Identification of $G\alpha_{11}$ as the phospholipase C-activating G-protein of turkey erythrocytes

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A 43 kDa phospholipase C-activating protein has been purified previously from turkey erythrocytes and shown to express immunological properties expected of that of the G_q family of G-protein α-subunits [Waldo, Boyer, Morris and Harden (1991) J. Biol. Chem. **266**, 14217–14225]. Internal amino acid sequence has now been obtained from this protein which shares 50–100% sequence identity with sequences encoded by mammalian $G\alpha_{11}$ and $G\alpha_q$ cDNAs. To identify the purified protein unambiguously, it was necessary to compare its amino acid sequence with the sequence encoded by avian G-protein α-subunit cDNA. As such, mouse $G\alpha_q$ was used as a probe to screen turkey brain and fetal-turkey blood cDNA libraries. A full-length cDNA was identified that encodes avian $G\alpha_{11}$, on the basis of its 96–98% amino acid identity with mammalian $G\alpha_{11}$. All eight peptides sequenced from the turkey erythrocyte phospholipase C-activating protein are completely contained within the deduced amino acid sequence of the avian $G\alpha_{11}$ cDNA. Expression of this cDNA in Sf9 cells by using a baculovirus expression system resulted in the production of a 43 kDa protein that reacts strongly with antisera to the G_q family of G-protein α-subunits and activated purified avian phospholipase C in an AlF_4^--dependent manner. Taken together, these results unambiguously identify the protein purified from turkey erythrocytes, on the basis of its capacity to activate avian phospholipase C, as $G\alpha_{11}$.

INTRODUCTION

G-proteins are heterotrimeric signalling proteins that couple a broad range of cell-surface receptors to second-messenger-regulating enzymes and to ion channels [1,2]. Although involvement of G-proteins in receptor-regulated inositol lipid signalling has been indirectly suggested for the last decade, progress in identifying these proteins has been reported only recently.

Proteins have been purified from detergent extracts of bovine brain [3,4], bovine liver [5,6] and turkey erythrocyte [7] membranes that confer AlF_4^-- and guanine-nucleotide-mediated regulation to phospholipase C. Immunoreactivity of these proteins with G-protein-selective antisera suggests that they are members of the G_q family of proteins initially identified by molecular cloning by Simon and colleagues ([8,9]; for review see [10]). On the basis of partial amino acid sequence, the preparation from brain was shown to consist of $G\alpha_q$ and some $G\alpha_{11}$ [3]. The liver preparation contained 42 and 43 kDa proteins expressing immunoreactivity consistent with $G\alpha_q$ and $G\alpha_{11}$ respectively [5,6].

Turkey erythrocytes have provided a useful homogeneous cell preparation for the study of inositol lipid signalling [11]. We have purified the G-protein-regulated form of phospholipase C from this source [12,13] and have used this enzyme to purify a 43 kDa phospholipase C-activating protein [7]. We now report internal amino acid sequence and molecular cloning of the cDNA for the avian G-protein that unambiguously confirm that this phospholipase C-activating protein is $G\alpha_{11}$. Also, using an insect cell expression system, we demonstrate that the protein encoded by the cDNA is recognized by $G\alpha_{11}$-selective antisera and that it activates purified avian erythrocyte phospholipase C.

EXPERIMENTAL

Materials

Q-Sepharose Fast Flow, Sephacryl S-300HR, octyl-Sepharose, heparin–Sepharose, Protein A–Sepharose, Sephadex G-50F and Mono Q HR5/5 were obtained from Pharmacia (Piscataway, NJ, U.S.A.). Bio-Gel HTP hydroxyapatite and the HPTP column were from Bio-Rad Laboratories (Richmond, CA, U.S.A.). Vydac C-4 (214-TP546) and C-18 (218-TP546) reverse-phase h.p.l.c. columns were from Rainin (Emeryville, CA, U.S.A.). Phosphatidylserine and phosphatidylethanolamine were from Avanti Polar Lipids (St. Louis, MO, U.S.A.). CHAPS was purchased from Pierce (Rockford, IL, U.S.A.), and sodium cholate (Sigma, St. Louis, MO, U.S.A.) was purified as previously described [7]. [^{3}H]Inositol was from American Radiolabelled Chemicals, and [^{3}H]PtdIns4*P* and [^{3}H]PtdIns(4,5)P_2 were purified from [^{3}H]inositol-labelled turkey erythrocytes as previously described [12]. Unlabelled PtdIns(4,5)P_2 and PtdIns4*P* were purified from bovine brain as previously reported [12]. The cDNA synthesis and cloning systems (λ ZAP) were purchased from Stratagene, and the Sequenase DNA-sequencing kits were from U.S. Biochemical Corp. [^{32}P]dCTP and [^{35}S]dATP were from Amersham. Nitrocellulose filters were from Schleicher & Schuell (Keene, NH, U.S.A.). Strepavidin-conjugated paramagnetic beads were from Promega (Madison, WI, U.S.A.). Sea Kem GTG agarose was from FMC BioProducts (Rockland, ME, U.S.A.) and the Qiaex resin was from Qiagen Inc. (Chatsworth, CA, U.S.A.). Vector pVL1392 and linear AcNPV viral DNA were from Invitrogen (San Diego, CA, U.S.A.). Antisera W082 and E976 were generously provided by Dr. Paul Sternweis and Dr. John Exton, respectively. Rabbit polyclonal antiserum 118 was generated in our laboratory against the haemocyanin-coupled synthetic peptide CILQLNLKEYNL, which corresponds to the C-terminal sequence of both $G\alpha_q$ and $G\alpha_{11}$.

