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Cloning and expression of a cDNA encoding a bovine brain brefeldin A-sensitive guanine nucleotide-exchange protein for ADP-ribosylation factor

NAOKO MORINAGA*, JOEL MOSS, AND MARTHA VAUGHAN

Pulmonary-Critical Care Medicine Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892

Contributed by Martha Vaughan, September 15, 1997

ABSTRACT A 200-kDa guanine nucleotide-exchange protein (p200 or GEP) for ADP-ribosylation factors 1 and 3 (ARF1 and ARF3) that was inhibited by brefeldin A (BFA) was purified earlier from cytosol of bovine brain cortex. Amino acid sequences of four tryptic peptides were 47% identical to that of Sec7 from *Saccharomyces cerevisiae*, which is involved in vesicular trafficking in the Golgi. By using a PCR-based procedure with two degenerate primers representing sequences of these peptides, a product similar in size to Sec7 that contained the peptide sequences was generated. Two oligonucleotides based on this product were used to screen a bovine brain library, which yielded one clone that was a partial cDNA for p200. The remainder of the cDNA was obtained by 5′ and 3′ rapid amplification of cDNA ends (RACE). The ORF of the cDNA encodes a protein of 1,849 amino acids (≈208 kDa) that is 33% identical to yeast Sec7 and 50% identical in the Sec7 domain region. On Northern blot analysis of bovine tissues, a ≈7.4-kb mRNA was identified that hybridized with a p200 probe; it was abundant in kidney, somewhat less abundant in lung, spleen, and brain, and still less abundant in heart. A six-His-tagged fusion protein synthesized in baculovirus-infected Sf9 cells demonstrated BFA-inhibited GEP activity, confirming that BFA sensitivity is an intrinsic property of this ARF GEP and not conferred by another protein component of the complex from which p200 was originally purified.

ADP-ribosylation factor (ARF), initially identified as an activator of cholera toxin-catalyzed ADP ribosylation, is now known to function in vesicular transport in the endoplasmic reticulum and Golgi (1), as well as to activate phospholipase D (2, 3). ARFs are ubiquitous in eukaryotic cells (4). Six known mammalian ARFs are grouped into three classes based on phylogenetic analysis, amino acid sequence, size, and gene structure (5): ARF1, 2, and 3 in class I; ARF4 and 5 in class II; and ARF6 in class III.

All ARFs, like similar GTPases, work as molecular switches by cycling between inactive GDP-bound and active GTP-bound states (4). In cytosol, ARF exists in the GDP-bound form and replacement of GDP by GTP (i.e., activation) is accelerated by guanine nucleotide-exchange proteins or GEPs. ARF GEP activities have been identified in Golgi and cytosolic fractions (6–8). ARF GEP, partially purified from bovine brain cytosol, was not inhibited by brefeldin A (BFA), a fungal fatty acid derivative that reversibly causes apparent disintegration of Golgi structure (8). Cytosol also contained a BFA-sensitive GEP activity that was associated with an ≈670-kDa complex; a 200-kDa BFA-sensitive GEP was purified from this complex (9). Amino acid sequences of tryptic peptides suggested that the 200-kDa protein might be a mammalian analogue of yeast Sec7, which is critical in vesicular protein transport (10, 11). On the basis of the assumption that a Sec7 domain might possess ARF GEP activity, a gene found in human lymphocytes, termed B2–1, which contains a Sec7 domain (12–14), was expressed in *Escherichia coli*. Its protein product, known as cytohesin 1, exhibited BFA-insensitive ARF GEP activity (15). Cytohesin appeared to be similar in physical and kinetic properties to an ≈55-kDa BFA-insensitive GEP that had been purified previously from rat spleen (16).

Consonant with these observations, two yeast ARF GEPs, Gea1 and Gea2, which have Sec7 domains, were cloned as plasmids that in multiple copies suppressed the growth defect of cells expressing a mutant ARF1 (17). The proteins, which exhibited BFA-sensitive GEP activity, have a function in Golgi transport. Sec7 domains have also been found in plant cell and *Caenorhabditis elegans* proteins (13, 14). A human homologue of Gea1 was identified in an expressed sequence tag database and cloned. The 47-kDa protein named ARNO (18) has a Sec7 domain but its GEP activity was BFA-insensitive. Thus far, no gene for a BFA-sensitive mammalian ARF GEP has been described. As reported herein, cloning the cDNA for p200 from a bovine brain library has enabled us to demonstrate that the 200-kDa protein synthesized in Sf9 cells is a BFA-sensitive ARF GEP.

