Hormonal inhibition of adenylate cyclase is mediated by a guanine nucleotide regulatory protein (N), which is different from the one which mediates hormonal stimulation. There is substantial evidence that the active component of N (termed α) can be ADP-ribosylated by a toxin from Bordetella pertussis. We have found that there are three proteins of similar molecular weight (39,000–41,000) which are modified by pertussis toxin. We have purified these proteins and have resolved the 41,000-dalton protein from the 40,000/39,000-dalton doublet. All three forms of pertussis toxin substrate can be isolated in free form or together with a 36,000 β component. We have also purified this β component. ADP-ribosylation of the three pertussis toxin substrates is greatly enhanced by the addition of the purified β component. This makes possible an assay of β subunit activity based on its interaction with α2.

The three forms of pertussis toxin substrate which we have purified differ in two functions: susceptibility to ADP-ribosylation and GTPase activity. The 41,000-dalton protein is more readily ADP-ribosylated by pertussis toxin than the smaller forms. The 39,000-dalton protein has GTPase activity with a low $K_m$ (0.3 μM) for GTP. The GTPase activity can be doubled by phospholipids. The GTPase activity of the 41,000-dalton protein is almost undetectable. It is not yet known what the relationship is between these enzymes; each other. The smaller forms may be derived from the larger by proteolysis or it may be intrinsically different. It remains to be shown whether one of the forms represents a different type of regulatory protein which transmits a hormonal signal to effectors other than adenylate cyclase.

Hormonal inhibition of adenylate cyclase is mediated by a guanine nucleotide binding protein (Ni) which is different from the one which mediates hormonal stimulation. There is substantial evidence that the active component of Ni (termed αi) can be ADP-ribosylated by a toxin from Bordetella pertussis and a β component that seems to be similar or identical from both N and Ni (2–4). In addition, there may be a small molecular weight γ subunit of unknown function associated with β (5).

The conclusion that the protein ADP-ribosylated by pertussis toxin is indeed the αi component of adenylate cyclase rests on substantial evidence from studies with intact cells, with cell membranes, and with purified adenylate cyclase components. In intact cells, exposure to pertussis toxin attenuates or abolishes the ability of some hormones to decrease intracellular cyclic AMP levels and blocks their physiological effects (6,7). Treatment of cells or membranes with pertussis toxin diminishes or blocks the ability of inhibitory hormones to attenuate adenylate cyclase activity (8–11) or to increase GTPase activity (11). Pertussis toxin treatment can also reduce receptor affinity for an inhibitory agonist (12). These actions are correlated with ADP-ribosylation of 41-kDa plasma membrane protein (9–12). The 41-kDa pertussis toxin substrate, in association with a 35,000-dalton β subunit, has recently been purified from rabbit liver (2) and human erythrocytes (3,13).

In addition to covalently modifying the inhibitory guanine nucleotide regulatory protein of the adenylate cyclase system, pertussis toxin can also ADP-ribosylate a closely related protein from bovine retinal rods, the Ta component of transducin (14,15). This 39-kDa protein is structurally similar to the αi component (4). The Ta component of transducin is necessary for the GTP-dependent stimulation of retinal cyclic GMP phosphodiesterase (reviewed in Ref. 16) and is itself a GTPase (17). Like αi, the Ta component of transducin is associated with a 36-kDa β component (15–18).

In the studies reported here, we have used ADP-ribosylation by pertussis toxin as a means of identifying the αi component. We have found that in bovine cerebral cortex, there are three substrates for ADP-ribosylation by pertussis toxin, with molecular weights of 39,000, 40,000, and 41,000. The forms differ not only in size but in function. For simplicity, we have referred to all three as αi throughout the paper. However, this is a tentative assignment since some of these proteins may also couple hormone receptors to effectors other than adenylate cyclase (see “Discussion”). The approximately 40-kDa proteins have been purified both in association with the β component and alone. We have also purified the β component free of αi. Northup et al. (19) have recently described a unit. Heavy (αiH) and lighter (αiL) forms of αi are found in some cells; β, a polypeptide component of both Ni and N, with a molecular weight of 55,000–36,000; αN, the polypeptide component of N, which can be ADP-ribosylated by pertussis toxin; Gpp(NH)p, guanosine 5'-β,γ-iminodiphosphosphate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