Purification and trypsin treatment of the 43 kDa turkey erythrocyte phospholipase C-activating protein

G-protein-regulated phospholipase C [12,13] and the 43 kDa phospholipase C-activating protein [7] were purified from turkey

Abbreviation used: TFA, trifluoroacetic acid.
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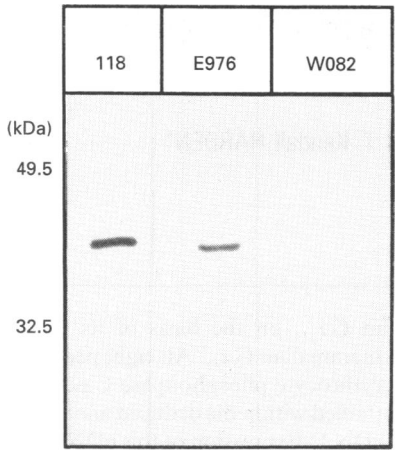


Figure 1 Immunoblot analysis of the 43 kDa phospholipase C-activating protein purified from turkey erythrocytes

Purified phospholipase C-activating protein (40 ng/lane) was subjected to SDS/PAGE and transferred to nitrocellulose for Western-blot analysis (see the Experimental section). Antiserum 118 was raised against a sequence common to $G\alpha_q$ and $G\alpha_{11}$; E976 was raised against a sequence unique to $G\alpha_{11}$; W082 was raised against a sequence unique to $G\alpha_q$. The 27–60 kDa region for each immunoblot is shown and the antisera used for immunodetection are listed at the top. Mobility of molecular-mass standards (kDa) is indicated on the left.

erythrocytes. Protease inhibitors and minor contaminants including $\beta\gamma$-subunits were removed from the 43 kDa phospholipase C-activating protein by C-4 reverse-phase h.p.l.c. before trypsin treatment. Briefly, the purified phospholipase C-activating protein pool was exchanged from buffer A (25 mM Tris, pH 7.5, 1 mM dithiothreitol, 50 μM $AlCl_3$, 10 mM NaF, 3 mM $MgCl_2$, 30 μM GDP, 0.1 mM benzamidine, 20 μg/ml phenylmethanesulphonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml aprotinin, 1 μg/ml pepstatin and 0.6% CHAPS) to buffer B (20 mM Tris, pH 7.5, and 0.1% Lubrol) by chromatography on a Mono-Q 5/5 f.p.l.c. column and elution of the protein with buffer B containing 400 mM NaCl. After addition of 0.1% trifluoroacetic acid (TFA), the Mono-Q eluate was loaded on a Vydac C-4 h.p.l.c. column at 1 ml/min and eluted with a linear gradient of water (+0.1% TFA) to acetonitrile/propan-2-ol (3:1, v/v) (+0.1% TFA) over 60 min. Fractions containing the 43 kDa protein, as determined by silver staining of SDS/PAGE and Western-blot analysis using antiserum 118, were pooled (approx. 10 μg) and dried in a polypropylene microfuge tube by rotary evaporation under vacuum. The protein was resuspended in 100 μl of 1% NH_4HCO_3, pH 8.0, and digested for 10 h at 37 °C at a trypsin/protein ratio of 1:50 (w/w). Tryptic peptides were resolved by h.p.l.c. on a Vydac C-18 h.p.l.c. column developed with a 180 min linear gradient of water (+0.1% TFA) to 100% acetonitrile (+0.1% TFA) at 0.5 ml/min. Peaks of absorbance at 214 and 280 nm selected for amino acid analysis were further purified by C-18 chromatography, with elution with a linear gradient of water (0.1% TFA) to 50% acetonitrile (0.1% TFA) over 180 min at a flow rate of 1 ml/min. Purified fractions were concentrated by rotary evaporation and subjected to sequence analysis on an Applied Biosystems 475A Protein Sequencer. The yields of amino acids during peptide sequencing ranged from 20 to 80 pmol.