MATERIALS AND METHODS

Materials. [α-^{32}P]dATP (6,000 Ci/mmol; 1 Ci = 37 GBq), [γ-^{32}P]ATP (6,000 Ci/mmol), and guanosine 5′-[γ-[^{35}S]thio]-triphosphate ([^{35}S]GTP[γS]) (1,250 Ci/mmol) were purchased from Dupont/NEN.

Cloning of the cDNA. Amino acid sequences of peptides from the 200-kDa ARF GEP that had been purified from bovine brain (9), EVMYAYVDQHDFSGK (pk57) and PEEYLSAIYNEIAGK (pk118), which were present also in yeast Sec7, were used to design degenerate oligonucleotides for PCR. Primer 1, 5′-ATGTA(C/T)GCITA(C/T)GTI-GA(C/T)CA(A/G)CA(C/T)GA(C/T)TT and primer 2, 5′-TTICCIGC(T/A/G)AT(T/C)TC(G/A)TT(G/A)TA(T/A/G)AT were used to amplify cDNA in a bovine brain library in λgt11 (CLONTECH). PCR, in 100 μl, of a mixture containing all four dNTPs (each at 200 μM), 200 pmol of each of the primers, and *Taq* polymerase was initiated with incubation at 94°C for 4 min, followed by 35 cycles of 95°C for 1 min, 50°C for 1 min, and 72°C for 1 min, and finally incubation at 72°C

Abbreviations: ARF, ADP-ribosylation factor; GEP, guanine nucleotide-exchange protein; BFA, brefeldin A; RACE, rapid amplification of cDNA ends.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AF023451).

*To whom correspondence should be addressed at: Room 5N-307, Building 10, 10 Center Drive, MSC 1434, National Institutes of Health, Bethesda, MD 20892-1434.

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for 15 min. Amplification products (≈350 bp) were inserted into pCR2.1 (Invitrogen) by T-A ligation and sequenced by using T7 and M13 reverse primers. Sequences identical to those of peptides pK57 and pK118 were found in the deduced amino acid sequences.

Two 50-mer oligonucleotides with sequences from this clone were end-labeled with [γ-^{32}P]ATP and used as probes to screen the λgt11 bovine brain cDNA library (3×10^5 plaque-forming units). Hybridization was done in 5× Denhardt's solution/0.25% SDS/1× SSPE (0.15 M NaCl/10 mM sodium phosphate, pH 7.4/1 mM EDTA) containing denatured salmon sperm DNA (100 μg/ml) and radiolabeled probe (5×10^5 cpm/ml) at 55°C overnight. Filters were finally washed with 2× standard saline citrate (SSC)/0.05% SDS at 55°C for 15 min. One positive plaque, which hybridized with both probes, was obtained. A 3.4-kb cDNA from the single purified clone was excised with *Bsi*W1, gel-purified, made blunt-ended with the Klenow fragment of DNase polymerase I, and ligated into pUC18, which had been digested with *Sma*I. This clone, named 200A, was used to transform DH5α competent cells.

5′ Rapid Amplification of cDNA Ends (RACE). The 5′ end of the clone was obtained by using a Marathon cDNA amplification kit (CLONTECH). A library of adaptor (company supplied)-ligated double-stranded cDNA was made from bovine brain poly(A)$^+$ RNA by using the gene-specific primer (5′-TCATACCAAGTTCCTGACTGCCTCGTCC). Primers for 5′ RACE (19) were a gene-specific reverse primer, GSP1 (5′-CACTCTGAGCATCTGCACAAATCC), and an AP1 primer (company supplied). PCR using Advantage KenTaq polymerase mixture (CLONTECH) was carried out with incubation at 94°C for 1 min; five cycles of 94°C for 30 sec and 72°C for 4 min; five cycles of 94°C for 30 sec and 70°C for 4 min; 28 cycles of 94°C for 20 sec and 68°C for 4 min. The largest (≈1.7 kb) of several 5′ RACE products detected by Southern blot analysis using the oligonucleotide 5′-CCACAAATGGATGGTTATTCAG as a probe, was excised from the gel and subcloned in pCR2.1 (Invitrogen) by T-A ligation. As this RACE product did not contain an initiating methionine codon, a second 5′ RACE was done using the AP1 primer and another gene-specific reverse primer, 5′-CTAACGCCACCTCGCACGTTTG. Subsequently, a nested PCR was carried out with AP2 (company supplied) and the gene-specific reverse primer 5′-ATGCGCCTTCTTCACTTCCTTGTCG, which yielded a product of about 350 bp that was purified and subcloned in the same vector.