2 E. J. Neer and L. G. Wolf, manuscript in preparation.

Purification and Properties of the Inhibitory Guanine Nucleotide Regulatory Unit of Brain Adenylate Cyclase*

Eva J. Neer, Josephine M. Lok, and Lisa G. Wolf

From the Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115

(Received for publication, June 11, 1984)
scribed an assay for activity of the β subunit based on its association with α, the guanine nucleotide binding subunit of N. We now show that the activity of β can also be measured based on its interaction with α.

**MATERIALS AND METHODS**

ADP-ribosylation of Purified N.—N. was identified by [32P]ADP-ribosylation with pertussis toxin under the following conditions: 5 μM NAD containing 0.3-0.5 μCi of [32P]NAD, 2.5 mM ATP, 2 mM GTP, 10 mM isoniazide, 10 mM thymidine, 60 ng of pertussis toxin, 0.2-0.5 μg of substrate protein in a total volume of 25-30 μl. Some earlier experiments also included 0.2 mM Gpp(NH)p. After 45 min at 37 °C, the reaction was stopped by the addition of 2% SDS in Laemmli sample buffer (20) and boiling for 1 min. For reconstitution of α and β, the two components were incubated together for 10 min at 23 °C before the assay reagents were added. The entire reaction mixture was centrifuged for approximately 450 ml, was taken up in 2.5 liters of homogenizing buffer and recentrifuged for 20 min at 10,000 g for 70 min. The total volume of supernatant was 1700 ml.

Preparation of N.—The procedure was a modification of the method of Northup et al. (25) and Sternweis et al. (26). Fresh bovine cerebral cortex (500 g) was homogenized in 3 liters of buffer of the following composition: 50 mM Tris-Cl, pH 8.1, 5% sucrose (w/v), 6 mM MgCl₂, 1 mM EDTA, 1 μg/ml soybean and lima bean trypsin inhibitors, 3 mM benzamidine-Cl, 1 mM dithiothreitol. The homogenate was centrifuged for 20 min at 10,000 × g at 4 °C. The pellet, which measured approximately 450 ml, was taken up in 2.5 liters of homogenizing buffer and recentrifuged for 20 min at 10,000 × g. The pellet from this centrifugation, which measured approximately 350 ml, was brought up to 500 ml of homogenizing buffer and frozen at −70 °C. Before solubilization, it was thawed, brought to 1 liter with homogenizing buffer and recentrifuged. The final pellet of 250 ml was brought up to 1 liter with homogenizing buffer. To this was added 1 liter of homogenizing buffer without sucrose containing 2% cholate to give a final cholate concentration of 1%. The homogenate was allowed to solubilize at 4 °C for 90 min and was then centrifuged at 32,000 × g for 70 min. The total volume of supernatant was 1700 ml.

The cholate supernatant was made 10 mM in ACl₃ and 10 mM in NaF and applied to a 1500-ml column of DEAE-Sephacel (Pharmacia) equilibrated with 50 mM Tris-Cl, pH 8, 75 mM sucrose, 6 mM MgCl₂, 1 mM dithiothreitol, 1 mM EDTA, 3 mM benzamidine-Cl, 1 mg/liter soy bean and lima bean trypsin inhibitor (Sigma), 10 μM ACl₃, 10 μM NaF, and 0.9% cholate (Buffer A). The column, termed DEAE-Sephacel I in Table I, was eluted with a 1800-ml linear NaCl gradient from 0 to 0.25 M NaCl in Buffer A. The column was assayed for N. activity by reconstitution with resolved catalytic unit from bovine caddate nucleus as described previously (27). Most of the approximately 40 and 36 kDa proteins were included in the peak of N., activity. This was pooled, concentrated in an Amicon concentrator with a XM-50 or YM-10 membrane to 1/4 its volume, and applied to a 1700-ml column of Sepharose 6B equilibrated with Buffer A. N. activity, as well as the approximately 40 and the 36-kDa proteins, eluted from the column with a distribution coefficient of 0.5-0.6.

