Antibodies Directed against Synthetic Peptides Distinguish between GTP-binding Proteins in Neutrophil and Brain*

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Antiseras AS/6 and 7, raised against a synthetic peptide KENLKDCCGLF corresponding to the carboxyl-terminal decapeptide of transducin-α, react on immunoblots with purified transducin-α and with proteins of 40–41 kDa in all tissues tested. The latter represent one or more forms of Gi, but not Goα, since a synthetic peptide, KNNLKDCGLF, corresponding to the carboxyl-terminal decapeptide of two forms of Gi blocks AS/6 and 7 reactivity with transducin-α and Giα on immunoblots, whereas the corresponding Go-related peptide, ANNLRGCGLY, does not. Antisera LE/2 and 3, raised against the synthetic peptide LERIAQSDYI, corresponding to an internal sequence predicted by one form of Giα cDNA (Giα-2) and differing by 3 residues from the sequence of another form, Giα-1, react strongly with a 40-kDa protein abundant in neutrophil membranes and with the major pertussis toxin substrate purified from bovine neutrophils. LE/2 and 3 reveal a relatively faint 40-kDa band on immunoblots of crude brain membranes or of purified brain Giα/Goα. LE/2 and 3 do not react with transducin-α or Giα nor with the 41-kDa form of pertussis toxin substrate in brain, Giα-1. These antisera distinguish between the major pertussis toxin substrates of brain and neutrophil and tentatively identify the latter as Giα-2.

A family of guanine nucleotide-binding proteins (G-proteins) couples diverse receptors to effectors. G-proteins are heterotrimeric; the α GTP-binding subunit is distinct for each member of the G-protein family and is thought to confer specificity in both receptor and effector interactions (1–3). α Subunits are also substrates for mono-ADP-ribosylation by bacterial toxins.

G-proteins have been identified by function, by purification, and more recently by cloning and sequencing of cDNAs. Thus, Gi is the G-protein associated with stimulation of adenylate cyclase, and transducin is a G-protein coupling visual pigments to cGMP phosphodiesterase in retinal photoreceptor cells (1–3). Distinct transducin-α subunits are found in rods and cones (4, 5). Giα is a G-protein abundant in brain (6–8). Its function is still unclear but may be related to regulation of calcium channels (9).

Gi was originally defined as the G-protein associated with inhibition of adenylate cyclase (1, 10). Unlike stimulation of adenylate cyclase by Gi, inhibition of adenylate cyclase has not been linked to a structurally defined G-protein. Gi has also been identified as a substrate for pertussis toxin; ADP-ribosylation of a cysteine residue near the carboxyl-terminus of Giα (11) causes uncoupling of Gα from receptors (10, 12). An approximately 40–41-kDa pertussis toxin substrate has been identified in essentially all cells, but this cannot be assumed to represent "Giα," since multiple potential pertussis toxin substrates, including Giα and transducin (1–3), have been identified.

At least two distinct forms of "Giα" have been identified by cDNA cloning (13). One form of cDNA, identified by screening bovine (14) and human (13) brain libraries, encodes a 354-amino acid protein. The amino acid sequence predicted by this cDNA corresponds to the sequence obtained for a 41-kDa pertussis toxin substrate purified from bovine (14) and rat (15) brain. We refer to this form as Giα-1 simply because it was the first to be defined in terms of primary structure. The second form of cDNA, Giα-2, was cloned from rat C6 glioma cell (15), mouse macrophage (16), and human monocyte (17) libraries and encodes a 355-amino acid protein. The protein corresponding to this cDNA sequence has not been identified. In this report, we describe the production and characterization of antisera directed against two synthetic peptides that proved useful in distinguishing G-proteins in neutrophil and brain and tentatively identify the major pertussis toxin substrate of neutrophils as the G-protein encoded by Giα-2 cDNA.