3′ RACE. The adaptor-ligated oligo(dT)-primed double-stranded cDNA made with the CLONTECH kit was used for 3′ RACE. The first PCR primers were AP1 and a gene-specific primer, 5′-TGAAGTAGCCTCTCTTTGCCTGGAAGGT. Amplification with nested PCR primers, AP2 and a gene-specific primer, 5′-GAACAGTACGAGGCAAGAAGG, yielded a single amplified product (3.5 kb), which was gel-purified and inserted into pCR2.1.

DNA Sequencing. Plasmid DNA was purified by using Wizard Plus Minipreps (Promega) from 10 ml of LB broth culture containing ampicillin (50 μg/ml) incubated overnight at 37°C. An ABI373 DNA sequencer was used for DNA sequencing.

Northern Blot Analysis. A Northern blot containing poly(A)$^+$ RNA (2 μg) from several tissues was prepared and incubated at 42°C for 4 hr in 5× SSPE/7.5× Denhardt's solution/50% formamide/2% SDS containing salmon sperm DNA (100 μg/ml), before hybridization overnight at 42°C in the same solution containing [α-^{32}P]dATP-labeled p200 cDNA probe (≈1.3 kb) with sequence representing an N-terminal part of the coding region (bases 43–1,329). Membranes were washed with 2× SSC/0.05% SDS at room temperature for 1 hr and 0.1% SSC/0.05% SDS at 42°C for 30 min followed by autoradiography at −80°C overnight. After stripping to remove labeled probe, the same blot was hybridized with a β-actin DNA.

Construction of a Six-His-Tagged Fusion Protein in pAcHLT-C. DNA sequences encoding amino acids 1,071–1,849 and 580–1,079, respectively, were amplified by PCR from 50 ng of purified plasmid DNA from 3′ RACE using primers A (5′-GGAACAGTACGAGGCAGAGAAGGATCA) and B (5′-AAAAATCGTCGCGGCCGCTCATTGCTTGTTTATTCCAA) and from 200A using primers C (5′-CAAACATATTCATACGACTAGTAAATGATCTGTC) and D (5′-TGATCCTTCTCTGCCTCGTACT). Then, a DNA sequence encoding amino acids 580–1,849 was amplified by PCR from the two PCR products (1 μl of each) using primers C and B. Primer C and reverse primer B were designed to contain, respectively, sites for *Nde*I and *Not*I restriction enzymes. The PCR product was subcloned into vector pAcHLT-C, which had been digested with *Nde*I and *Not*I and treated with alkaline phosphatase before it was used to transform DH5α cells. The resulting plasmid B was purified and sequenced. To amplify the remainder of the cDNA encoding amino acids bases 1–601, primer E (5′-CCTCCTTCTCCATATGCATGTATGAGGGGAAGAAGACGAAGAACATGTTCCTGACCCGGGCCCTGGAGAAGATTTTG), which included a *Nde*I site, and reverse primer GSP1 (5′-CACTCTGAGCATCTGCACAAATCC) were used to amplify DNA from 80 ng of plasmid DNA from the first 5′ RACE clone. The PCR product (1 μl) and the 200A DNA were used for PCR with primer E and reverse primer 5′-TTACTCATACCAAGTTCC. The product was gel-purified and inserted into pCRScript AmpSK(+) (Stratagene), which was used to transform DH5α cells. The resulting plasmid A was purified and sequenced.

To construct the full-length p200 DNA, plasmid B was digested with *Spe*I and *Not*I and plasmid A digested with *Spe*I and *Nde*I. Each insert was gel-purified before the two were ligated into baculovirus transfer vector pAcHLT-C (PharMingen) that had been digested with *Nde*I and *Not*I and treated with alkaline phosphatase. This construct was used to transform DH5α cells. Colonies were selected on LB agar/ampicillin plates; the presence of inserts was verified by digestion of purified plasmids with *Nde*I and *Not*I.