Reconstitution assay

G-protein-regulated phospholipase C activity was measured by a modification of the method described by Waldo et al. [7]. Briefly, lipids {200 μM phosphatidylserine, 800 μM phosphatidylethanolamine and 200 μM [^{3}H]PtdIns(4,5)P_2} were dispersed by sonication in a buffer containing 20 mM Hepes, pH 7.4, 2 mM $MgCl_2$, 100 mM NaCl, 1 mM dithiothreitol and 1.2% sodium cholate. Extracts from Sf9-cell membranes (in 1.2% cholate) were added to the resuspended lipids and vesicles were formed by dialysis against the above buffer (minus cholate). Assays were initiated by addition of phospholipase C (10 ng) in a buffer containing 57.5 mM Hepes, pH 7.0, 75 mM NaCl, 4 mM $MgCl_2$, 2 mM EGTA, 0.5 mg/ml BSA and $CaCl_2$ to yield a free [Ca^{2+}] of 3.4 μM. Reactions were terminated by addition of chloroform/methanol/HCl (20:40:1, by vol.)

Gel electrophoresis and immunoblots

SDS/PAGE was performed on gels of 8.5% acrylamide/0.5% bisacrylamide as previously described [7].

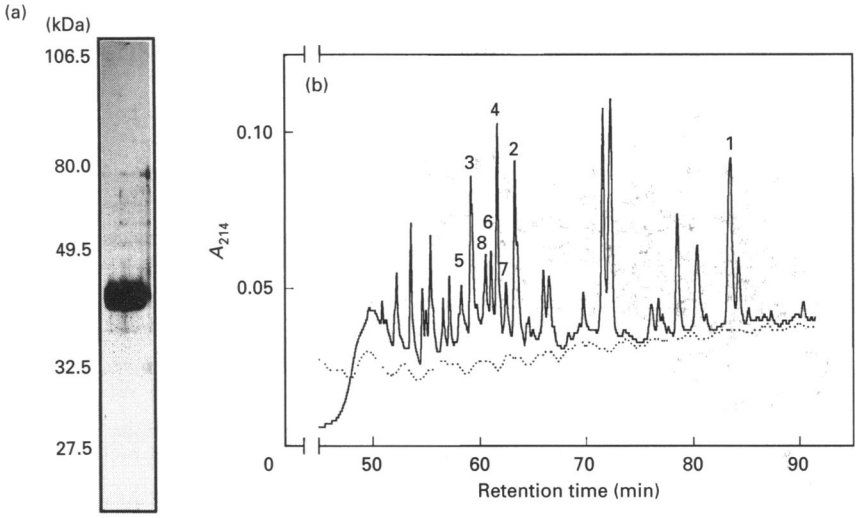
Construction of fetal turkey blood and turkey brain cDNA libraries

The tissues were prepared as follows. Fifty 9–11-day-incubated turkey eggs were obtained, the eggs were opened and the chorionic membrane and its associated vasculature were dissected, minced and placed in ice-cold isotonic saline. The minced tissue was strained through cheesecloth, and blood cells were collected from the filtrate by centrifugation. The cells were washed twice in ice-cold isotonic saline. The brain of an adult female turkey was removed, cleaned and immediately placed in liquid nitrogen. The frozen tissue was then powdered. RNA was isolated from fetal turkey blood and turkey brain by the method of Chomczynski and Sacchi [14]. Briefly, RNA was extracted from the pelleted blood cells and frozen brain tissue, and polyadenylated RNA was isolated from the total RNA by using a biotinylated oligo(dT) probe and a magnetic separation system employing strepavidin-conjugated paramagnetic beads, by following the manufacturer's suggested procedures, except that the annealing reaction was performed in 3.5 × SSC (1 × SSC = 0.15 M NaCl/15 mM sodium citrate).

