

Purification and properties of double-stranded RNA-specific adenosine deaminase from calf thymus

(inosine/RNA modification/RNA editing/RNA-protein interaction)

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ABSTRACT A double-stranded RNA-specific adenosine deaminase, which converts adenosine to inosine, has been purified to homogeneity from calf thymus. The enzyme was purified $\approx 340,000$ -fold by a series of column chromatography steps. The enzyme consists of a single polypeptide with a molecular mass of 116 kDa as determined by electrophoresis on a SDS/polyacrylamide gel. The native protein sediments at 4.2 s in glycerol gradients and has a Stokes radius of 42 Å upon gel-filtration chromatography. This leads to an estimate of $\approx 74,100$ for the native molecular weight, suggesting that the enzyme exists as a monomer in solution. Enzyme activity is optimal at 0.1 M KCl and 37°C. Divalent metal ions or ATP is not required for activity. The K_m for double-stranded RNA substrate is $\approx 7 \times 10^{-11}$ M. The V_{max} is $\approx 10^{-9}$ mol of inosine produced per min per mg and the K_{cat} is 0.13 min $^{-1}$.

Double-stranded RNA-specific adenosine deaminase (dsRNA adenosine deaminase) was first discovered in extracts from *Xenopus laevis* (1, 2). The enzyme was originally thought to be an RNA helicase and was called dsRNA unwinding/modifying enzyme. Subsequent studies have shown that the enzyme does not actively unwind dsRNAs but destabilizes them by converting adenosine (A) to inosine (I), which results in inosine-uracil (I-U) base pairing (3, 4). The I-U base pairing is less stable than the Watson-Crick A-U base pairing; therefore, the RNA duplex becomes increasingly unstable as the enzyme modifies it. The mechanism for converting A to I is hydrolytic deamination (5). The enzyme is ubiquitous in metazoans; it is found in mammalian tissues as well as in tissue culture cells (6) and in the silkworm *Bombyx mori* (7). The other enzymes that can convert A to I, adenosine deaminase and 5'-adenylic acid deaminase, cannot use dsRNA as substrate for the reaction (4).

The physiological function of dsRNA adenosine deaminase is not known. The enzyme has been implicated in modifying a specific single A residue in the transactivation response element of human immunodeficiency virus 1 (HIV-1) RNA at position +27, where Tat binds to RNA folded into a stem-loop structure (8, 9). It is not certain whether this modification has any effect on the infection of mammalian cells by HIV-1. In other instances, the enzyme appears to act non-specifically in that it will deaminate many A residues present in a stretch of duplex RNA sequence. This mechanism has been proposed to occur in persistent measles virus infection (10), where 50% of the A residues in the viral matrix protein mRNA are replaced by guanosine (G) residues in the viral minus-strand RNA after replication (11). The matrix protein is necessary for virus assembly and budding from the cell, and, when it is not expressed in the brain, fatal neuropathic measles infection can result. In this case, the modification of the mRNA prevents translation of functional protein (10).

dsRNA adenosine deaminase may also be responsible for editing the mRNA of glutamate-gated ion-channel proteins in the brain (12). This alteration changes the permeability of the ion channels to calcium (12). In the case of the GluR-B ion-channel subunit a glutamine codon (CAG) is converted to an arginine codon (CGG), and this editing is dependent on the formation of a short intramolecular duplex between exonic and intronic sequences at the site of editing (13). The conversion of CAG to CIG by dsRNA adenosine deaminase would appear as CGG in the cDNA derived from edited mRNA.

We have purified dsRNA adenosine deaminase to homogeneity from calf thymus by chromatography over seven columns. Ion-exchange chromatography was the principal method used, and the final purification was achieved by chromatography on a dsRNA affinity column. Enzyme activity copurifies with a single polypeptide of 116 kDa.

