, 46, 47). In PKR the two R copies differ quantitatively in relative importance. Although the N-proximal R_I copy within PKR is necessary and sufficient for dsRNA-binding activity, both R_I and R_{II} are required for the maximal RNA-binding activity of PKR (11, 26).

Our results obtained with the single and double mutants of dsRAD revealed that the C-proximal copy of the three repeats, R_{III}, was most essential for both dsRNA-binding activity and deaminase activity. By contrast, the middle copy of the repeats, R_{II}, was dispensable for both activities. Although the R_{III} motif was necessary for deaminase activity, it was not sufficient. In addition to the requirement for the wild-type form of the R motif at the R_{III} position, the presence of the wild-type form was also required at either R_I or R_{II}, but not both, in order to demonstrate dsRAD enzyme activity. Conceivably, the distance between the RNA-binding subdomains and the catalytic C domain as specified by R_{III} is critical, and in addition to R_{III}, another R motif is required for directing the RNA substrate correctly to the catalytic center; R_I, in this case, appears to be more efficient than R_{II}. This is consistent with the finding that the R_{II} mutant is fully, and the R_I mutant is partially, active as an enzyme.

Our findings regarding the relative importance of the three R motifs of dsRAD as established by the analysis of point mutants are similar to those of Lai et al. (22), who recently described an analysis of five deletion mutants in which one or more of the R motifs of dsRAD were deleted. Two deletion mutants of dsRAD, one with a 73-aa deletion that removed R_{III} and one with a 72-aa deletion that removed R_I, both lacked enzyme activity, whereas a mutant with a 79-aa deletion that removed R_{II} retained enzyme activity comparable to that of the wild-type protein. Because we have examined point mutants in our study, and not mutants with large deletions, it seems less likely that major conformational changes that would possibly abrogate either binding or catalytic activity without the mutated region per se being significant would have occurred. The apparent inconsistency between our results and those of Lai et al. (22) regarding mutants affecting R_I may conceivably be due to such conformational differences. We observed about 50% of wild-type enzymatic activity with the R_I(K554E) point mutant, whereas Lai et al. (22) did not report any deaminase activity with their M1 mutant, in which a 72-aa region encompassing R_I was deleted.

The deaminase activity that we observed for the seven different R-motif point mutants correlated reasonably well with the dsRNA-binding activity of the mutants, except for the R_I mutant. For example, $R_IR_{II}{}^*R_{III}$, the R_{II} mutant which had a relatively high level of dsRNA-binding activity, was as active as wild-type dsRAD. All of the other mutants which possessed point mutations in one or more of the R motifs, except the $R_I{}^*R_{II}R_{III}$ mutant with the single substitution in R_I, displayed a reduced level of dsRNA-binding activity and no detectable deaminase activity. Although the R_I motif appeared essential for dsRNA binding, site-directed replacement of the lysine in this motif curiously did not completely abolish enzyme activity. Conceivably, the apparent inconsistency is due to the assays employed and the nature of the interaction between dsRAD and dsRNA required in the assays. Interaction between dsRAD and dsRNA may be more transient in the context of a substrate-enzyme interaction, but in order to measure the interaction in the binding assay with dsRNA-Sepharose beads, a more stable interaction may be required. Furthermore, preliminary evidence suggests that regions N proximal of the R motifs may participate in the RNA-binding activity of dsRAD (33a). This may provide an explanation for the results of Lai et al. (22), who examined for RNA-binding activity a limited set of single R-motif mutants (ΔM1, ΔM2, and ΔM3) but not mutants with alterations in two or more R motifs; they found that all of the single-motif mutants possessed binding activity as measured by a Millipore filter assay.

The catalytic activity of the protein kinase PKR is activated by phosphorylation following the binding of RNA (41). We considered the possibility that dsRNA binding serves two functions for dsRAD: to activate the catalytic domain by mediating a conformational change in the protein and to bind the substrate RNA that will be deaminated. So far, we have no evidence in support of this possibility. For example, dsRAD does not undergo detectable phosphorylation following RNA binding that results in activation of deaminase activity (24a), and there is no evidence that dsRAD truncated to lack the N terminus and R motifs possesses deaminase activity (21, 33).