The peak of N. activity from the Sepharose 6B column was pooled and applied to a second column of DEAE-Sephacel (DEAE-Sephacel II, Table I) equilibrated with Buffer A. The volume of this column was 350 ml and it was eluted with a 1400-ml linear gradient of NaCl (0-0.25 M) in Buffer A. N. activity eluted from this column of two peaks as shown in Fig. 1A. N. eluted between the two N. peaks. The distribution of polypeptides in the column effluent is shown in Fig. 1B. The subsequent steps of the purification are described under "Results."

**RESULTS**

Purification of α.—A flow chart for the purification of the α and β components is shown in Table I. Two routes led to the preparation of pure components. The first used fractions separated from N. activity by the DEAE-Sephacel column shown in Fig. 1, A and B. Fractions 136-148 were pooled and applied to a column of heptylamine-Sepharose synthesized by the method of Shaltiel (28). The 50-ml heptylamine-Sepharose column was equilibrated with Buffer A which contained 100 mM NaCl and 0.4% Trit-cholate; lima bean and soybean trypsin inhibitors were omitted from buffers for this and

![Fig. 1. DEAE-Sephacel chromatography of N. and N. (DEAE-Sephacel II) (A) and polypeptide distribution in the fractions from DEAE-Sephacel II (B). A. A cholate extract of bovine cerebral cortex that had been purified through the Sepharose 6B step (see "Materials and Methods" and Table I) was the starting material for this column. A 150-ml sample containing 360 mg of protein was applied to a 350-ml column and eluted as described under "Materials and Methods." The fraction size averaged 6 ml. Samples of 0.5 μl were assayed for N. activity by reconstitution with resolved catalytic unit from bovine caddate nucleus as described by Bender and Neer (27). The reconstitution assay contained 20 μM Gpp(NH)p and 50 μM forskolin (●). Protein in 50-μl samples (○) was measured by the method of Lowry (22, 23). B. Samples of 40 μl from the designated column fractions were applied to an 11% acrylamide Laemmli gel (20). The gel was stained with Coomassie Blue.](image-url)
The 39/40-kDa protein eluting in heptylamine-Sepharose under the same conditions as Fig. 2, the 30-kDa contaminant eluted in the flow-through volume. The 39-kDa protein eluted at the position of peak 2B in Fig. 2. Most of the 36-kDa component bound firmly to the hydrophobic resin and was eluted in the cholate gradient. The 39-kDa protein could be separated from the remaining contaminants by gel filtration over Ultrogel AcA-44 (LKB) equilibrated with 0.05 M Tris, pH 7.6, 6 mM MgCl₂, 1 mM EDTA, 1 mM dithiothreitol, and no detergent. This preparation, called α₁-II (Table I), contained only a trace of the 40-kDa and no 41-kDa protein. An overloaded SDS-PAGE pattern of a peak fraction is shown in Fig. 4.

Even without detergent, the 39-kDa protein eluted from the Ultrogel AcA-44 gel filtration column with the same distribution coefficient as hemoglobin, indicating that the 39-kDa protein does not aggregate in the absence of detergent. This suggests that the protein is not strongly hydrophobic and is consistent with the observation that it can be eluted from heptylamine-Sepharose with buffer or 0.5 M NaCl.

We have also purified the 39–41-kDa proteins in association with the 36-kDa β component (N₁-I in Table I). Fractions 125–135 from the DEAE-Sephacel column shown in Fig. 1, A and B, were diluted to decrease the salt concentration and applied to a 130-ml DEAE-Sephacel column equilibrated with 0.05 M Tris-Cl, 75 mM sucrose, 6 mM MgCl₂, 1 mM dithiothreitol, 3 mM benzamidine, 0.6% Lubrol 12A9 without AlCl₃ or NaF. The column was eluted with an NaCl gradient from 0 to 0.25 M NaCl in the equilibrating buffer. Although the 36-kDa component was present, the cluster of approximately 40-kDa proteins distributed similarly to the column profile shown in Fig. 3. Lanes 2 and 3 of Fig. 5 show the protein distribution from the leading edge of the peak and from the back edge. The 41-kDa protein elutes ahead of the 39/40-kDa proteins.