MATERIALS AND METHODS

Preparation of dsRNA Substrate. The dsRNA substrate was prepared by *in vitro* transcription of both the sense and antisense RNA of a shortened form of chloramphenicol acetyltransferase (1). The plasmid pSP65 was digested with *Bam*HI and *Eco*RI and the 548-nucleotide insert was subcloned into the polylinker of Bluescript KS. The sense RNA was transcribed with T7 RNA polymerase (Stratagene) after linearizing the plasmid with *Hind*III to give a 605-nucleotide transcript. The antisense RNA was transcribed with T3 RNA polymerase (Stratagene) after linearizing the plasmid with *Bam*HI to give a 594-nucleotide transcript. Either the sense or antisense transcript was internally labeled with [α - 32 P]ATP (3000 Ci/mmol; 1 Ci = 37 GBq) (Amersham), which was diluted with 0.4 mM unlabeled ATP as described in ref. 1. The amount of dsRNA substrate routinely used in an assay was RNA containing 45 fmol of labeled adenosine, with only one strand of the duplex being labeled. Therefore, the inosine measured came only from the labeled RNA strand, so in theory probably twice the amount of inosine was produced.

Enzyme Assay. The enzyme was assayed by thin-layer chromatography (TLC) of nuclease P1-digested RNA products (3, 4) with minor modifications. The reaction volume was 25 μ l and contained 0.15 mg of tRNA per ml, 5 mM EDTA, and dsRNA, which contained 45 fmol of labeled adenosine and 1–12.5 μ l of enzyme fraction; buffer A [50 mM Tris-HCl (pH 7.9)/50 mM KCl/5 mM EDTA/10% (vol/vol) glycerol/1 mM dithiothreitol (DTT)/0.5 mM phenylmethylsulfonyl fluoride containing 0.7 μ g of pepstatin per ml and 0.4 μ g of leupeptin per ml] was used to make up the remaining volume. If the final salt concentration in the dsRNA adenosine deaminase assay would be higher than 150 mM, aliquots

of the column fractions were dialyzed before assaying. To increase the stability of the enzyme 0.2 mg of bovine serum albumin (BSA) per ml was added to the reaction mixture when assaying the pure column fractions. The assay was performed at 30°C for 1 hr (all values for purification in Table 1 were determined at 30°C). Pure protein was assayed at 37°C, as RNases present in the cruder fractions were more active at the higher temperature. After incubation, 8.3 μ l of 7.5 M ammonium acetate and 300 μ l of ethanol were added and the samples were centrifuged for 30 min at 4°C; the pellets were washed with 70% ethanol and vacuum dried. The pellets were resuspended in 10 μ l of P1 buffer (30 mM KOAc, pH 5.3/10 mM ZnSO₄) and digested with 1.5 μ g of nuclease P1 (Boehringer Mannheim) for 1 hr at 50°C. Unlabeled 5' inosine monophosphate (pI) (Sigma) was added to the reaction mixture as an internal standard, and the digestion products were separated on a cellulose NM 300 TLC plate (Macherey & Nagel) and dried. The chromatographic solvent was saturated (NH₄)₂SO₄/100 mM NaOAc, pH 6/isopropanol (79:19:2). The TLC plates were autoradiographed overnight and then the spots corresponding to 5 pI and 5' adenosine monophosphate (pA) were cut out and assayed in a scintillation counter. One unit of enzyme is defined as the amount required to produce 1 fmol of inosine in 1 min. Since only one strand of the dsRNA is labeled, the units indicate only the 5' pI coming from the labeled strand.