cDNA synthesis was performed by using 27 μg of fetal turkey blood RNA and 15 μg of turkey brain RNA. The cDNA synthesis reactions essentially followed the method of Gubler and Hoffman [15], with the substitution of methyl-dCTP for CTP, and were primed with an oligo(dT)-containing adaptor–primer which introduced a *Xho*I restriction site into the 3′ end of the newly synthesized double-stranded cDNA. After ligation with *Eco*RI adaptors and phosphorylation of the adaptor ends, the cDNA was cleaved with *Xho*I and resolved from unligated adaptors and size-fractionated by electrophoresis on a 1% low-melting-agarose gel. cDNA of the desired size class (1–2 kb for the fetal turkey blood cDNA and > 1 kb for the turkey brain cDNA) was recovered from the melted gel slices by using Qiaex resin. Approx. 200 ng of each cDNA preparation was ligated with 1 μg of *Eco*RI/*Xho*I-digested λ ZAP and the resulting preparations were packaged to yield functional cDNA libraries. The unamplified fetal turkey blood 1–2 kb cDNA library contained 1.5×10^6 plaque-forming units, and when amplified contained 94% recombinant bacteriophage. The unamplified turkey brain > 1 kb cDNA library contained 1.4×10^6 plaque-forming units, and when amplified contained 96% recombinant bacteriophage.

Isolation of cDNA

Approx. 10^6 recombinants were screened by high-stringency plaque hybridization. Nitrocellulose filters were washed with



(c)

Peptide 1: LVYQNIFTAMQSMIR		Peptide 5: ALFR
α11: ----------A-V-		α11: ----
αq : ----------A---		αq : ----
α14: ----------A---		α14: ----
Peptide 2: VMTFEQPYVSAIK		Peptide 6: EFILKM
α11: -T---HQ--N---		α11: ------
αq : -SA--N---D---		αq : ------
α14: -TALSRDQ-A---		α14: ------
Peptide 3: YYLSDVDR		Peptide 7: FVFAAVK
α11: ---I----		α11: -------
αq : ---N-L--		αq : -------
α14: ---T-IE-		α14: -------
Peptide 4: IATPGYLPTQQDVL		Peptide 8: EYNLV
α11: ---V----------		α11: -----
αq : V-D-S---------		αq : -----
α14: --M-SFV-------		α14: -----

Figure 2 Trypsin treatment and amino acid analysis of the 43 kDa phospholipase C-activating protein purified from turkey erythrocytes

(**a**) Silver-stained SDS/PAGE of 60 ng of the purified turkey erythrocyte phospholipase C-activating protein before trypsin treatment. The mobility of molecular-mass standards (kDa) is indicated. (**b**) H.p.l.c. of peptides generated by trypsin treatment of 10 μg of purified turkey erythrocyte phospholipase C-activating protein (see the Experimental section). Absorbance of trypsin alone is also indicated (· · · ·). Peptides for which the amino acid sequence was determined are numbered. (**c**) Partial amino acid sequence comparison of turkey erythrocyte phospholipase C-activating protein and α-subunits of members of the G_q family of G-proteins. Identical amino acids are indicated by (-), and different amino acids are shown.

5 × SSC/0.5 % SDS/1 mM EDTA at 42 °C for 2 h. Filters were incubated at 42 °C for 2 h in 100 ml of a prehybridization solution that consisted of 0.8 M NaCl, 0.02 M Pipes, pH 6.8, 0.25 % SDS, 50 % formamide and 100 μg/ml denatured salmon sperm DNA. Filters were probed with 25 ng of [^{32}P]dCTP-labelled mouse $G\alpha_q$ that contained the entire coding sequence as well as 3′ and 5′ untranslated sequences. The probe (approx. 1×10^9 c.p.m./μg) was hybridized to the nitrocellulose filters for 16–24 h at 42 °C in 50 ml of a solution identical with that used in the prehybridization step. Filters were washed in 2 × SSC/0.1 % SDS for 4 × 5 min at room temperature and then for 2 × 1 h in 1 × SSC/0.1 % SDS at 68 °C. Positive plaques were purified and pBluescript was rescued from λ-phage DNA according to the manufacturer's recommendations. Inserts were subcloned into bacteriophage M13 (mp18 and mp19), and both strands were sequenced by using primers which yielded overlapping sequences.