Fig. 5 shows a comparison of the mobility on a single SDS-PAGE gel of α₁ purified without-the β unit on heptylamine-Sepharose. While the 36-kDa protein was eluted at the position of peak 2B in Fig. 2. Most of the 36-kDa component bound firmly to the hydrophobic resin and was eluted in the cholate gradient. The 39-kDa protein could be separated from the remaining contaminants by gel filtration over Ultrogel AcA-44 (LKB) equilibrated with 0.05 M Tris, pH 7.6, 6 mM MgCl₂, 1 mM EDTA, 1 mM dithiothreitol, and no detergent. This preparation, called α₁-II (Table I), contained only a trace of the 40-kDa and no 41-kDa protein. An overloaded SDS-PAGE pattern of a peak fraction is shown in Fig. 4.

Even without detergent, the 39-kDa protein eluted from the Ultrogel AcA-44 gel filtration column with the same distribution coefficient as hemoglobin, indicating that the 39-kDa protein does not aggregate in the absence of detergent. This suggests that the protein is not strongly hydrophobic and is consistent with the observation that it can be eluted from heptylamine-Sepharose with buffer or 0.5 M NaCl.

We have also purified the 39–41-kDa proteins in association with the 36-kDa β component (N₁-I in Table I). Fractions 125–135 from the DEAE-Sephacel column shown in Fig. 1, A and B, were diluted to decrease the salt concentration and applied to a 130-ml DEAE-Sephacel column equilibrated with 0.05 M Tris-Cl, 75 mM sucrose, 6 mM MgCl₂, 1 mM dithiothreitol, 3 mM benzamidine, 0.6% Lubrol 12A9 without AlCl₃ or NaF. The column was eluted with an NaCl gradient from 0 to 0.25 M NaCl in the equilibrating buffer. Although the 36-kDa component was present, the cluster of approximately 40-kDa proteins distributed similarly to the column profile shown in Fig. 3. Lanes 2 and 3 of Fig. 5 show the protein distribution from the leading edge of the peak and from the back edge. The 41-kDa protein elutes ahead of the 39/40-kDa proteins.

Fig. 5 shows a comparison of the mobility on a single SDS-PAGE gel of α₁ purified without-the β unit on heptylamine-Sepharose.
**The N1 Component of Adenylate Cyclase**

14225

**FIG. 2. Heptylamine/Sepharose chromatography of αi and β subunits.** Fractions 136-148 from the DEAE-Sephacel column shown in Fig. 1A were pooled and applied to a 50-ml column of heptylamine-Sepharose equilibrated and eluted as described in the text. The distribution of the 39/40-kDa (●) and 36-kDa (△) polypeptides was determined by SDS-PAGE of samples from the fractions followed by densitometry of the silver-stained, dried gel. The area under the peaks, measured by densitometry, is reported in arbitrary units.

Sepharose and Ultrogel AcA 44 gel filtration (αi-II, lanes 1 and 5), αi purified together with the β unit as described in the preceding paragraph (N-I, lanes 2 and 3), and the Tα subunit of transducin from bovine retinal rods. (Transducin Tα and Tβ components were the kind gift of Dr. Bernard K.-K. Fung, University of Rochester Medical Center, Rochester, NY) The mobility of the 39-kDa brain proteins is identical to that of the Tα component of transducin. The larger, 41 kDa, protein is readily distinguishable from the others.

**Purification of the β Subunit—** Purified 36-kDa β component could be obtained from two sources: it could be separated from αi, by heptylamine-Sepharose chromatography as shown in Fig. 2, and it could be further purified by gel filtration over an Ultrogel AcA 44 column in 50 mM Tris·Cl, 6 mM MgCl2, 75 mM sucrose, 3 mM benzamidine·CL, 1 mM dithiothreitol, and 0.4% cholate. This is preparation β-II on the chart in Table I. An overloaded SDS-PAGE gel of such a preparation is shown in Fig. 3. A small amount of a 35-kDa protein is also found in all the preparations. Fig. 4 (lanes 6 and 7) shows that the pure brain β component has the same electrophoretic mobility as the Tβ component of transducin.