Purification of dsRNA Adenosine Deaminase. All manipulations were carried out at 4°C. Fractions were frozen in liquid nitrogen and stored at -80°C. The principal buffer used in the purification of dsRNA adenosine deaminase was buffer A. Changes in the KCl concentration and other components added are indicated below. The dsRNA adenosine deaminase was purified from 2 kg of calf thymus that had been stored at -80°C. One kilogram of frozen thymus was partially thawed in 2 liters of buffer A, which contained 100 mM KCl, homogenized in a Waring Blendor for 1.5 min, and centrifuged for 1 hr at 15,000 \times g. The supernatant was poured through cheese cloth and loaded directly onto a 4-liter column of DEAE-Sepharose Fast Flow (Pharmacia). The column was washed with 1 column vol of buffer A and the protein was eluted with a 10-liter gradient from 100 to 500 mM KCl in buffer A. The enzyme activity eluted in a broad peak around 300 mM KCl. The second kilogram of thymus was treated similarly and the conductivity of the two pools was adjusted to 300 mM KCl and loaded separately onto a 1-liter Blue Sepharose column preequilibrated at 300 mM KCl in buffer A. The column was washed with 1 column vol of buffer A and protein was eluted with a 4-liter gradient from 300 to 1000 mM KCl in buffer A. The deaminase activity eluted in a broad peak between 480 and 770 mM KCl. The active pools from both Blue Sepharose columns were combined; 10 mM CaCl₂ was added to the pooled fractions and loaded directly onto a 40-ml Ultrogel hydroxyapatite column. The hydroxyapatite column had been preequilibrated with buffer B, which contained 20 mM Tris-HCl (pH 7.9), 50 mM KCl, 10% glycerol, 1 mM DTT, 0.5 mM phenylmethylsulfonyl fluoride, 0.7 μ g of pepstatin per ml, and 0.4 μ g of leupeptin per ml. After loading, the column was washed with 2 column vol of buffer B and protein was eluted with a 10 column vol gradient of 0-500 mM potassium phosphate (pH 7.9) in buffer A. The deaminase eluted between 90 and 185 mM potassium phosphate. The active pool was precipitated with ammonium sulfate (55% saturation) and dialyzed extensively against buffer A. The activity was then loaded onto a 10-ml heparin Sepharose column (Pharmacia), washed with 2 column vol of buffer A, and eluted with an 8 column vol gradient of 50-500 mM KCl in buffer A. The activity eluted at 150-220 mM KCl. The conductivity of the active pool was adjusted to 50 mM KCl by dilution with buffer A minus KCl. At this point 0.02% Nonidet P-40 (NP-40) was added to all buffers and to the

active pool to increase protein stability. The active pool was loaded on a 1-ml Mono Q column. The column was washed with 13 ml of buffer A after loading and the protein was eluted with a 30-ml gradient from 50 to 500 mM KCl in buffer A. The deaminase activity eluted at 150-230 mM KCl. The final column was an affinity column of poly(G)-poly(C) (see below for details of preparation). The active pool was diluted to 50 mM KCl and loaded on a 7-ml poly(G)-poly(C) column, washed with 2 column vol of buffer A at 1.5 column vol per hr, and then developed with a 63-ml gradient from 50 to 1000 mM KCl. The dsRNA adenosine deaminase eluted at \approx 600 mM KCl.

Protein concentration was determined by the Bradford method (14) with BSA as the reference standard, except for the pure protein, which was precipitated with trichloroacetic acid (final concentration, 15%), and electrophoresed on an SDS/8% polyacrylamide gel with different amounts of BSA as reference standard.

dsRNA Affinity Column. One milligram of polycytidylic acid [poly(C)] (Pharmacia) was annealed to 1 mg of short polyguanylic acid [poly(G)] (Boehringer Mannheim) by heating to 85°C followed by slow cooling to 37°C. The poly(G)-poly(C) was coupled to 2 g of cyanogen bromide-activated Sepharose 4B (Pharmacia) in 0.1 M NaHCO₃, pH 8.3/0.5 M NaCl for 2 hr at room temperature. Unreacted sites were blocked by washing with 0.1 M Tris-HCl (pH 8) for 2 hr at room temperature. The resin was ready to use after washing with three cycles of alternating pH: 0.1 M acetate, pH 4/0.5 M NaCl followed by 0.1 M Tris-HCl, pH 8/0.5 M NaCl. The resin was washed at 4°C with 10 column vol of buffer A containing 50 mM KCl before applying the protein sample.

Glycerol Gradient Sedimentation. dsRNA adenosine deaminase was purified in the absence of NP-40 and 500 μ l was concentrated 2:1 in the presence of 0.5 mg of BSA per ml on a Centricon 10 microconcentrator (Millipore). One hundred microliters was applied to a 4.2-ml 15-30% glycerol gradient in buffer A containing 500 mM KCl. One hundred microliters of partially purified dsRNA adenosine deaminase from the Mono Q step was applied to a parallel gradient. Protein standards were applied to parallel gradients: cytochrome *c* (1.9 s), ovalbumin (3.5 s), BSA (4.5 s), aldolase (7.35 s), and catalase (11.3 s). The gradients were spun for 22 hr at 4°C in a Kontron TST 60 rotor at 50,000 rpm (340,000 \times g), 22-24 fractions were collected per gradient, and the standard proteins were identified by their UV absorbance at 280 nm. Three microliters of each fraction was assayed for dsRNA adenosine deaminase activity.