EXPERIMENTAL PROCEDURES

Peptide Synthesis—Decapeptides were assembled stepwise by the Merrifield solid-phase method (18) using an Applied Biosystems 430A automated peptide synthesizer. The peptide resins were cleaved with anhydrous hydrogen fluoride, and the crude peptides were purified by preparative liquid chromatography on reverse-phase C18. The purified peptides were homogeneous by analytical high-performance liquid chromatography and gave amino acid compositions consistent with those theoretically expected.

Peptide Conjugation and Immunization—10 mg of keyhole limpet hemocyanin (Sigma) and 3 mg of peptide were dissolved in 1.0 ml of 1.0 M mg of keyhole limpet hemocyanin (Sigma) and 3 mg of peptide were dissolved in 1.0 ml of...
performed weekly, beginning 2 weeks after booster immunization. The 1.5-ml solution was mixed with an equal volume of adjuvant. Preimmune sera were collected, and subsequent bleeds were performed weekly, beginning 2 weeks after booster immunization. Affinity purification of antibodies from antisera AS16 and AS/7 was done using immobilized holotransducin as previously described (19).

Other Antisera—Additional antisera used in this study, including AS/1 (20), CW/6 (20, 21), G1/2 (4, 21), PG/1 (22), and RV/3 (19) have been previously characterized. Rabbit polyclonal antisera (numbers 1 and 3) raised against the retinal "48k" protein were the kind gift of Igal Gery (National Eye Institute).

Membrane Preparations—Human neutrophils were isolated and plasma membrane-enriched fractions prepared as previously described (23). C6 glioma cells were cultured and membrane preparations performed as described in Ref. 24. Bovine brain membrane fractions and cholate extracts were made as described (6, 19).

Protein Purification—Transducin was purified from bovine rod outer segment membranes described as in Ref. 20. A mixture of G, and G, was purified from bovine brain as described in Refs. 8 and 19. The "48k" protein was purified from bovine retinas as described in Ref. 25 with the kind assistance of Samuel Zigler, Jr. (National Eye Institute). The major pertussis toxin substrate of bovine neutrophils was purified as described in Ref. 26.

Other Methods—SDS-PAGE and immunoblotting were performed as described previously (19-21). Incubations with first antibody solutions were for about 24 h at room temperature. Dilutions of antisera used in the first antibody solutions are indicated in each figure legend. The second antibody, peroxidase-conjugated goat anti-rabbit Ig (Kierkegaard and Perry, Gaithersburg, MD), was used at a 1:300 dilution, and incubation was for 2 h at room temperature. 4-Chloronaphthol (Sigma) was used as substrate for peroxidase. Pertussis toxin (List Biochemicals) was activated and used in ADP-ribosylation reactions with either nonradioactive NAD (1 mM) or [α-32P]NAD (10 μM) as described (22).

RESULTS

We synthesized the decapeptide, KENLKDCGLF, corresponding to the carboxyl-terminus of both rod and cone transducin-α (5). This sequence includes the cysteine residue that is the site of pertussis toxin-catalyzed ADP-ribosylation (11). The synthetic peptide was conjugated to KLH and three rabbits designated AS/6, 7, and 8 immunized with the peptide-KLH conjugate. Preimmune and postimmunization bleeds from each animal were tested for specific reactivity on immunoblots of purified holotransducin. For comparison, we also tested an immune bleed of a rabbit, AS/1, immunized with holotransducin. Fig. 1 shows that all three peptide-immunized rabbits developed antibodies against transducin-α; preimmune sera showed no reactivity. By comparison, AS/1, as previously shown (20), recognizes all three transducin subunits, α, β, and γ.

The carboxyl-terminal decapeptide of transducin-α shows some homology to the carboxyl-terminus of transducin-γ (27), as well as to an internal sequence of the "48k" protein of rod outer segments (28, 29). The latter homology may reflect the involvement of this domain in receptor interaction. We tested the reactivity of antisera AS/6 and AS/7 against these related sequences by performing immunoblots with holotransducin and the purified 48k protein. AS/6 and AS/7 reacted exclusively with transducin-α; AS/1, a holotransducin antiserum, readily recognized transducin-γ (as well as β) in this experiment, and both anti-48k sera tested reacted strongly with this protein (data not shown).