Protein Expression and Purification. Sf9 cells (1×10^6 cells) were cotransfected with 1 μg of Baculogold DNA (PharMingen) and 3 μg of recombinant baculovirus vector DNA containing full-length DNA (total volume, 3 ml). After incubation at 27°C for 5 days, the supernatant was collected; 1 ml was transferred to a plate with 1.2×10^7 cells in 15 ml of medium. Three days later, cells were collected and dispersed in 1 ml of ice-cold 10 mM sodium phosphate, pH 8.0/100 mM NaCl containing 8 μg of benzamidine hydrochloride, 0.5 mM phenylmethylsulfonyl fluoride, and 5 μg of phenanthroline, 5 μg of aprotinin, 5 μg of leupeptin, and 5 μg of pepstatin A. All subsequent procedures were done at 4°C. Cells were lysed (freezing and thawing twice) and cellular debris was removed by centrifugation (16,000 × *g*, 15 min). After verifying the presence of recombinant protein by SDS/PAGE, the clear lysate was incubated (1 hr) with 0.5 ml of Ni-nitrilo-triacetic acid agarose, which was then washed extensively with 20 mM imidazole in 50 mM sodium phosphate, pH 8.0/300 mM NaCl/10% glycerol/0.5 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride until no protein was detected by Bio-Rad protein assay of 200- to 500-μl samples. Bound protein was eluted with 100 mM imidazole in the same buffer at pH 6.0 and dialyzed against 20 mM Tris·HCl, pH 8.0/1 mM EDTA/1 mM NaN_3/1 mM dithiothreitol (DTT)/0.25 M sucrose/5 mM $MgCl_2$/0.5 mM aminoethylbutylsulfonyl fluoride/30 mM NaCl.

Assay of BFA-Sensitive GEP Activity. GEP was assayed by its effect on ARF stimulation of cholera toxin A subunit (CTA)-catalyzed ADP ribosylagmatine synthesis (9). Each sample, with or without BFA (6 μg) and with 10 μg of

Table 1. Amino acid sequences of nine tryptic peptides from p200 present in the deduced sequence of the cloned cDNA

Peptide	Sequence	Positions
pk60	AQGRGSQELGMSN	590–602
pk118	PETINRYGSLN	656–666
pk82	EIIEQGIDLFTK	700–711
pk57	EVMYAYVDQHDFSGK	761–775
pk118	PEEYLSAIYNEIAGK	866–880
pk68	QRRLLYNLEM	906–915
pk54	GELANFRFQK	1,225–1,234
pk80	DFLRPFEHIMK	1,235–1,245
pk63	VDIHDSIQPR	1,570–1,579

Sequences of peptides from purified p200 and their positions in the predicted amino acid sequence of the cloned cDNA are shown.

partially purified mixed ARF (primarily ARF1 and ARF3) prepared from rat spleen cytosol by gel filtration (20), was incubated at room temperature for 10 min in 20 mM Tris·HCl, pH 8.0/1 mM EDTA/1 mM NaN_3/10 mM DTT/ 0.25 M sucrose/5 mM $MgCl_2$/15 μg of bovine serum albumin/20 μg of phosphatidylserine/0.1 μg of leupeptin/0.1 μg of aprotinin/0.1 μg of lima bean trypsin inhibitor/0.1 μg of soybean trypsin inhibitor (total volume, 90 μl). For the second step, 10 μl of 100 μM GTPγS was added, and after incubation at 37°C for 40 min, CTA ADP-ribosyltransferase activity was quantified.

GEP was also assayed by its effect on GTPγS binding to partially purified ARF proteins using a rapid filtration procedure (21). Briefly, samples were incubated without or with BFA as indicated for 20 min at 30°C with 4 μM [^{35}S]GTPγS ($\approx 5 \times 10^6$ cpm) in 20 mM Tris·HCl, pH 8.0/1 mM DTT/5 mM $MgCl_2$/1 mM EDTA containing 50 μg of bovine serum albumin and 20 μg of phosphatidylserine (total volume, 100 μl). Samples were transferred to nitrocellulose filters, which were washed six times with 1 ml of ice-cold buffer (20 mM Tris·HCl, pH 8.0/100 mM NaCl/1 mM DTT/1 mM EDTA/5 mM $MgCl_2$). Filters were dissolved in scintillation fluid for radioassay. Data presented are means ± SEM of values from triplicate determinations.