Gel-Filtration Chromatography. A Sephacryl S-300 gel filtration column (1 \times 32 cm) (Pharmacia) was used to determine the Stokes radius of the dsRNA adenosine deaminase. Five hundred microliters of a partially purified fraction from a Mono Q step, which was prepared in the absence of NP-40, was loaded on the column. The column was equilibrated with buffer D (50 mM Tris-HCl, pH 7.9/50 mM KCl/0.2 mM EDTA/2 mM MgCl₂/10% glycerol/1 mM DTT/protease inhibitors). Sixty-two fractions of 0.4 ml were collected and assayed for enzyme activity. Marker proteins were run separately or pairwise and were as follows: ferritin (61 Å), catalase (52.5 Å), aldolase (48.1 Å), ovalbumin (30.5 Å), and chymotrypsinogen (20.9 Å); they were detected by UV absorbance at 280 nm. The void volume was determined with blue dextran.

RESULTS AND DISCUSSION

The dsRNA adenosine deaminase was purified to homogeneity >340,000-fold from 2 kg of calf thymus by chromatography over seven columns (Table 1). Ion-exchange chromatography was the principal method used and the final puri-

Table 1. Purification of dsRNA adenosine deaminase from calf thymus

Fraction	Protein, mg	Activity, units*	Specific activity, units/mg [†]	Purification factor	Recovery, %
Calf thymus extract	72,930	176,974	2.43	1	100
DEAE pool	4,470	149,440	33.43	14	84
Blue Sepharose pool	114.6	62,974	549.5	226	35.6
Hydroxyapatite pool	36.5	29,706	813.4	335	16.7
Heparin Sepharose pool	12.6	35,650	2,820	1,163	20.2
Mono Q pool	2.9	23,354	8,053	3,314	13.2
ds poly(G)-poly(C) pool	0.003	2,491	830,407	341,731	1.4

Enzyme was purified from 2 kg of calf thymus as described.

*One unit = 1 fmol of 5' pI produced per min.

[†]RNA substrate concentration used in the standard assay is below the K_m . Therefore, specific activity of the pure enzyme indicated in the table is underestimated by ≈ 2.6 -fold (see text for further discussion).

fication was achieved by chromatography on an RNA affinity column. The amount of dsRNA adenosine deaminase in the crude calf thymus extract could not be accurately measured because of high levels of RNases; therefore, there may be more activity present in this tissue than is apparent. The

order of the first three columns was chosen to avoid dialysis of the active fractions, which was an advantage considering the large volumes involved. After the ammonium sulfate precipitation there was a decrease in dsRNA adenosine deaminase activity that is probably due to inhibition by