It was of interest to determine if antisera raised against the synthetic peptide, KENLKDCGLF, could recognize this sequence after ADP-ribosylation on cysteine by pertussis toxin. To test this we performed two types of experiments. In the first, we treated intact C6 glioma cells with pertussis toxin, cholera toxin, or no toxin and then tested the reactivity on immunoblots of AS/7 with a membrane preparation from each set of cells. Treatment of intact C6 glioma cells with pertussis toxin abolished subsequent ADP-ribosylation of a 40-kDa protein in membranes from treated cells incubated with pertussis toxin and [α-32P]NAD (not shown). Antisera raised against KENLKDCGLF react with 40-41-kDa protein(s) in most cells tested (see later), including C6 glioma cells. Fig. 2 shows the reactivity of AS/7 with a 40-kDa protein in C6 glioma cell membranes from cells incubated without toxin or with cholera toxin. In cell membranes from cells

0.1 M phosphate buffer, pH 7.0. 0.5 ml of 21 mM glutaraldehyde (also in 0.1 M phosphate buffer, pH 7.0) was then added dropwise with stirring, and the combined 1.5 ml were incubated for 24 h at room temperature. The 1.5-ml solution was mixed with an equal volume of complete Freund's adjuvant, and 1-ml aliquots of the resulting emulsion were injected in multiple intradermal sites in three New Zealand White rabbits. Four weeks later each animal received a booster immunization with material prepared identically except that one-half as much peptide and KLH were injected in incomplete Freund's adjuvant. Preimmune sera were collected, and subsequent bleeds were performed weekly, beginning 2 weeks after booster immunization. Affinity purification of antibodies from antisera AS/6 and AS/7 was done using immobilized holotransducin as previously described (19).

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incubated with pertussis toxin, immunoreactivity is not reduced, but the migration of the reactive protein is slightly reduced as expected after ADP-ribosylation (22). In a second type of experiment, we incubated purified holotransducin with pertussis toxin for varying times under conditions leading to increasing degrees of ADP-ribosylation. PG/1, an antisera raised against chemically conjugated ADP-ribose (22), reacts with ADP-ribosylated, but not unmodified, transducin-α and showed an increase in ADP-ribosylation of the transducin-α subunit with increasing time of incubation with pertussis toxin. In agreement with the results of the experiment shown in Fig. 2, AS/7 reactivity with transducin-α is not affected by pertussis toxin-catalyzed ADP-ribosylation (data not shown).

Sequencing of cDNA clones encoding G-protein α subunits (5, 13-17) allows comparison of the amino acid sequence of the synthetic peptide, KENLKDCGLF, with that predicted by the cDNA clones. Table I shows such a comparison and indicates that both G<sub>α1</sub> and G<sub>α2</sub> differ from the synthetic peptide sequence by a single residue. In contrast, G<sub>α0</sub> differs by 5 of 10 residues. G<sub>α0</sub> (not shown) shares only 2 residues in common with the synthetic peptide. Based on this comparison, we predicted that antisera raised against KENLKDCGLF might recognize G<sub>α1</sub> and G<sub>α2</sub> subunits, but not G<sub>α0</sub>.

To test this we performed the immunoblots shown in Fig. 3. A crude brain membrane preparation or a cholate extract of such membranes contains both G<sub>α</sub> and G<sub>β</sub> (6419). Antisera used were: affinity-purified RV/3 antisera, KENLKDCGLF, with 1:lOO dilution. The positions of G<sub>α</sub> subunits of Gi, Go, and of the Gi<sub>α</sub>, Gi<sub>β</sub> and the common subunits of Gi<sub>α</sub> and G<sub>β</sub> are indicated.