The enzymatic activity of purified natural dsRAD is inhibited by *O*-phenanthroline, a chelator of zinc ions (19). The His residue thought to be important in the binding of Zn^{2+} and a Glu residue believed to be important in proton transfer functions as established from the crystal structures of murine adenosine deaminase (50) and *Escherichia coli* cytidine deaminase (5) are conserved in dsRAD (21, 32, 33). The conserved CHAE motif is present at amino acid positions 909 to 912 within the C-terminal region of the 1,226-aa dsRAD protein sequence, as deduced from the nucleotide sequence of the human cDNA clone (33). Our observation that dsRAD enzyme activity was destroyed by replacement of His-910 with Gln and of Glu-912 with Ala is in full agreement with the recent report of Lai et al. (22), and together the studies provide

direct evidence in support of the notion that the CHAE motif may indeed be involved in the catalytic mechanism of dsRAD.

dsRAD is detected ubiquitously in primary tissues and cell lines (20, 32, 33, 49) and is inducible by IFN (34). The recent finding that this enzyme is induced by IFN raises the possibility that the enzyme may play a role in the antiviral (35, 40, 45) or cell-growth-regulatory (24, 35) actions of IFN. It has been proposed that dsRAD is involved in the biased hypermutation observed in some viruses, most commonly in negative-stranded RNA viruses (6) as exemplified by modification of measles virus RNA (7), but also in other virus types as exemplified by modification of retroviral RNA (13). Although preferential deamination of certain adenosine residues has been demonstrated in vitro with synthetic RNA substrates (30, 36), the basis of the site selectivity presumed to occur in vivo, as illustrated by the postulated modification of hepatitis delta virus and human immunodeficiency virus RNAs (6, 44) and also the cellular mRNAs encoding glutamate-activated cation channel subunits in which one or a few adenosine residues are modified (16, 52), has not been resolved. Curiously, synthetic DNA in the Z conformation also has been demonstrated recently to interact with dsRAD (14). It is tempting to speculate that the repeated nature of the R-motif subdomain found within dsRAD may be important in determining the relative substrate selectivity of the enzyme. However, as demonstrated for the RNA-dependent protein kinase PKR (18, 41), the activity of the enzyme may also be modulated by interaction with other proteins within the cell.

It is now of importance to confirm in vitro with purified dsRAD enzyme preparations that certain viral and cellular RNAs postulated from in vivo studies are indeed substrates for dsRAD and then to examine the requirement for the individual R motifs of dsRAD for the modification of these natural RNAs compared with synthetic dsRNAs. So far this has not been successful. For example, it has not been possible to demonstrate the editing in vitro of GluR-B pre-mRNA by using wild-type dsRAD purified from bovine tissue (29). This may be due to different requirements in the reaction mixture for these highly structured single-stranded RNAs postulated to be substrates of dsRAD, in contrast to established dsRNA substrates. Alternatively, multiple forms of dsRAD enzyme which differ in their substrate specificities and accessory protein requirements may exist. Indeed, biochemical and cell biological evidence consistent with the hypothesis that there are two or more forms of dsRAD in mammalian cells has been obtained (33, 52), although only a single type of cDNA clone has been reported for the enzyme from human, bovine, and rat sources (21, 32, 33).