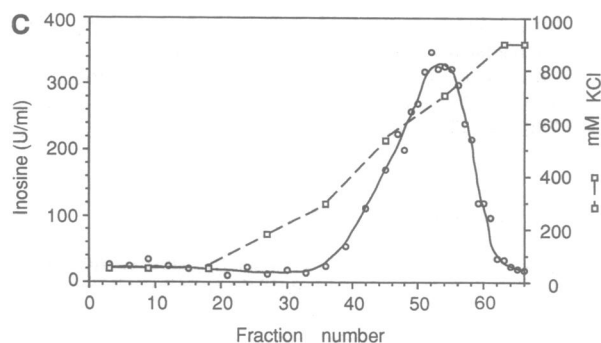
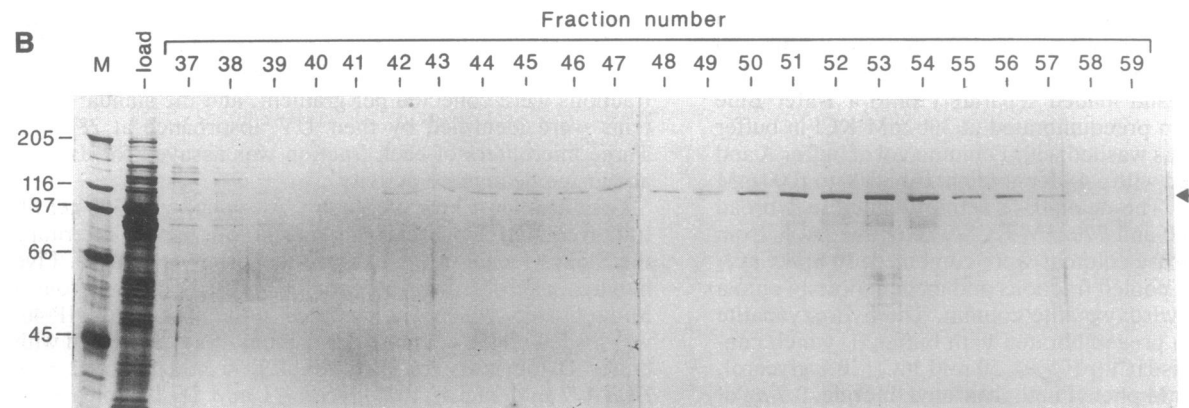
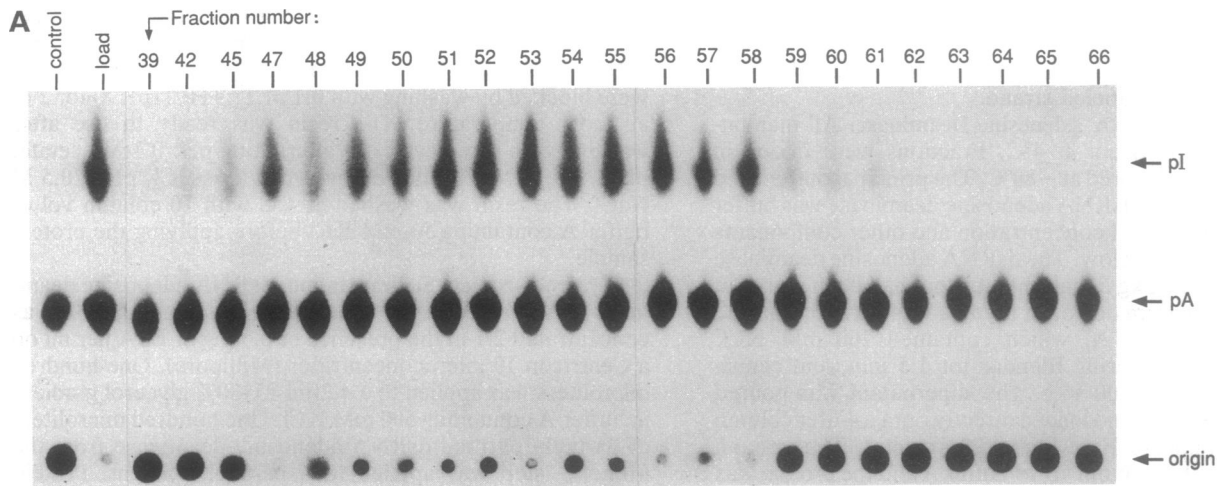


FIG. 1. Chromatography of dsRNA adenosine deaminase on poly(G)-poly(C). (A) Two microliters per fraction from the poly(G)-poly(C) column was assayed for adenosine deaminase activity and the products were chromatographed on TLC plates. Spot at the bottom is the origin; spot in the middle corresponds to 5' pA; spot on the top corresponds to 5' pI. Lane 1, control reaction mixture incubated without protein. (B) SDS electrophoresis. Column fractions (150 μ l) were precipitated with trichloroacetic acid (15%); precipitates were washed twice with cold acetone and dissolved in SDS loading buffer (15). Proteins were electrophoresed on SDS/8% polyacrylamide gels and visualized by staining with Coomassie brilliant blue followed by silver staining. Arrow on right points to 116-kDa polypeptide band. (C) Activity profile of the final poly(G)-poly(C) column; units are $\text{fmol}\cdot\text{min}^{-1}\cdot\text{ml}^{-1}$.

ammonium sulfate, which was still present despite extensive dialysis. This inhibition was reversible as shown by the increase in activity units after the heparin-Sepharose column.