![Fig. 3](image)

**Fig. 3. Detection of brain "Giα" with AS/6 and 7.** 100 μg/lane of a cholate extract of bovine cerebral cortical membranes was separated by SDS-PAGE and immunoblotted as described in experimental procedures except that samples were treated with N-ethylmaleimide as described in ref. 6 before SDS-PAGE. Antiserum used were affinity-purified RV/3 (panels A and B) at lanes 1 and 2, 1:20 dilution, and lane 3, 1:40 dilution; AS/6 (panel A) and AS/7 (panel B) at lane 2, 1:400, lane 3, 1:200 dilution, and lane 4, 1:100 dilution. The positions of α subunits of G<sub>α</sub> of Gi<sub>α</sub> and of the common β subunit (19) reveals 39- and 36-kDa immunoreactive bands, as expected (lane 1). By mixing either AS/6 and RV/3 or AS/7 and RV/3, we were ready to show (lanes 2 and 3) that AS/6 and AS/7 recognize protein(s), presumably G<sub>α</sub>, distinct from those recognized (G<sub>α</sub> and G<sub>β</sub>) by RV/3. Identical results were obtained with purified G<sub>α</sub>/G<sub>β</sub> preparations (not shown, but see Figs. 4 and 7).

To obtain more rigorous and at least semiquantitative evidence that antisera raised against the synthetic peptide KENLKDCGLF also recognize the sequence predicted by Gα cDNAs, KNNLKDCGLF, we performed an experiment, the results of which are shown in Fig. 4. In panel A, we performed immunoblots of holotransducin with an antisera, GI/2, monospecific for transducin-α (4, 21), and with AS/6 (lanes 5-17). 100 μg of KENLKDCGLF or of KNNLKDCGLF or of the unrelated peptide RLKIDGESA had no effect on GI/2 reactivity. In contrast, the KENLKDCGLF peptide and the KNNLKDCGLF peptide, each to an essentially similar extent, blocked AS/6 reactivity with transducin-α. The unrelated peptide, RLKIDGESA, had no effect at any concentration on AS/6 reactivity. Similar results are shown in panel B for immunoblots of purified brain G<sub>α</sub>/G<sub>β</sub>. GI/2 (lanes 1-4) does not cross-react with G<sub>α</sub> (21), but as shown earlier (Fig. 3), AS/6 does, and this reactivity is equivalently blocked in a concentration-dependent manner by KENLKDCGLF and KNNLKDCGLF, but not RLKIDGESA. In an experiment shown in panel C, we compared the synthetic peptide KNNLKDCGLF to the corresponding decapeptide predicted by G<sub>α</sub> cDNA, ANNLRGCGGLY (Table I), in terms of ability to block AS/6 and AS/7 reactivity with brain membrane proteins on immunoblots. KNNLKDCGLF at 1 and 10 μg effectively blocked AS/6 and AS/7 binding to 40-41-kDa proteins in brain membranes; identical amounts of the G<sub>α</sub> peptide, ANNLRGCGGLY, failed to block AS/6 or AS/7 binding.

Antiseras raised against KENLKDCGLF thus react with transducin-α and G<sub>α</sub> but not G<sub>α0</sub>. These antisera, however, cannot discriminate between G<sub>α1</sub> and G<sub>α2</sub> which share the use of peptide antisera to identify G-proteins.
To develop a reagent capable of discriminating between these two closely related (88% homologous) sequences, we chose a sequence in a region similar to that used by Lerea et al. (5) to prepare antisera capable of differentiating rod and cone transducin-α subunits. The sequence of the decapeptide synthesized, LRERQSDYI, corresponds exactly to that predicted by G_{α_{i}}, cDNAs cloned from human (17), rat (15), and mouse (16) libraries. This sequence is compared to the homologous sequences of other G-protein α subunits, including bovine (14) and human (13) G_{α_{i}}, in Table II. Note that G_{α_{i}} differs in sequence from the synthetic peptide at 3 residues and that rod and cone transducin-α and G_{α_{i}} show further differences.