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REFERENCES

1. **Alberts, B., D. Bray, J. Lewis, M. Raff, K. Roberts, and J. D. Watson.** 1994. Molecular biology of the cell, 3rd ed. Garland Publishing Co., New York.
2. **Bass, B. L.** 1991. The dsRNA unwinding/modifying activity: fact and fiction. Semin. Dev. Biol. **3:**425–432.
3. **Bass, B. L., and H. Weintraub.** 1988. An unwinding activity that covalently modifies its double-stranded RNA substrate. Cell **55:**1089–1098.
4. **Bass, B. L., H. Weintraub, R. Cattaneo, and M. A. Billeter.** 1989. Biased hypermutation of viral RNA genomes could be due to unwinding/modification of double-stranded RNA. Cell **56:**331.
5. **Betts, L., S. Xiang, S. A. Short, R. Wolfenden, and C. W. Carter, Jr.** 1994. Cytidine deaminase. The 2.3 Å crystal structure of an enzyme: transition-state analog complex. J. Mol. Biol. **235:**635–656.
6. **Cattaneo, R.** 1994. Biased (A→I) hypermutation of animal RNA virus genomes. Curr. Opin. Genet. Dev. **4:**895–900.
7. **Cattaneo, R., and M. A. Billeter.** 1992. Mutations and A/I hypermutations in measles virus persistent infections. Curr. Top. Microbiol. Immunol. **176:**63–74.
8. **Cattaneo, R., A. Schmid, D. Eschle, K. Baczko, V. ter Meulen, and M. A. Billeter.** 1988. Biased hypermutation and other genetic changes in defective measles viruses in human brain infections. Cell **55:**255–265.
9. **Chang, H.-W., and B. L. Jacobs.** 1993. Identification of a conserved motif that is necessary for binding of the vaccinia virus E3L gene products to double-stranded RNA. Virology **194:**537–547.
10. **Gatignol, A., C. Buckler, and K.-T. Jeang.** 1993. Relatedness of an RNA-binding motif in human immunodeficiency virus type 1 TAR RNA-binding protein TRBP to human P1/dsI kinase and *Drosophila Staufen*. Mol. Cell. Biol. **13:**2193–2202.
11. **Green, S. R., L. Manche, and M. B. Mathews.** 1995. Two functionally distinct RNA-binding motifs in the regulatory domain of the protein kinase DAI. Mol. Cell. Biol. **15:**358–364.
12. **Green, S. R., and M. B. Mathews.** 1992. Two RNA binding motifs in the double-stranded RNA activated protein kinase, DAI. Genes Dev. **6:**2478–2490.
13. **Hajjar, A. M., and M. L. Linial.** 1995. Modification of retroviral RNA by double-stranded RNA adenosine deaminase. J. Virol. **69:**5878–5882.
14. **Herbert, A., K. Lowenhaupt, J. Spitzner, and A. Rich.** 1995. Chicken double-stranded RNA adenosine deaminase has apparent specificity for Z-DNA. Proc. Natl. Acad. Sci. USA **92:**7550–7554.
15. **Higuchi, M., F. N. Single, M. Kohler, B. Sommer, R. Sprengel, and P. H. Seeburg.** 1993. RNA editing of AMPA receptor subunit GluR-B: a base-paired intron-exon structure determines position and efficiency. Cell **75:**1361–1370.
16. **Hollmann, M., and S. Heinemann.** 1994. Cloned glutamate receptors. Annu. Rev. Neurosci. **17:**31–108.
17. **Hough, R. F., and B. L. Bass.** 1994. Purification of the *Xenopus laevis* double-stranded RNA adenosine deaminase. J. Biol. Chem. **269:**9933–9939.
18. **Katze, M. G.** 1995. Regulation of the interferon-induced PKR: can viruses cope? Trends Microbiol. **3:**75–78.
19. **Kim, U., T. L. Garner, T. Sanford, D. Speicher, J. M. Murray, and K. Nishikura.** 1994. Purification and characterization of double-stranded RNA adenosine deaminase from bovine nuclear extracts. J. Biol. Chem. **269:**13480–13489.
20. **Kim, U., and K. Nishikura.** 1993. Double-stranded RNA adenosine deaminase as a potential mammalian RNA editing factor. Semin. Cell Biol. **4:**285–293.
21. **Kim, U., Y. Wang, T. Sanford, Y. Zeng, and K. Nishikura.** 1994. Molecular cloning of cDNA for double-stranded RNA adenosine deaminase, a candidate enzyme for nuclear RNA editing. Proc. Natl. Acad. Sci. USA **91:**11457–11461.
22. **Lai, F., R. Drakas, and K. Nishikura.** 1995. Mutagenic analysis of double-stranded RNA adenosine deaminase, a candidate enzyme for RNA editing of glutamate-gated ion channel transcripts. J. Biol. Chem. **270:**17098–17105.
23. **Lasky, S. R., B. L. Jacobs, and C. E. Samuel.** 1982. Characterization of sites of phosphorylation in the interferon-induced phosphoprotein P1 from mouse fibroblasts: evidence for two forms of P1. J. Biol. Chem. **257:**11087–11093.
24. **Lengyel, P.** 1993. Tumor suppressor genes: news about the interferon connection. Proc. Natl. Acad. Sci. USA **90:**5893–5895.