The elution profile of dsRNA adenosine deaminase on the Mono Q column was not always reproducible. Often the activity split, with some appearing in the flow-through and the remainder binding to the column. This was not due to overloading, as when the activity in the flow-through was reapplied to the Mono Q column it again eluted in the flow-through. When both activities were applied separately to dsRNA affinity columns, the same 116-kDa protein was purified (data not shown). There was no significant difference in the specific activity of the dsRNA deaminase purified from the Mono Q flow-through and the bound fraction.

The dsRNA affinity column purified the dsRNA adenosine deaminase 100-fold. The activity profile of this column (Fig. 1C) shows that the enzyme activity coeluted with a protein of 116 kDa (Fig. 1B). This was the major protein present in these fractions when visualized on a SDS/polyacrylamide gel stained with silver. The pure deaminase was very active as 2 μ l was used to assay the column fractions (Fig. 1A) but 150 μ l was precipitated with trichloroacetic acid before being electrophoresed on a SDS/polyacrylamide gel (Fig. 1B). The yield of homogeneous enzyme is extremely low, 3–10 μ g per kg of calf thymus.

The poly(G) used to prepare this column was very short, \approx 20 nucleotides. This was important, as when longer poly(G) was used the enzyme did not bind with the same high affinity. The poly(C) used seemed not to be as critical as the poly(G) and was heterogeneous in length, with an average length of 500 nucleotides. Therefore, this poly(G)·poly(C) column probably had both double-stranded and single-stranded regions and it may be this structure the enzyme recognized and bound to. The RNA affinity column used by Hough and Bass (16) to purify the dsRNA adenosine deaminase from *X. laevis* was also a poly(G)·poly(C) column but differed from the one we prepared in that the poly(G) and poly(C) were coupled to the cyanogen bromide-activated Sepharose 4B separately and were then annealed. When we used this method to prepare the dsRNA affinity column, the dsRNA adenosine deaminase eluted at the same KCl concentration, \approx 600 mM, as it had with the other poly(G)·poly(C) affinity column.

Reaction Requirements. The pure dsRNA adenosine deaminase alone can convert A to I, A is the only nucleotide that is deaminated, and the activity is dependent on dsRNA and will not modify single-stranded RNA (data not shown). The deaminase does not require ATP or any other cofactor. In competition experiments with 100 ng of poly(A), poly(U), poly(C), and poly(G), only poly(G) inhibited the dsRNA adenosine deaminase by 93%, suggesting that some higher-order structure of the poly(G) is recognized by the dsRNA adenosine deaminase. It is known that poly(G), unlike other homopolymers, rarely exists as single strands but tends to form four-stranded helical hydrogen-bonded complexes (17). Either long or short poly(G) can compete with the dsRNA adenosine deaminase. The other polynucleotides [poly(A), poly(C), poly(U)] inhibit the enzyme by only 10–20% (data not shown).

The temperature optimum of the dsRNA adenosine deaminase is 37°C. The enzyme has a broad pH optimum between pH 7 and 8 and the optimal KCl concentration is between 75 and 125 mM (data not shown). Below 50 mM there is a sharp reduction in activity as the salt is probably necessary to keep the RNA substrate in double-stranded conformation. At higher KCl concentrations (>200 mM), there is >90% inhibitory effect on the enzyme; this is also observed with 200 mM NaCl and 200 mM (NH₄)₂SO₄ (data not shown). The deaminase is very sensitive to *N*-ethylmaleimide (NEM) and is completely inhibited at 5 mM NEM. This inhibition could be

prevented by prior addition of 5 mM DTT, which suggests that sulfhydryl bonds are important for enzyme activity.

Physical Properties and Kinetic Constants. On an SDS/polyacrylamide gel, the dsRNA adenosine deaminase protein has a molecular mass of 116 kDa (Fig. 1B). There are two minor bands near 96 kDa that varied in intensity in different protein preparations and are probably degradation products. The sedimentation coefficient was determined by centrifugation through glycerol gradients with pure detergent-free protein (Fig. 2). The glycerol gradients contained 0.5 M KCl to prevent protein aggregation. The sedimentation coefficient of 4.2 *s* was similar to that obtained with partially purified protein. A sedimentation coefficient of 4.2 *s* corresponds to a globular protein of \approx 60 kDa. This is much lower than the value obtained by gel filtration (see below) and by denaturing gel electrophoresis. It suggests that dsRNA adenosine deaminase has an asymmetrical shape in solution.