Fig. 5 shows the results of immunoblots of human neutrophil membranes (panel A) and of bovine brain membrane cholate extract (panel B) performed with antisera against KNLKDCGLF, AS/6, and AS/7, and with antisera, LE/1, 2, and 3, raised against a conjugate of the synthetic peptide LERIAQSDYI and carrier protein, KLH. As reported earlier (23), AS/6 and AS/7 detect the abundant 40–41-kDa pertussis toxin substrate(s) in neutrophil membranes and in brain. The three preimmune LE sera showed no specific reactivity in either neutrophil membranes or brain cholate extract. All three LE immune sera recognized a band of similar mobility to that revealed by AS/6 and AS/7 in neutrophil membranes, with LE/1 showing weaker reactivity at equivalent dilution. In brain cholate extract, LE/1 immune antiserum failed to detect specific immunoreactivity, whereas LE/2 and LE/3 revealed bands of similar mobility, but much weaker reactivity, as those seen with AS/6 and AS/7.

These results suggested that LE antisera detect a protein identical to or closely related to G_{α_{i}}, that is particularly abundant in neutrophils and a similar or identical protein in brain that is relatively lower in abundance (compare the ratio of AS/LE immunoreactivity in neutrophil and brain). To provide more quantitative evidence for this difference, we performed immunoblots of neutrophil membranes and of brain cholate extracts at varying dilutions of AS/6 and LE/3 antisera (Fig. 6). At a 1:2500 dilution, AS/6 gave reactivity in neutrophil membranes similar to that seen with 1:100 and 1:250 dilutions of LE/3 (panel A). At the same dilutions, the results in brain cholate extracts were very different. AS/6 (1:2500) showed greater and broader reactivity in brain than did LE/3 (1:100 and 1:250 dilutions). The immunoreactive band detected with LE/3 comigrated with the bottom of the broad band detected by AS/6.

We next compared the reactivity of LE/3 and AS/6 with purified G-protein preparations, including the major pertussis toxin substrate purified from bovine neutrophils (26). Fig. 7A shows the pattern of protein staining of the purified preparations from bovine neutrophil (lane 1, α subunit only), bovine brain (lane 2), and bovine rod outer segments (lane 3). Panel B shows the results of immunoblots of these proteins with three distinct antisera, AS/6, LE/3, and CW/6, a unique antiserum raised against holotransducin (29) and shown to cross-react (21) with G_{i}, but not G_{α_{i}}, in brain. CW/6 as expected detects the common β subunit in the two holoprotein preparations, reacts strongly with transducin-α, and with a more slowly migrating (about 41-kDa) G_{i} from brain. CW/6 also cross-reacts with the purified bovine neutrophil protein, but this reactivity is lower than with the brain protein (note

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**TABLE II**

Comparison of the amino acid sequence of the synthetic peptide corresponding to the G_{α_{i}} subunit to homologous sequences of other G-protein α subunits

The single letter amino acid code is used.

<table>
<thead>
<tr>
<th>Transducin cone (159-168):</th>
<th>L D R I T A P D Y L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transducin rod (155-164):</td>
<td>L E R L V T P G Y V</td>
</tr>
<tr>
<td>G_{α_{i}} (160-168):</td>
<td>L E R I A Q S D Y I</td>
</tr>
<tr>
<td>G_{α_{i}} (159-168):</td>
<td>L D R I A Q I N Y I</td>
</tr>
<tr>
<td>G_{i}</td>
<td>L D R I G A A D Y Q</td>
</tr>
</tbody>
</table>

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**Fig. 6. Reactivity of LE/3 and AS/6 with “G_{α_{i}}” in brain and neutrophil.** In panel A, 50 μg/lane highly purified human neutrophil plasma membranes and in panel B, 150 μg/lane bovine brain membrane cholate extract were separated by SDS-PAGE on a 10% gel, and immunoblotting was performed as described. In lane 1, a 1:2500 dilution of AS/6 was used as first antibody; in lanes 2 and 3, 1:100 and 1:250 dilutions, respectively, of LE/3.