24a. **Liu, Y., and C. E. Samuel.** Unpublished data.

25. **Lomeli, H., J. Mosbacher, T. Melcher, T. Hoger, J. R. P. Geiger, T. Kuner, H. Monyer, M. Higuchi, A. Bach, and P. H. Seeburg.** 1994. Control of kinetic properties of AMPA receptor channels by nuclear RNA editing. Science **266:**1709–1712.
26. **McCormack, S. J., L. G. Ortega, J. P. Doohan, and C. E. Samuel.** 1994. Mechanism of interferon action: motif I of the interferon-induced RNA-dependent protein kinase PKR is sufficient to mediate RNA-binding activity. Virology **198:**92–99.
27. **McCormack, S. J., D. C. Thomis, and C. E. Samuel.** 1992. Mechanism of interferon action: identification of a RNA binding domain within the N-terminal region of the human RNA-dependent P1/eIF-2α protein kinase. Virology **188:**47–56.
28. **McMillan, N. A. J., B. W. Carpick, B. Hollis, W. M. Toone, M. Zamanian-Daryoush, and B. R. G. Williams.** 1995. Mutational analysis of the double-stranded RNA binding domain of the dsRNA-activated protein kinase, PKR. J. Biol. Chem. **270:**2601–2606.
29. **Melcher, T., S. Maas, M. Higuchi, W. Keller, and P. H. Seeburg.** 1995. Editing of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor GluR-B pre-mRNA in vitro reveals site-selective adenosine to inosine conversion. J. Biol. Chem. **270:**8566–8570.
30. **Nishikura, K., C. Yoo, U. Kim, J. M. Murray, P. A. Estes, F. E. Cash, and S. A. Liebhaber.** 1991. Substrate specificity of the dsRNA unwinding/modifying activity. EMBO J. **10:**3523–3532.
31. **O'Connell, M. A., and W. Keller.** 1994. Purification and properties of double-stranded RNA-specific adenosine deaminase from calf thymus. Proc. Natl. Acad. Sci. USA **91:**10596–10600.
32. **O'Connell, M. A., S. Krause, M. Higuchi, J. J. Hsuan, N. F. Totty, A. Jenny,**