The Stokes radius determined by gel filtration was 42 Å (Fig. 3). The protein sample used in Fig. 3 was partially purified but the same result was obtained with pure protein. Using the above value and the sedimentation constant, the dsRNA adenosine deaminase was calculated to have a native molecular weight of 74,100 (18). This value is considerably lower than the denatured molecular weight of the polypeptide obtained upon electrophoresis. The underestimate is due to the aberrantly low sedimentation constant, in comparison to the sedimentation of the protein standards. The *s* value and the Stokes radius indicate that the enzyme is a monomer in solution.

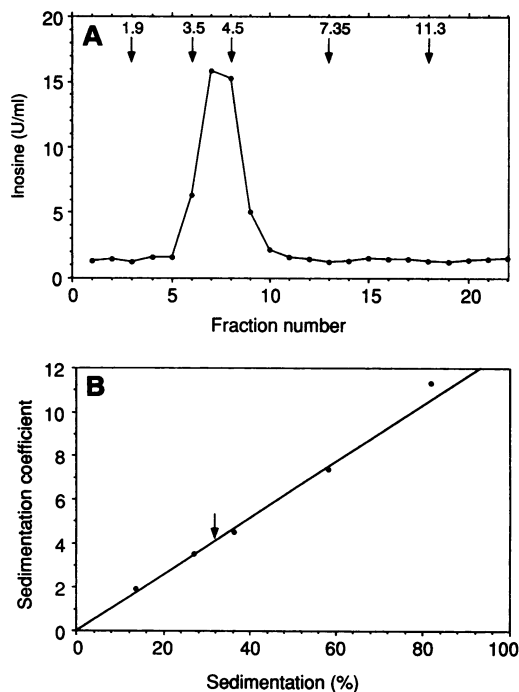


FIG. 2. Sedimentation coefficient of dsRNA adenosine deaminase. One hundred microliters of pure detergent-free dsRNA adenosine deaminase was concentrated 2:1 in the presence of BSA (0.5 mg/ml) with a Centricon 10 microconcentrator and applied to a 4.2-ml 15–30% glycerol gradient in buffer A containing 0.5 M KCl. (A) Sedimentation pattern of dsRNA adenosine deaminase. The enzyme was detected by activity assay and units are $\text{fmol}\cdot\text{min}^{-1}\cdot\text{ml}^{-1}$. Protein standards were applied to parallel gradients (cytochrome *c*, ovalbumin, BSA, aldolase, and catalase) and were detected by UV absorbance. Arrows indicate positions of markers and their sedimentation coefficients. (B) Data from A were replotted and position of each protein is expressed as a percentage of the total number of fractions recovered from the gradient. Position of dsRNA adenosine deaminase is indicated by an arrow.