RESULTS

Cloning of p200 ARF GEP. The complete cDNA of 7,016 bases contains an ORF of 5,547 bases encoding 1,849 amino acids, which include sequences of all nine peptides obtained from tryptic digestion of the purified bovine brain 200-kDa protein (Table 1). The context of the proposed initiator ATG (CGTCC*ATG*T) is not a Kozak consensus sequence (22). It is reported (10) that the first methionine codon in yeast Sec7 similarly does not reside in a consensus sequence for eukaryotic translational initiation. There is an in-frame termination codon (TGA) 153 bp upstream of the ATG. Several attempts with 5′ RACE to find more upstream cDNA failed, with the sequence terminating between the TGA and ATG. In the 3′ noncoding region, a polyadenylylation signal, AATAAA, is present 1,262 bp downstream from TGA, followed after 16 more bases by a 26-bp poly(A)$^+$ tail.

The encoded protein is predicted to have a molecular mass of 207.8 kDa with an amino sequence that is 33% identical to that of yeast Sec7 with which it is aligned in Fig. 1. Near the center, there is a sequence of ≈170 amino acids that is 50% identical to the so-called Sec7 domain of the yeast protein (Fig. 1). On either side are regions that are only 27% identical, followed by segments of >40% identity. The N- and C-terminal regions are less similar (Fig. 1). A major difference between the amino acid sequences of yeast Sec7 and p200 is the presence in yeast Sec7 of a highly acidic domain near the N terminus that is lacking in p200; of amino acids 89–186, 58% are acidic. In this region, the identity of the two proteins is lowest.

Amino acid sequences of Sec7 domains of proteins known to have ARF GEP activity are aligned in Fig. 2. Yeast Sec7 has not been reported to have GEP activity, although the Sec7 domains of ARNO and cytohesin 1 have (15, 18). Gea1 is a yeast protein with ARF GEP activity that is sensitive to BFA (17). ARNO and cytohesin 1 are smaller human proteins and are not inhibited by BFA. Gea1 has 22 amino acids (amino acids 661–682) that are not found in the other proteins. In these Sec7 domains, 35 amino acids are identical (18%); with 40 conservative differences, overall similarity is 39%. Results of individual comparisons are shown in Table 2. The ARNO and cytohesin l Sec7 domains are clearly most similar. The distinctly lower percentage identity (similarity) of Gea1 to the other proteins reflects the presence of 22 extra amino acids.

Tissue Distribution of p200 mRNA. On Northern blots a single transcript of ≈7.4 kb was found in each bovine tissue (Fig. 3). The mRNA was more abundant in kidney than in brain, spleen, or lung and still less abundant in heart.

Function of Recombinant p200 Synthesized in Sf9 Cells. To verify that the cDNA sequence encodes a functional ARF GEP, it was used to synthesize a six-His fusion protein in Sf9 cells. The soluble fraction from lysates of transfected cells contained a faint protein band of ≈200 kDa that was not seen in lysates from non-transfected cells. The lysate containing GEP enhanced ARF activation of CTA-catalyzed ADP-ribosylation of agmatine, and this effect was inhibited by BFA. Control cell lysate did not have ARF GEP activity (data not shown).

The fusion protein purified from lysate by Ni-nitrilotriacetic acid agarose affinity (Fig. 4*A*) exhibited one major band of ≈200 kDa after SDS/PAGE. The purified protein enhanced ARF stimulation of CTA ADP-ribosyltransferase activity in a concentration-dependent manner and this effect was inhibited by 6 μg of BFA (Fig. 4*B*). The purified fusion protein did not itself bind GTPγS but increased GTPγS binding to ARF (Fig. 4*C*). BFA inhibition of GEP activity in this assay appeared somewhat greater than that in the CTA assay for reasons that are not clear but may be due to differences in assay conditions, e.g., the presence in the latter assay of proteins with which BFA can interact resulting in a

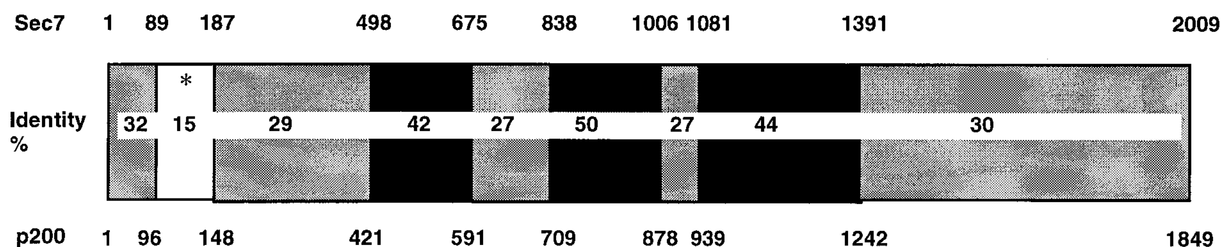

FIG. 1. Identity of amino acid sequences of Sec7 (10) and the 200-kDa protein. An optimized alignment of the amino acid sequence of Yeast Sec7 and p200 is shown diagramatically. Above and below are numbers of amino acid positions in each protein. Percentage identity of amino acids in the boxed area is shown. The asterisk indicates the highly acidic region of Sec7.