and W. Keller. 1995. Cloning of cDNAs encoding mammalian double-stranded RNA-specific adenosine deaminase. Mol. Cell. Biol. **15:**1389–1397.
33. **Patterson, J. B., and C. E. Samuel.** 1995. Expression and regulation by interferon of a double-stranded-RNA-specific adenosine deaminase from human cells: evidence for two forms of the deaminase. Mol. Cell. Biol. **15:**5376–5388.
33a. **Patterson, J. B., and C. E. Samuel.** Unpublished data.
34. **Patterson, J. B., D. C. Thomis, S. L. Hans, and C. E. Samuel.** 1995. Mechanism of interferon action: double-stranded RNA-specific adenosine deaminase from human cells is inducible by alpha and gamma interferons. Virology **210:**508–511.
35. **Pestka, S., J. A. Langer, K. C. Zoon, and C. E. Samuel.** 1987. Interferons and their actions. Annu. Rev. Biochem. **56:**727–777.
36. **Polson, A. G., and B. L. Bass.** 1994. Preferential selection of adenosines for modification by double-stranded RNA adenosine deaminase. EMBO J. **13:** 5701–5711.
37. **Saiki, R. K., S. Scharf, F. Faloona, K. B. Mullis, G. T. Horn, H. A. Erlich, and N. Arnheim.** 1985. Enzymatic amplification of b-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science **230:**1350–1354.
38. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
39. **Samuel, C. E.** 1979. Mechanism of interferon action: phosphorylation of protein synthesis initiation factor eIF-2 in interferon-treated human cells by a ribosome-associated protein kinase possessing site specificity similar to hemin-regulated rabbit reticulocyte kinase. Proc. Natl. Acad. Sci. USA **76:** 600–604.
40. **Samuel, C. E.** 1991. Antiviral actions of interferon: interferon-regulated cellular proteins and their surprisingly selective antiviral activities. Virology **183:**1–11.
41. **Samuel, C. E.** 1993. The eIF-2α protein kinases, regulators of translation in eukaryotes from yeasts to humans. J. Biol. Chem. **268:**7603–7606.
42. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
43. **Schumacher, J. M., K. Lee, S. Edelhoff, and R. E. Braun.** 1995. Tenr, an RNA-binding protein that is distributed in a lattice-like network within the spermatid nucleus. Biol. Reprod. **52:**1274–1283.
44. **Scott, J.** 1995. A place in the world for RNA editing. Cell **81:**833–836.
45. **Sen, G. C., and P. Lengyel.** 1992. The interferon system: a bird's eye view of its biochemistry. J. Biol. Chem. **267:**5017–5020.
46. **St. Johnston, D., N. H. Brown, J. G. Gall, and M. Jantsch.** 1992. A conserved double-stranded RNA-binding domain. Proc. Natl. Acad. Sci. USA **89:**10979–10983.
47. **Thomis, D. C., J. P. Doohan, and C. E. Samuel.** 1992. Mechanism of interferon action: cDNA structure, expression and regulation of the interferon-induced, RNA-dependent P_1/eIF-2α protein kinase from human cells. Virology **188:**33–46.
48. **Wagner, R. W., J. E. Smith, B. S. Cooperman, and K. Nishikura.** 1989. A double-stranded RNA unwinding activity introduces structural alterations by means of adenosine to inosine conversions in mammalian cells and Xenopus eggs. Proc. Natl. Acad. Sci. USA **86:**2647–2651.
49. **Wagner, R. W., C. Yoo, L. Wrabetz, J. Kamholz, J. Buchhalter, N. F. Hassan, K. Khalili, S. U. Kim, B. Perussia, F. A. McMorris, and K. Nishikura.** 1990. Double-stranded RNA unwinding and modifying activity is detected ubiquitously in primary tissues and cell lines. Mol. Cell. Biol. **10:**5586–5590.
50. **Wilson, D. K., F. B. Rudolph, and F. A. Quiocho.** 1991. Atomic structure of adenosine deaminase complexed with a transition-state analog: understanding catalysis and immunodeficiency mutations. Science **252:**1278–1284.
51. **Wilson, R., R. Alnscough, K. Anderson, C. Baynes, M. Berks, J. Bonfield, J. Burton, M. Connell, T. Copsey, J. Cooper, A. Coulson, M. Craxton, S. Dear, Z. Du, R. Durbin, A. Favello, A. Fraser, L. Fulton, A. Gardner, P. Green, T. Hawkins, L. Hillier, M. Jier, L. Johnston, M. Jones, J. Kershaw, J. Kirsten, N. Laisster, P. Latrelille, J. Lightning, C. Lloyds, B. Mortimore, M. O'Callaghan, J. Parson, C. Percy, L. Rifken, A. Roopra, D. Saunders, R. Shownkeen, M. Sims, N. Smaldron, A. Smith, M. Smith, E. Sonnhammer, R. Staden, J. Suston, J. Thlerry-Mleg, K. Thomas, M. Vaudin, K. Vaughan, R. Waterston, A. Watson, L. Weinstock, J. Wilkinson-Sproat, and P. Wohldman.** 1994. 2.2 Mb of contiguous nucleotide sequence from chromosome III of *C. elegans*. Nature (London) **368:**32–38.
52. **Yang, J.-H., P. Sklar, R. Axel, and T. Maniatis.** 1995. Editing of glutamate receptor subunit B pre-mRNA in vitro by site-specific deamination of adenosine. Nature (London) **374:**77–81.