Expression of avian $G\alpha_{11}$ in Sf9 insect cells

cDNA B4 was subcloned into the *Eco*RI/*Sma*I sites of the pVL1392 transfer vector. After co-transfection of Sf9 cells with this construct and linear *Autographa californica* nuclear-polyhedrosis-virus DNA, recombinant viral stock was made by established procedures [16], after selection on the basis of the *occ*$^-$ phenotype. The plaque pure virus was used to infect adherent Sf9 cells at a multiplicity of approx. 1. Adherent cells were grown at 27 °C for 72–96 h, suspended and collected by centrifugation (500 ***g***, 5 min). Lysis was by resuspension in ice-cold lysis buffer containing 1 mM $NaHCO_3$, 2 mM $CaCl_2$, 5 mM $MgCl_2$, 10 mM Tris, pH 7, 0.1 mM benzamidine, 0.1 mM phenylmethanesulphonyl fluoride and homogenization with 15–20 strokes in a Wheaton-type Dounce homogenizer. Intact nuclei and non-lysed cells were pelleted by centrifugation (1500 ***g***; 5 min). Membranes were collected by centrifugation

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                                GCA CGA GGA GGA CCG GAG CGT CCC GGC GGC GTC TTT ACC
1/1                                                     31/11
ATG ACT CTG GAG TCC ATG ATG GCT TGT TGC CTG AGC GAC GAG GTG AAA GAG TCG AAA CGA
Met thr leu glu ser met met ala cys cys leu ser asp glu val lys glu ser lys arg
61/21                                                   91/31
ATC AAT GCC GAG ATC GAA AAG CAG CTG CGG AGG GAC AAA CGC GAC GCA CGG CGA GAG CTG
ile asn ala glu ile glu lys gln leu arg arg asp lys arg asp ala arg arg glu leu
121/41                                                  151/51
AAA CTG CTG CTG TTG GGC ACT GGG GAG AGT GGA AAA AGC ACG TTC ATT AAG CAG ATG CGT
lys leu leu leu leu gly thr gly glu ser gly lys ser thr phe ile lys gln met arg
181/61                                                  211/71
ATC ATT CAT GGC TCA GGC TAC TCT GAA GAG GAC AAA AAA GGT TTT ACC AAG CTG GTA TAT
ile ile his gly ser gly tyr ser glu glu asp lys lys gly phe thr lys leu val tyr
241/81                                                  271/91
CAA AAC ATC TTC ACT GCC ATG CAG TCC ATG ATC AGG GCC ATG GAA ACC CTG AAG ATT CTG
gln asn ile phe thr ala met gln ser met ile arg ala met glu thr leu lys ile leu
301/101                                                 331/111
TAC AAA TAC GAG CAG AAC AAG GCC AAC GCA GTC CTG ATC CGG GAG GTG GAT GTA GAA AAG
tyr lys tyr glu gln asn lys ala asn ala val leu ile arg glu val asp val glu lys
361/121                                                 391/131
GTC ATG ACA TTT GAG CAG CCC TAC GTA AGT GCA ATT AAA ACC TTG TGG AAC GAC CCT GGA
val met thr phe glu gln pro tyr val ser ala ile lys thr leu trp asn asp pro gly
421/141                                                 451/151
ATA CAG GAG TGT TAT GAC AGA AGA AGA GAA TAC CAA CTT TCT GAT TCA GCT AAA TAC TAT
ile gln glu cys tyr asp arg arg arg glu tyr gln leu ser asp ser ala lys tyr tyr
481/161                                                 511/171
CTC AGC GAC GTG GAT CGT ATC GCT ACC CCA GGA TAT CTA CCA ACT CAG CAA GAT GTG CTA
leu ser asp val asp arg ile ala thr pro gly tyr leu pro thr gln gln asp val leu
541/181                                                 571/191
CGG GTT CGA GTT CCT ACG ACT GGG ATC ATA GAG TAC CCC TTT GAC CTA GAG AAT ATT ATC
arg val arg val pro thr thr gly ile ile glu tyr pro phe asp leu glu asn ile ile
601/201                                                 631/211
TTC AGA ATG GTG GAT GTT GGA GGT CAG AGA TCA GAA CGG AGG AAG TGG ATC CAT TGC TTT
phe arg met val asp val gly gly gln arg ser glu arg arg lys trp ile his cys phe
661/221                                                 691/231
GAA AAT GTG ACT TCC ATC ATG TTT TTA GTA GCA CTT AGT GAA TAT GAC CAA GTT CTG GTG
glu asn val thr ser ile met phe leu val ala leu ser glu tyr asp gln val leu val
721/241                                                 751/251
GAG TCT GAT AAT GAG AAC CGG ATG GAA GAG AGT AAA GCC CTC TTC CGA ACC ATT ATC ACC
glu ser asp asn glu asn arg met glu glu ser lys ala leu phe arg thr ile ile thr
781/261                                                 811/271
TAT CCC TGG TTC CAA AAC TCA TCA GTT ATC CTC TTT CTG AAC AAG AAG GAT CTG TTG GAA
tyr pro trp phe gln asn ser ser val ile leu phe leu asn lys lys asp leu leu glu
841/281                                                 871/291
GAC AAG ATC CTC TAT TCC CAT CTC GTT GAC TAT TTC CCA GAG TTT GAT GGC CCG CAG AGG
asp lys ile leu tyr ser his leu val asp tyr phe pro glu phe asp gly pro gln arg
901/301                                                 931/311
GAC GCG CAG GCA GCC CGC GAG TTC ATC CTC AAG ATG TTT GTG GAT TTG AAT CCA GAC AGT
asp ala gln ala ala arg glu phe ile leu lys met phe val asp leu asn pro asp ser
961/321                                                 991/331
GAC AAA ATC ATC TAC TCC CAC TTC ACG TGT GCC ACG GAC ACC GAG AAC ATC CGC TTC GTC
asp lys ile ile tyr ser his phe thr cys ala thr asp thr glu asn ile arg phe val
1021/341                                                1051/351
TTC GCG GCC GTG AAG GAC ACC ATC CTA CAG CTC AAC CTG AAG GAA TAC AAC CTG GTT TGA
phe ala ala val lys asp thr ile leu gln leu asn leu lys glu tyr asn leu val