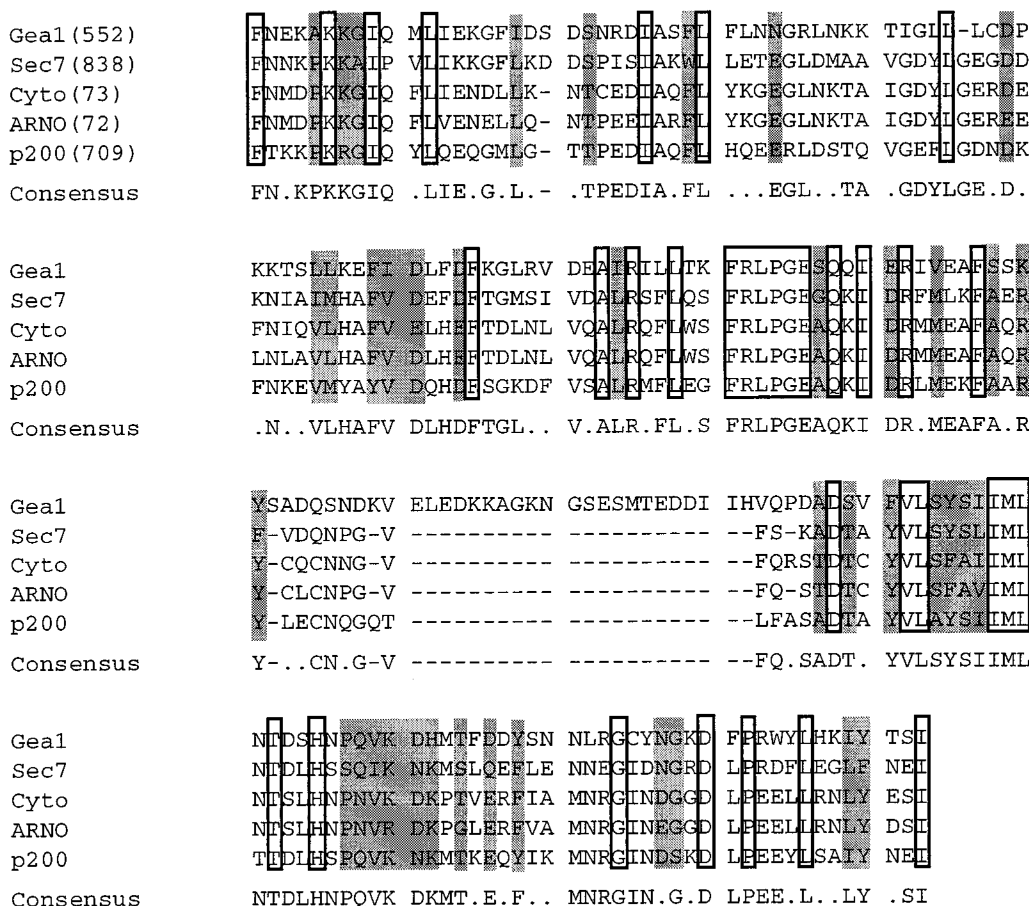

FIG. 2. Amino acid sequences of Sec7 domains of five ARF GEPs. The sequence of p200 is aligned with Gea1, cytohesin 1 (cyto), ARNO, and Sec7. Boxed residues are identical. Conservative differences are shaded. Consensus residues are identical in three or more sequences. Hyphens denote gaps introduced to maximize identity. Numbers in parentheses refer to position of the first amino acid in each Sec7 domain.

lower effective concentration of the inhibitor. In either assay, ARF activity was enhanced by <0.1 pmol of GEP.