**Fig. 7. Reactivity of antisera with purified GTP-binding proteins.** The major pertussis toxin substrate (α subunit only) purified from bovine neutrophils (lane 1), G_{i}/G_{α_{i}} purified from bovine brain (lane 2), and holotransducin (lane 3) purified from bovine rod outer segments were separated by SDS-PAGE on a 10% gel. In panel A, the gel was stained for protein, and in panel B immunoblotting was performed with the indicated antisera (LE/3 and CW/6, 1:100 dilution; AS/6, 1:250 dilution). In lane 1 panel A, 0.5 μg was loaded; in lane 1 panel B, 1.0 μg was loaded. In lane 2 (A and B), 4 μg were loaded, and in lane 3 (A and B) 2 μg were loaded. The positions of α and β subunits are indicated.
that 1.0 µg of bovine neutrophil protein was loaded on im-
munoblot lanes compared with 0.5 µg on the lane stained with
Coomassie Blue shown in panel A). AS/6 reacts equally well
with transducin-α, brain Go, and neutrophil “Gi,” as expected
given its ability to recognize the KENLKDCLGF and
KNNLKDCGLFL sequences. Note the subtle but definite dif-
fferences in migration of the immunoreactive bands detected
with AS/6. In contrast, as expected (Table II), LEI/3 fails to
react with either transducin-α or Gi,w. Consistent with the
results seen in neutrophil membranes, LEI/3 strongly reacts
with the purified neutrophil major pertussis toxin substrate.
Interestingly, LEI/3 reveals a faintly reactive band (lane 2) in
the purified brain Gx/Gw lane that comigrates with the purified
neutrophil protein. It is highly likely that this represents
reactivity of LEI/3 with a protein similar if not identical to
that in neutrophils, rather than weak cross-reactivity with
the 41-kDa form of Gi,a abundant in brain that is readily
detected by AS/6. This statement is based on the clear differ-
ences in mobility of the bands detected on immunoblot (Fig.
7).

We have further assessed the specificity of LE/2 and LE/5
antisera with reagents kindly provided by James Hurley (Uni-
versity of Washington). Peptides corresponding to the se-
quencies of rod and cone transducin-α subunits from the region
homologous to LERIAQSDYI (these are defined in Ref. 5 and
see also Table II) failed to block LE/2 or LE/3 reactivity with
the neutrophil membrane protein. Comparable amounts (1
and 9 µg) of the LERIAQSDYI synthetic peptide effectively
blocked LE antigen reactivity (not shown). Also, antisera
raised to the synthetic peptides corresponding to rod and cone
transducin-α sequences failed to react with the 40–41-kDa
protein in neutrophil membranes, although these antisera
readily react with rod and cone transducin-α (5).

**DISCUSSION**

Peptides have successfully been used as immunogens to
generate antisera directed against many proteins (30), includ-
ing G-protein subunits (31). Useful antisera directed against
Gi,a, however, have until recently not been produced either by
injection of synthetic peptides (31) or by injection of purified
Gi,a protein (19,32). A recent report (33) briefly described
production of antisera directed against the 41-kDa form of
Gi,a from rat brain. These antisera were produced by injection
of the purified protein, and the epitope(s) against which they
are directed have not been defined.

To obtain antisera directed against Gi,a and transducin-α,
we immunized rabbits with the synthetic peptide KENL
KDCLGF equivalent to the carboxyl-terminal decapetide of
transducin-α and differing by only 1 residue from the corre-
sponding sequences of Gx,Gw and Gi,w. We chose this se-
quence also because it may correspond to a domain critical for
G-protein-receptor interaction.