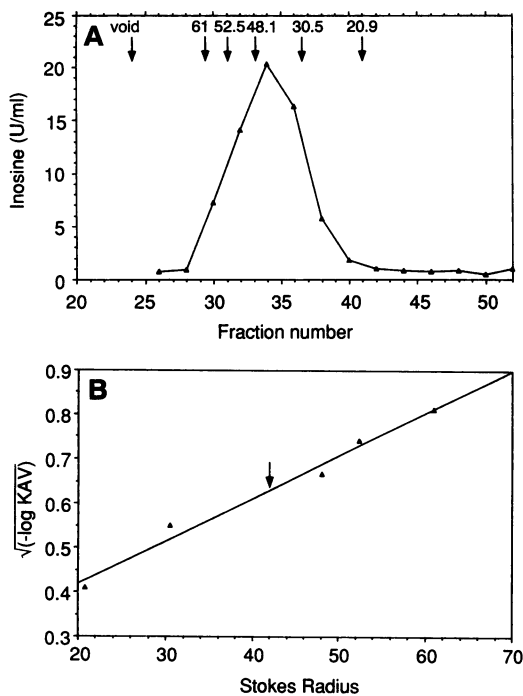


FIG. 3. Determination of Stokes radius by gel filtration. Partially purified dsRNA adenosine deaminase from a Mono Q step that was purified in the absence of detergent was loaded on a Sephacryl S-300 gel-filtration column (1 × 32 cm). (A) Elution profile of the dsRNA adenosine deaminase, which was detected by an activity assay. Units are $\text{fmol} \cdot \text{min}^{-1} \cdot \text{ml}^{-1}$. Arrows indicate positions of marker proteins and their radii. They were run separately or pairwise and were ferritin, catalase, aldolase, ovalbumin, and chymotrypsinogen. (B) Data from A were plotted according to Siegel and Monty (18); position of dsRNA adenosine deaminase is indicated by an arrow and positions of marker proteins are indicated by triangles.

The K_m and the V_{max} were determined by varying the dsRNA concentration in the assay from 0.09 to 3.6 fmol of dsRNA and the units were taken as the average of four independent experiments. The K_m for dsRNA was measured to be $\approx 7 \times 10^{-11}$ M. Therefore, the substrate concentration used in the standard assay (0.36 fmol of dsRNA) during the purification was below the K_m . The true specific activity of the pure dsRNA adenosine deaminase is probably 2.6-fold higher than is indicated in Table 1. The V_{max} was determined to be $\approx 10^{-9}$ mol of inosine produced per min per mg and the catalytic constant K_{cat} is $\approx 0.13 \text{ min}^{-1}$. These values are only approximate as the protein concentration in the highly purified enzyme fractions could not be measured precisely. Therefore, the values calculated for V_{max} and K_{cat} could differ by a factor of ≈ 3 .

Comparison to the dsRNA Adenosine Deaminase from *Xenopus* and from Bovine Liver. A dsRNA adenosine deaminase has recently been described from *X. laevis* (16) and has properties similar to the protein we have purified. The *Xenopus* enzyme has a denatured molecular mass of 120 kDa. Although the assay conditions of both proteins are different (the standard assay of the *Xenopus* enzyme was performed at 25°C for 1–3 hr with 2–50 fmol of dsRNA substrate), the specific activity of both proteins is similar; the dsRNA adenosine deaminase from *X. laevis* has a specific activity of 120,000 pmol of inosine per hr per mg and the bovine enzyme has a specific activity of 129,500 pmol of inosine per hr per mg. Both proteins have similar activity requirements—they are inhibited by high salt, either KCl or NaCl, and by NEM but are unaffected by addition of BSA, EDTA, or any nucleotide.

dsRNA adenosine deaminase has also been purified from bovine liver nuclear extracts (19). The most purified fractions contained three polypeptides of 93, 88, and 83 kDa, which are probably the result of partial proteolysis of a larger protein. These polypeptides were not seen in our pure fractions but as this dsRNA adenosine deaminase was purified from a different organ it could be that different tissues contain different forms of the enzyme. This issue will be resolved when cDNA clones coding for the enzyme become available. The purification procedure is different from the one we used and also the specific activity of the pure liver enzyme is approximately half the specific activity of both the *X. laevis* and the calf thymus proteins. But it must be stressed that a different substrate was used (c-myc) and that the assay conditions were different as the dsRNA adenosine deaminase purified from bovine liver extracts was measured in the presence of 200 mM KCl, which inhibits the thymus enzyme by >90%.

Until now we have used a total of 7 kg of calf thymus to purify sufficient protein to obtain a peptide sequence. These peptide sequences were used to isolate a partial clone from a bovine cDNA library. A fusion protein was made with this cDNA clone carrying a histidine-affinity tag. Rabbit polyclonal antibodies were raised against fusion protein expressed in *Escherichia coli*. This antibody recognizes a 116-kDa protein in pure enzyme preparations and in crude calf thymus extracts (unpublished data). The antibody also recognizes a 116-kDa protein that is present only in the peak activity fractions from the gel-filtration column and in the glycerol gradient fractions. Since this antibody can also inhibit the deaminase activity, we are confident that the 116-kDa protein we have purified corresponds to the dsRNA adenosine deaminase.

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