The 36-kDa protein could also be purified from peak 3 in Fig. 2, by chromatography over DEAE-Sephacel in 0.6% Lubrol 12A9 under the same conditions as were described in the legend to Fig. 3. When the buffer contained Al"+ and F- free 36-kDa protein eluted in the void volume of the column (preparation β-I in Table I). The β subunit from liver also does not bind to DEAE-Sephacel in Lubrol 12A9 (26). The subunit obtained from both sources is active in the functional assay that will be described below.

**Properties of N1—** Pertussis toxin predominantly ADP-ribosylates a 41-kDa protein in crude preparations of membranes from many cell types (9-12). Fig. 6A shows that only one radioactive band is found when a relatively crude preparation of solubilized brain proteins is incubated with [32P]NAD and pertussis toxin. However, this band is rather broad, suggesting that there may be heterogeneity in the brain substrates for pertussis toxin. This heterogeneity is confirmed by the experiment shown in Fig. 6B. Preparations enriched in either the 41- or the 39-kDa proteins were ADP-ribosylated with pertussis toxin as described under “Materials and Methods.” The fractions used in the experiment are the same ones shown in lanes 2 and 3 of Fig. 5. The larger and smaller proteins can all be ADP-ribosylated by pertussis toxin but the larger seems to be the better substrate. Although the two assays contained equal amounts of 41- and 39-kDa substrate, and of β subunit, there was 2–5 times as much radioactivity incorporated into the largest protein in lane 1 as into the sum of the three bands seen in lane 2 of Fig. 6B. By Coomassie staining, the fraction enriched in the 39-kDa protein shown...
protein only in those gradient fractions that also contained poorly if at all. The reason for this difficulty became apparent

After centrifugation, the fractions were incubated with per-

detergent as described in the text (B-II in Table I). 16

... were loaded onto the gel. The figure shows a 9% Laemmli gel (20) stained with Coomassie Blue.

in lane 2 contained no detectable 41-kDa protein, yet its presence in trace amounts was revealed by ADP-ribosylation. Fig. 6A shows that we were able to ADP-ribosylate the αi component in rather crude preparations. However, we found that the pure preparations of 39–41-kDa proteins labeled poorly if at all. The reason for this difficulty became apparent from the results of the experiment shown in Fig. 7. The figure shows a sucrose density gradient sedimentation pattern of the 39/40-kDa doublet and 36-kDa polypeptides taken from peak 2A of the heptamidine-Sepharose column shown in Fig. 2. After centrifugation, the fractions were incubated with pertussis toxin and [32P]NAD as described under “Materials and Methods.” Radioactivity was incorporated into the 39/40-kDa protein only in those gradient fractions that also contained β component. Similar results were obtained after gel filtration over columns of Ultrogel AcA 34 resin (not shown). These results might be explained by two populations of 39/40-kDa proteins: one which is a heterodimer and which can be ADP-ribosylated, one which is not associated with the β subunit and cannot be ADP-ribosylated. Addition of excess pure β component allowed ADP-ribosylation of αi in all the fractions. This shows that the centrifugation had not separated a pool of 39/40-kDa proteins which could not be ADP-ribosylated. Heat-inactivated β component did not enhance ADP-ribosylation.

The amount of [32P]ADP-ribose incorporated into the 39-kDa protein depends on the amount of β added (Fig. 8). In the experiment shown, 0.2 mol of ADP-ribose are incorporated per mol of 39-kDa protein at saturation. We used αi-II (Table I) for the experiment. In this preparation there were no detectable 40- or 41-kDa ADP-ribosylated bands. Despite a variety of maneuvers with several preparations of αi, we have not been able to incorporate more than 0.3 mol of [32P] ADP-ribose/mol of 39-kDa protein. The reason for this is not yet clear.

Enhancement of αi ADP-ribosylation by the β subunit can form the basis for an assay of β activity which depends on its
interaction with $\alpha_1$. This would be complementary to assays described by Northup et al. which measure the function of the $\beta$ unit based on its interaction with $\alpha_1$. (19). The finding that $\alpha_1$ needs $\beta$ for ADP-ribosylation by pertussis toxin is consistent with the observation of Codina et al. (3) that after ADP-ribosylation, the $\alpha_1$ and $\beta$ subunits of N, from human red cells are associated into a heterodimer.