Antisera AS/6 and AS/7, raised against the peptide
KENLKDCLGF, reacted not only with purified transducin-α
but also with a 40–41-kDa protein in essentially all tissues
and cells we have tested including brain, neutrophils, and Cé-
glioma cells. The proteins recognized by AS/6 and AS/7
presumably represent multiple forms of Gi,a since the antisera
reacted with purified Gi,a (41 kDa) from brain and with the
purified 40-kDa pertussis toxin substrate from neutrophils.
AS/6 and AS/7 do not react with purified Gi,w.

Although AS/6 and AS/7 are clearly useful reagents for
identification of Gi,a in multiple tissues and for discrimination
between Gi,a and Gi,w, these antisera cannot distinguish be-
tween putative forms of Gi,a such as Gi,c and Gi,y. Two of these
forms of Gi,a have been identified by cDNA cloning (13–17),
as has at least one additional form of Gi,a.

To obtain reagents capable of distinguishing closely related
proteins such as Gi,c and Gi,w, we have immunized rabbits
with synthetic peptides corresponding to regions of amino
acid sequence divergence between the two proteins. The pep-
tide used to produce antisera LE/1, 2, and 3, LERIAQSDYI,
corresponds to an internal sequence of Gi,c (15–17) and differs
by 3 residues from the corresponding sequence of Gi,w (13,14).

We had seen previously that antisera specific for trans-
ducin, Gi,a and Gi,w fail to react with the major pertussis toxin
substrate in neutrophils. LE/2 and LE/3 react strongly and
specifically with this 40-kDa protein in human neutrophil
membranes, as well as with the 40-kDa protein purified from
bovine neutrophils (26). LE/2 and LE/3 do not react with
purified transducin-α or Gi,w nor do they react with the 41-
kDa protein purified from bovine brain, Gi,w. The faint, but
nonetheless specific, 40-kDa immunoreactive band revealed
by LE/2 and LE/3 in crude brain membranes as well as in
purified preparations of brain Gi,Gx may represent a protein
similar, if not identical to the neutrophil protein. These data
do not allow us to conclude that the abundant protein in
neutrophils recognized by LE/2 and LE/3 is in fact Gi,w, but
they are highly suggestive of this possibility. It remains pos-
sible that the immunoreactive protein in neutrophils corre-
sponds to an as yet unidentified G-protein with a sequence
similarly sufficient to that of Gi,w to lead to cross-reactivity.
A similar consideration applies in brain. The relatively faint
band revealed by LE/2 and LE/3 in brain may reflect low
abundance of Gi,w or a related protein. In this regard, it is
interesting that an approximately 40-kDa pertussis toxin
substrate, in addition to Gi,w and the 41-kDa protein, was
detected in one of the original reports on purification of
pertussis toxin substrates from brain (7) and has recently
been purified (33). Two-dimensional gel electrophoresis com-
bined with immunoblotting using peptide and other antisera
has revealed marked heterogeneity of brain pertussis toxin
substrates.

Notwithstanding the qualifications concerning the identity
of proteins detected by LE/2 and LE/3, it is clear that these
reagents provide a very useful tool for discrimination of closely
related forms of Gi,a. The studies described here demonstrate
directly that the major pertussis toxin substrate of neutrophils
differs from the two major forms found in brain, Gi,a and
Gi,w. A neutrophil G-protein couples the fMet-Leu-Phe re-
ceptor to phospholipase C (55). This form of Gi,a is pertussis
toxin-sensitive, unlike Gi,a in many other tissues (36). Our
data are compatible with the suggestion that the neutrophil
form of Gi,a is the protein detected by LE/2 and LE/3, possibly
Gi,w. Use of these highly specific antisera to identify G-
protein(s) associated with the fMet-Leu-Phe receptor and/or
phospholipase C will allow this hypothesis to be tested.

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