TCC CCC CCC CCC GCC CCG CCC CTT GGA AAG GAA TCG TTC CTG AAA AGA AAA AGA CAA AAA
AAG GAG AAA AAA AAA AAA AAA AAA GGA ATT AAT TTT AAA CAT GAC CGG AAA AAA AAA CAA
ACA AAA AAA AAA AAA AAA...
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Figure 3 Sequence of clone B4

The entire cDNA sequence was derived from one clone isolated from an oligo(dT)-primed turkey brain cDNA library, by using mouse $G\alpha_q$ as a probe (see the Experimental section). The regions of deduced amino acid sequence that are identical with the peptide sequence obtained by tryptic digestion of the purified 43 kDa turkey erythrocyte phospholipase C-activating protein are underlined.

of the supernatant obtained after cell lysis at 100000 *g* for 30 min.

RESULTS AND DISCUSSION

We have recently reported the purification of a 43 kDa protein from turkey erythrocytes that, when reconstituted in phosphoinositide-substrate-containing phospholipid vesicles, confers AlF_4^-- and guanosine 5′-[γ-thio]triphosphate-sensitivity to purified avian phospholipase C [7]. The guanosine 5′-[γ-thio]-triphosphate-stimulated activation of the avian phospholipase C, though small, was blocked by guanosine 5′-[β-thio]diphosphate supporting the concept that a G-protein α-subunit is involved [we have recently purified avian 43 kDa protein that is activated by GTP[S] and AlF_4^- to similar extents (G. L. Waldo, unpublished work)]. This purified avian phospholipase C-activating protein shows strong immunoreactivity with antisera X384 [7] and 118 (Figure 1), which were generated against the C-terminal dodecapeptide of $G\alpha_q$ and $G\alpha_{11}$. Conversely, antiserum W082, generated against a sequence in $G\alpha_q$ that is not well conserved in $G\alpha_{11}$ and has been shown to be selective for $G\alpha_q$ [17], recognized the turkey erythrocyte protein only very weakly (ref. [7]; Figure 1). Antiserum E976 [18] generated against a sequence (amino acids 160–172) in $G\alpha_{11}$ that is not well conserved in $G\alpha_q$ reacted strongly with the purified 43 kDa turkey erythrocyte protein (Figure 1). Although the absolute selectivity of E976 has not

Table 1 Reconstitution of purified turkey erythrocyte phospholipase C with sodium cholate extracts from recombinant-baculovirus-infected Sf9-cell membranes

Membranes from baculovirus-infected Sf9 cells were prepared as described in the Experimental section. Purified avian 43 kDa phospholipase C-activating protein (60 ng), or sodium cholate extracts of membranes from Sf9 cells infected with recombinant baculoviruses expressing either β-galactosidase (1.29 μg) or avian $G\alpha_{11}$ (1.29 μg), were combined with dispersed phospholipids containing [^{3}H]PtdIns4P, and vesicles were formed by dialysis as described in the Experimental section. The vesicles were reconstituted with purified turkey erythrocyte phospholipase C (10 ng) and incubated with either 10 mM Hepes, pH 7.4 (vehicle), or 10 mM NaF/20 μM $AlCl_3$ (AlF_4^-). Assays were for 10 min at 30 °C, and the reactions were terminated by addition of chloroform/methanol/HCl (20:40:1, by vol.). Values are means ± S.E.M. from three separate determinations. Qualitatively similar results were obtained in three other experiments.