**GTPase Activity of N.**—The purified 39-kDa protein and the 39/40-kDa doublet have GTPase activity with a high affinity for GTP. Fig. 9 shows the GTP concentration dependence of the $\alpha_1$ GTPase activity. The apparent $K_a$ for GTP is $3.0 \pm 0.1 \mu M$ GTP ($n = 4$). The GTPase activity distributes with the 39-kDa protein or the 39/40-kDa doublet through a variety of separation procedures. An example is shown in Fig. 10, which shows the correlation of GTPase activity with the amount of 39/40-kDa protein on DEAE-Sephacel column. In other experiments, we found that GTPase activity also coincided with the peak of 39/40-kDa protein on sucrose density gradient centrifugation in Lubrol 12A9-containing buffers, and with the peak of 39-kDa protein on Ultrogel AcA 44 gel filtration in buffers with no detergent (not shown). This consistent distribution suggests that the GTPase activity is indeed a property of these proteins and not of a contaminant. Pure $\beta$ component has no detectable GTPase activity.

The ability to separate the 41- and 39-kDa forms of N, allowed us to compare their GTPase activity. We used the fractions shown in lanes 2 and 3 of Fig. 4 for this experiment. Surprisingly, the GTPase activity of the 41-kDa protein was much lower than that of 39-kDa one. The specific activity of the former was $0.2 \pm 0.1 \text{ nmol of Pi/(mg x min); } n = 4$ while that of the latter was 1.2-4 nmol of Pi/(mg x min), $n = 4$. In both cases we tested the activity at three concentrations of protein. The activity was linear with protein and with time. The specific activity of the 39-kDa protein was similar whether it was measured in the presence of the $\beta$ subunit (as in the experiment described above) or without the $\beta$ unit. The pure 39-kDa protein ($\alpha_1$-II, Table I; lane 2, Fig. 3) had a specific GTPase activity of $2.4 \pm 0.4 \text{ nmol of Pi/(mg x min); } n = 5$.

Although the GTPase activity of the $\alpha_1$ protein is easily measurable, its turnover number is quite low: 0.08-0.20 mol of P, product/(mol of enzyme x min) in four different preparations. The reason for the low turnover number is not clear. The GTPase activity associated with $\alpha_1$ can be increased 2-fold by the addition of mixed soybean phospholipids (Sigma, P3644) which contain predominantly phosphatidylycholine (Fig. 11). This suggests that the activity might be higher if measured in a membrane environment.
DISCUSSION

The methods described here lead to the purification of three substrates for ADP-ribosylation by pertussis toxin from bovine brain as well as purification of a 36-kDa β component. The latter can interact both with the stimulatory guanine nucleotide regulatory protein, αs, and, as we now show, with the inhibitory unit αi. The β unit enhances ADP-ribosylation of all three pertussis toxin substrates. The capacity to interact with both αs and αi components is consistent with the role proposed for the β subunit by Sterweis et al. (26) and Northup et al. (19). The observation that the amount of [32P]ADP-ribose incorporated into αi depends on the concentration of β subunit, means that the ADP-ribosylation can only be used to quantitate αi under conditions where the amount of β is known to be saturating.

The substrate for ADP-ribosylation by pertussis toxin differs in brain from that reported in other tissues. Instead of being a single polypeptide, there appear to be three proteins of very similar molecular weight which can be modified by the toxin. These proteins can be separated by chromatography over DEAE-Sephacel. The protein present in greatest quantity is a 39-kDa one which can be obtained either in free form or associated with a 36-kDa β component. This protein is less effective as a substrate for pertussis toxin than the 41-kDa protein which is also found in association with a 36-kDa subunit. In addition to these two, we can detect a 40-kDa ADP-ribosylated protein which we have not yet obtained free of the 39-kDa polypeptide. The relationship among these proteins is not yet known. It is tempting to speculate that the proteins may be derived from each other by proteolysis but this must await analysis of the separated subtypes. The molecular weight of the larger ADP-ribosylated protein is the same as that reported for purified αi from rabbit liver (2). The smaller molecular weight is the same as that reported for N, purified from human red blood cells (3). It also corresponds to that of the Tα subunit of transducin from bovine retinal rods, a guanine nucleotide binding protein which can also be ADP-ribosylated by pertussis toxin (15), and the two molecules migrate identically in our SDS-PAGE gels. It has previously been shown that the 41-kDa αi subunit from rabbit liver and the Tα component of transducin share some peptides, suggesting that they have a structural similarity (4). Extrapolation from that data suggests that the three approximately 45-kDa brain proteins may be related.