DISCUSSION

We had earlier found that ARF GEP activity behaved as an ≈670-kDa complex on gel filtration. A 200-kDa protein separated from the complex by SDS/PAGE exhibited BFA-inhibited GEP activity after electroelution and renaturation by dialysis. Cloning of p200 and synthesis of the recombinant fusion protein in Sf9 cells now provide further confirmation that it is a BFA-inhibited ARF GEP. The data support the conclusion that in mammalian systems, as in yeast, a single protein possesses both the catalytic site responsible for guanine nucleotide-exchange activation and the BFA-inhibitory domain. Sequence comparison with yeast Sec7 is consistent with the conclusion that this protein may represent a mammalian homologue. The region of greatest similarity is the Sec7 domain. If the primary functional similarity between the two proteins is their ability to serve as guanine nucleotide-exchange factors for ARF, conservation of the Sec7 functional domain among eukaryotes is not surprising, because ARF structures are so highly conserved across species.

Table 2. Percentage similarity and identity of amino acid sequences of Sec7 domains of five proteins with ARF GEP activity

	p200	Geal	ARNO	Cytol	Sec7
p200	—	44	70	70	69
Geal	30	—	54	51	54
ARNO	51	34	—	95	70
Cytol	55	36	88	—	67
Sec7	50	35	50	50	—

Percentage similarity (considering G, A, S, T, P/V, L, I, M/K, R, H/F, Y, W/D, E, N, Q as conservative differences) is above the diagonal and identity is below.

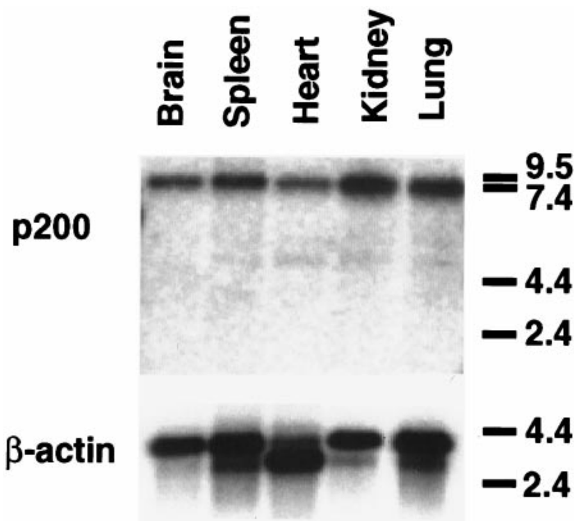

FIG. 3. Northern blot analysis of p200 mRNA in bovine tissues. The blot with poly(A)$^+$ RNA (2 μg) from the indicated bovine tissues was hybridized with a 1.3-kb cDNA representing nucleotides 43–1,329 from p200 and then after stripping, with β-actin cDNA. Positions of size markers (kb) are indicated.