Addition	AlF_4^-	Phospholipase C activity [pmol of Ins(1,4)P_2]
Purified turkey erythrocyte α_{11}	−	5.6 ± 5.3
	+	35.1 ± 5.5
Cholate extract from Sf9 cells expressing β-galactosidase	−	23.5 ± 4.2
	+	27.8 ± 4.3
Cholate extract from Sf9 cells expressing avian α_{11}	−	16.3 ± 6.4
	+	62.6 ± 6.2

been assessed, these immunological data are consistent with the hypothesis that the purified turkey erythrocyte G-protein is avian $G\alpha_{11}$. However, to identify this protein unequivocally, we obtained an internal amino acid sequence and cloned its cDNA. Both of these approaches were considered necessary, since the members of the G_q family of G-proteins share greater than 80% amino acid sequence identity [10].

The avian phospholipase C-activating protein was purified as previously reported [7], with the inclusion of an additional C-4 column step as described in the Experimental section. The purified 43 kDa protein (Figure 2a) was subjected to cleavage by trypsin and peptides were purified by reverse-phase h.p.l.c. (Figure 2b). Sequences from eight peptides were obtained. All eight sequences were highly (50–100%) similar to sequences predicted from the deduced amino acid sequences of G-protein α-subunit cDNA cloned from mouse brain cDNA libraries (Figure 2c). However, it was not possible to identify the 43 kDa protein conclusively from this information, or to conclude categorically that peptides generated from tryptic digests of the phospholipase C-activating protein were from a single polypeptide. Thus, to establish the identity of the avian protein unambiguously, it was imperative that the avian peptide sequence be compared with avian cDNA.

A probe made from mouse $G\alpha_q$ cDNA was used to screen an oligo(dT)-primed turkey brain cDNA library. High-stringency screening of approx. 10^6 phages yielded four positive plaques. Sequence analysis revealed that these four cDNAs were of different lengths, and that they encoded part of a G-protein α-subunit that was similar to, though distinct from, $G\alpha_q$. Further high-stringency screening of the turkey brain cDNA library with the largest of these cDNAs, 16a, yielded 17 more clones. cDNA B4 was isolated from this second screening. B4 was shown to contain the entire coding sequence of a $G\alpha_q$-like protein, in addition to 5′ and 3′ untranslated sequences (Figure 3). All remaining cDNAs isolated from the brain library with clone 16a had nucleic acid sequences identical with that of B4, though they were not full length. Similarly, high-stringency screening of an oligo(dT)-primed fetal turkey blood cDNA library with cDNA 16a resulted in the isolation of seven additional cDNAs. These cDNAs had nucleic acid sequences identical with those isolated from the brain library, though none were full length. The longest of the cDNAs isolated from the blood library comprised nucleotides 125–1259 of clone B4. Since the brain and blood libraries both yielded cDNAs with identical nucleic acid sequences, and a full-length version had been isolated from the brain library, no further attempt was made to isolate a full-length clone from the blood library.

cDNA B4 encodes a 359-amino-acid protein (M_r 42031) (Figure 3) that is most similar in sequence to mouse $G\alpha_{11}$ [9] and its bovine homologue, $GL\alpha_2$ [19]. The similarity between the amino acid sequence deduced from cDNA B4 and that of mouse and bovine $G\alpha_{11}$ is striking. There is only one amino acid difference in the last 119 amino acids of the C-terminus of B4 and bovine $G\alpha_{11}$, and the overall identity between the proteins encoded by these two sequences is 96%. The overall identity between clone B4 and bovine $GL\alpha_2$ was 98%, and mouse $G\alpha_{11}$ and bovine $GL\alpha_2$ share 97% sequence identity. Similarly, the deduced amino acid sequence from cDNA B4 shared 91% and 81% sequence identity with those of mouse $G\alpha_q$ and $G\alpha_{14}$ respectively. Although this similarity is also high, differences within specific regions of these G-proteins make it highly unlikely that the cDNA that we report here represents an avian version of these. The choice of initiating methionine (see Figure 3) was based entirely on the highly conserved nature of the sequences 5′ to the other two in-frame methionines in the avian clone and the sequences of mouse $G\alpha_{11}$ and bovine $GL\alpha_2$. Though Nakamura et al. [19] had not considered the first ATG as a likely initiation codon for translation of $GL\alpha_2$, the first six amino acids present in B4 are identical with those in mouse $G\alpha_{11}$ and bovine $GL\alpha_2$. Although the codons for these first six amino acids are conserved in all three cDNA sequences, we were unable to obtain N-terminal sequence from our purified protein, and as such have not confirmed that these six amino acids are present. On the basis of these sequence similarities, we conclude that clone B4 encodes a protein that is the avian equivalent of $G\alpha_{11}$. As with other members of this family [10], the lack of a cysteine at position 356 indicates that this protein would not be a substrate for pertussis toxin. G-protein-stimulated phospholipase C activity in turkey erythrocytes is insensitive to the effects of pertussis toxin.