Similar heterogeneity occurs in αs from brain and other tissues. In many tissues cholera toxin identifies only a 42-45-kDa form of the αs component of Nt. In brain (39), S49...
lymphoma cells (30), 3T3-L1 (31, 32), and other cell types (33, 34), two forms of $N_1$ can be identified, a heavier form $\alpha_{cat}$ with a molecular mass of 47–52 kDa and a lighter form $\alpha_{cat}$, with a molecular mass of 42–45 kDa. Hudson and Johnson (30) showed that the heavier and lighter forms of $\alpha_1$ from S49 lymphoma cells have common peptides. This observation has been confirmed in fat cells (33) and liver (34).

The larger and smaller forms of $\alpha_1$ from cerebral cortex differ in their GTPase activity. The larger form has barely detectable GTPase activity. In contrast, the 39-kDa protein has a clearly measurable GTPase activity. Although the enzymatic activity is easily measured, the turnover number of the $\alpha_1$-associated GTPase is low (0.08–0.2 mol of P, formed per mol of enzyme $\times$ min). The activity is increased 2-fold by the addition of phospholipids. Unlike transducin, it is increased only slightly if at all by the addition of purified $\beta$ subunit (data not shown). The purified $N_1$ protein has no detectable GTPase activity unless it is reconstituted in lipid vesicles with a hormone receptor (34). The specificity of the hormone response argues that the GTPase is indeed an intrinsic activity of $N_1$ and not of a contaminant. However, even after hormonal stimulation, the turnover number is only 1 mol of P, formed per mol of enzyme/min. Brandt et al. (36) calculate that this value is similar to that observed in intact turkey erythrocyte membranes. The activity of the GTPase of $N_1$, $N_2$, and transducin may be limited by factors such as the rate of substrate binding and the slow rates measured may reflect the regulatory function of GTP turnover.

The heterogeneity of size, the differences in susceptibility to ADP-ribosylation by pertussis toxin and in GTPase activity which we find in the $\alpha_1$ proteins from brain may be due to a primary difference in protein structure or to secondary changes such as partial proteolysis or post-translational modifications. Whatever the molecular cause, the differences may reflect a physiologically important heterogeneity of function.

Acknowledgments—We are very grateful to Dr. Erik L. Hewlett at the University of Virginia Medical School, Charlottesville, VA and to Merck, Sharp and Dohme Research Laboratories, Rahway, NJ for their generous gift of purified pertussis toxin.