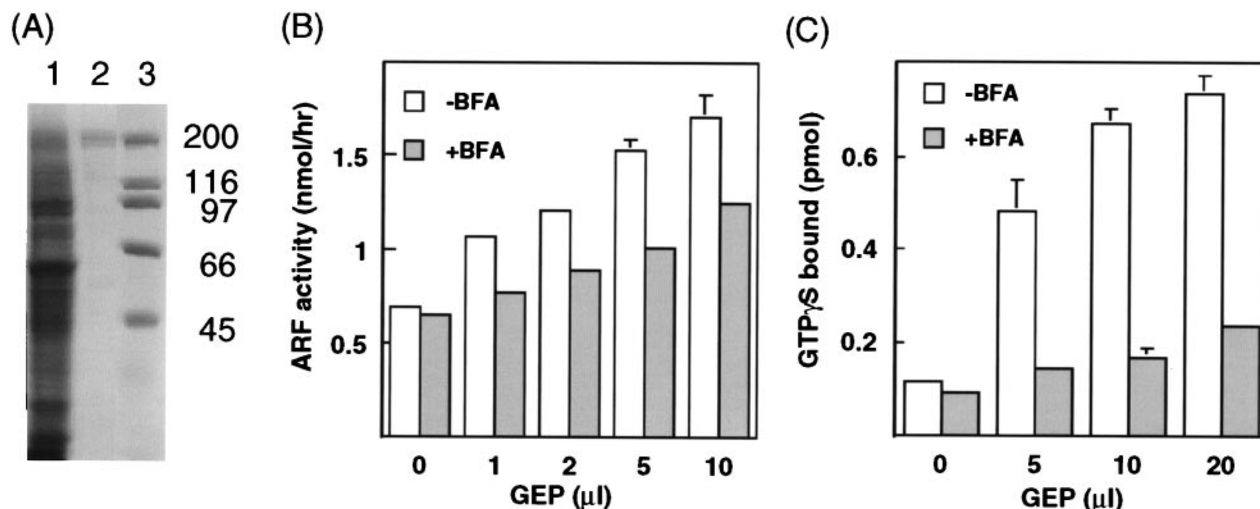

FIG. 4. (*A*) Purification of p200 synthesized as a six-His fusion protein in Sf9 cells. After separation by SDS/PAGE in 10% gel, proteins were stained with Coomassie blue. Lanes: 1, soluble proteins (30 μg) from lysate of induced Sf9 cells; 2, affinity-purified six-His-tagged p200 ($\approx$100 ng); 3, standard proteins, size (kDa) on the right. (*B*) Effect of recombinant six-His-tagged p200 on ARF activity. ARF activity is CTA activity (nmol of ADP-ribosylagmatine synthesized per hr) with ARF minus that without ARF. Assays with the indicated amounts of purified six-His-tagged-p200 ($\approx$3 μg/ml) were carried out without and with 6 μg of BFA. Data are means ± SEM of values of two samples. This experiment was replicated three times with different preparations. Activity of the partially purified ARF (10 μg) was equivalent to $\approx$0.1 μg of purified ARF3. (*C*) Effect of BFA on six-His-tagged p200 stimulation of GTPγS binding by ARF. Assays with the indicated amount of purified six-His-tagged-p200 ($\approx$3 μg/ml) and 10 μg of partially purified native ARFs without or with 6 μg of BFA were incubated for 20 min at 30°C. Data are means ± SEM of values from triplicate assays. This experiment was replicated twice.

ARF GEPs can be divided into two families based on their size and sensitivity to BFA. The larger $\approx$200-kDa proteins appear to be BFA sensitive, whereas the smaller $\approx$50-kDa proteins are insensitive. Of the other proteins with ARF GEP activity, Gea1 from yeast is inhibited by BFA (17). ARNO (18) and cytohesin 1 (15), human proteins that are very similar to each other and to members of a highly conserved family of cytohesins (23), have GEP activity that is not inhibited by BFA. As each of these ARF GEPs contains a Sec7 domain, it appears that BFA sensitivity is dependent on other parts of the proteins.

Yeast Sec7 (10), a 230-kDa protein of 2,008 amino acids contains near the N terminus an unusual highly charged acidic sequence (125 amino acids with 29% glutamate, 18% aspartate, and 21% serine) that is not present in the 200-kDa bovine brain GEP. Achstetter *et al.* (10) suggested that the acidic region may have a structural function, interacting with lipids or proteins on the cytoplasmic surface of the Golgi. Deitz *et al.* (11) later showed that the Sec7 protein moves between cytosol and membrane and to that extent may be similar to ARF, whose translocation between the cytosol and membrane is dependent on whether GDP or GTP is bound. Soluble ARF GEPs, both BFA-sensitive and insensitive, have been found by cDNA cloning to contain Sec7 domains. Presumably, the proteins can interact with soluble ARF GDP, accelerate nucleotide exchange, and thereby promote ARF binding to membrane. The identity of the BFA-sensitive Golgi-bound GEP has not been established. Perhaps the association of GEP with ARF can result in translocation of the complex to membranes as a prelude to interaction with a specific membrane receptor.

The report (11) that expression of human ARF4 rescued Sec7-defective *Saccharomyces* is consistent with the notion that Sec7 could be an ARF4 GEP, although this was not mentioned, and it was noted that ARF5, which is very similar to ARF4, was ineffective. Peyroche *et al.* (17) investigated genetic interactions between Gea1 and Sec7 in yeast. They found that multicopy expression of Sec7 did not suppress the temperature-sensitive growth defect of the Gea1–4 at nonpermissive temperatures. In addition, multicopy Gea1 expression failed to suppress the phenotype of a temperature-sensitive Sec7 mutant. They concluded, therefore, that Sec7 and Gea1 have non-overlapping functions; i.e., Sec7 appeared not to be an ARF GEP in yeast. Comparison of the activities of intact Sec7 domain proteins and their Sec7 domains under the same conditions should provide important information about structure–function relationships in ARF GEP molecules and may enable us to understand better the mechanism(s) of ARF action and its regulation.

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