The availability of cDNA encoding avian $G\alpha_{11}$ has allowed a direct comparison with the peptide sequence obtained from the purified avian phospholipase C-activating protein. Peptides 1–8 contain amino acid sequences that were identical with amino acids 78–92, 121–133, 159–171, 172–180, 253–256, 307–312, 339–345 and 355–359 of the deduced amino acid sequence of cDNA B4. The sequence generated by peptides 3 and 4 is very similar to the region in $G\alpha_{11}$ against which antiserum E976 was generated [18]. Peptide 2 represents the region in avian $G\alpha_{11}$ that corresponds to the $G\alpha_q$ sequence to which antiserum W082 was raised. Since avian $G\alpha_{11}$ and mammalian $G\alpha_q$ share only 69% identity in this region, it is perhaps not surprising that antiserum W082 reacts only very weakly with the purified avian protein. From the complete amino acid identity of the peptide sequence with that predicted from the avian $G\alpha_{11}$ cDNA clone, we conclude that the purified 43 kDa turkey phospholipase C-activating protein is $G\alpha_{11}$.

Although extensive analyses have not been carried out, the avian $G\alpha_{11}$ cDNA has been successfully expressed in a baculovirus insect-cell (Sf9) expression system. The expressed protein is recognized by antibodies 118 and E976 (results not shown).

Cholate extracts of membranes from recombinant $G\alpha_{11}$ baculovirus-infected Sf9 cells markedly stimulated the purified avian phospholipase C in an AlF_4^--dependent manner after co-reconstitution in substrate-containing phospholipid vesicles (Table 1). Cholate extracts from Sf9 cells infected with a β-galactosidase-expressing recombinant baculovirus did not contain significant amounts of protein that reacted with antiserum 118 (results not shown) and only activated the avian phospholipase C to a very small extent (Table 1).

In summary, purification of the components of the avian G-protein-regulated phospholipase C from a single homogeneous cell preparation eliminates complexities inherent in assessing the interactions between proteins purified from heterologous cell or tissue preparations in different species. Using this approach, we believe that we have purified and cloned the G-protein involved in the regulation of phospholipase C in turkey erythrocytes and have unequivocally demonstrated that it is an avian homologue of $G\alpha_{11}$. $G\alpha_{11}$ has high sequence similarity with $G\alpha_q$, and it is now clear that both proteins can activate phospholipase C [3–7,17,18,20–22]. However, it is not clear if they express different regulatory properties or interact with additional or different effector proteins. The availability of purified avian $G\alpha_{11}$ of defined composition as well as recombinant $G\alpha_{11}$ will allow us to begin to address some of these and other questions.

We thank Dr. Mel Simon for providing the $G\alpha_q$ cDNA and Dr. Paul Sternweis and Dr. John Exton for providing antibodies. We also thank Dr. Elmer M. Price for his invaluable assistance with the Sf9 expression experiments, and Dr. Gene Scarborough, Dr. U. S. Rao and Dr. Jose Luis Boyer for their help in the purification of the tryptic peptides. Thanks are also due to Dr. Russell Henry for the amino acid analysis, Goldsboro Milling (Goldsboro, NC, U.S.A.) for fertilized turkey eggs, and Carolina Turkeys (Mt. Olive, NC, U.S.A.) for turkey blood. This work was supported by U.S. Public Health Service Grants GM29536 and GM38213, American Heart Association Grant-in-Aid 91015240, a Patrick Mitchell Fellowship Award from the American Heart Association, North Carolina Affiliate (A.J.M.), and a Medical Research Council of Canada Fellowship Award (D.H.M.).

Received 28 September 1992/26 October 1992; accepted 2 November 1992

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