REFERENCES

1. Gilman, A. G. (1984) Cell 36, 577–579
2. Bokoch, G. M., Katada, T., Northup, J. K., Ui, M., and Gilman, A. G. (1984) J. Biol. Chem. 259, 3560–3567
3. Codina, J., Hildebrandt, J., Iyengar, R., Birnbaumer, L., Sekura, R. D., and Manclark, C. R. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 4276–4280
4. Manning, D. R., and Gilman, A. G. (1983) J. Biol. Chem. 258, 7059–7063
5. Hildebrandt, J. D., Codina, J., Risinger, R., and Birnbaumer, L. (1984) J. Biol. Chem. 259, 2039–2042
6. Hazeki, O., and Ui, M. (1980) J. Biol. Chem. 255, 3866–3862
7. Katada, T., and Ui, M. (1981) J. Biol. Chem. 256, 8310–8317
8. Murayama, T., and Ui, M. (1983) J. Biol. Chem. 258, 3319–3326
9. Katada, T., and Ui, M. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 3129–3133
10. Katada, T., and Ui, M. (1982) J. Biol. Chem. 257, 7210–7216
11. Burns, D. L., Hewlett, E. L., Moss, J., and Vaughan, M. (1983) J. Biol. Chem. 258, 1453–1458
12. Hisa, J. A., Moss, J., Hewlett, E. L., and Vaughan, M. (1984) J. Biol. Chem. 259, 1086–1090
13. Codina, J., Hildebrandt, J. D., Sekura, R. D., Birnbaumer, M., Bryan, J., Manclark, C. R., Iyengar, R., and Benbaumer, L. (1984) J. Biol. Chem. 259, 5871–5876
14. Van Dop, C., Yamazaki, G., Steinberg, F., Sekura, R. D., Manclark, C. R., Stryer, L., and Boume, H. R. (1984) J. Biol. Chem. 259, 23–26
15. Manning, D. R., Fraser, B. A., Kaha, R. A., and Gilman, A. G. (1984) J. Biol. Chem. 259, 749–756
16. Stryer, L., Hurley, J. B., and Fung, B. K.-K. (1981) Curr. Top. Membr. Transp. 15, 93–108
17. Fung, B. K.-K. (1985) J. Biol. Chem. 258, 10495–10502
18. Baehr, W., Morita, E. A., Swanson, R. J., and Applebury, M. C. (1982) J. Biol. Chem. 257, 15052–15055
19. Northup, J. K., Sternweis, P. C., and Gilman, A. G. (1983) J. Biol. Chem. 258, 11361–11366
20. Laemmli, U. K. (1970) Nature (Lond.) 227, 680–685
21. Merri, C. R., Goldman, D., Sedman, S. A., and Ebert, M. H. (1984) Nature (Lond.) 311, 147–148
22. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
23. Bailey, J. L. (1967) Techniques in Protein Chemistry, p. 340, Elsevier Publishing Co., New York
24. Neer, E. J., Echeverria, D., and Knox, S. (1980) J. Biol. Chem. 255, 9782–9789
25. Northup, J. K., Sternweis, P. C., Smigel, M. D., Schiefelbein, L. S., Ross, E. M., and Gilman, A. G. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 6519–6520
26. Sternweis, P. C., Northup, J. K., Smigel, M. D., and Gilman, A. C. (1981) J. Biol. Chem. 256, 11517–11526
27. Bender, J. L., and Neer, E. J. (1983) J. Biol. Chem. 258, 2432–2438
28. Schilts, B. (1974) Methods Enzymol. 34, 126–140
29. D'Alayer, J., Berthilier, G., and Munneron, A. (1983) Biochemistry 22, 3945–3953
30. Hudson, T. H., and Johnson, G. L. (1980) J. Biol. Chem. 255, 7480–7486
31. Watkins, P. A., Moss, J., Pekala, P. H., and Lane, D. M. (1982) J. Biol. Chem. 257, 14719–14722
32. Lai, E., Rosen, G. M., and Rubinson, C. S. (1981) J. Biol. Chem. 256, 12866–12874
33. Malbon, C. G. (1982) Biochim. Biophys. Acta 714, 429–434
34. Ward, W. H., and van Huytingen, S. (1982) Biochem. Biophys. Res. Commun. 108, 1181–1187
35. Aktories, K., and Jakobs, K. H. (1981) FEBS Lett. 13, 235–238
36. Brandt, D. R., Asano, T., Pedersen, S. E., and Ross, E. M. (1982) Circ. Res. 50, 482–489
37. Wright, G. B., Alexander, R. W., Ekstein, L. S., and Gimbrone, M. A. Jr. (1982) Circ. Res. 50, 482–489
38. Wright, G. B., Alexander, R. W. Ekstein, L. S., and Gimbrone, M. A. Jr. (1983) Mol. Pharmacol. 29, 213–221
39. Gunther, S. (1984) J. Biol. Chem. 259, 7622–7629
40. Godhardt, M., Perry, N., Gepnet, P., and Hanoune, J. (1982) J. Biol. Chem. 257, 11571–11575
41. Snavely, M. D., and Insel, P. A. (1983) Mol. Pharmacol. 22, 532–536
42. Extone, J. H. (1981) Mol. Cell. Endocrinol. 23, 233–264