Pertussis toxin substrate is a guanosine 5'-[β -thio]diphosphate-, *N*-ethylmaleimide-, Mg^{2+} - and temperature-sensitive GTP-binding protein

Stephen K. F. WONG, B. Richard MARTIN and Aviva M. TOLKOVSKY*

Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, U.K.

1. We compared the effects of guanine nucleotides and Mg^{2+} on ADP-ribosylation of rat brain and liver membrane proteins catalysed by *Bordetella pertussis* toxin (IAP) and cholera toxin (CT). Labelling of proteins in the presence of [α - ^{32}P]NAD⁺, ATP and CT required GTP or guanosine 5'-[γ -thio]triphosphate (GTP[S]). In contrast, labelling of one (liver) or two (brain) polypeptides by IAP was enhanced by guanosine 5'-[β -thio]diphosphate (GDP[S]) or GTP, but was blocked by GTP[S] or guanosine 5'-[β , γ -imido]triphosphate (p[NH]ppG). The order of labelling intensity was GDP[S] > GTP > no addition > GTP[S] = p[NH]ppG.
2. Mg^{2+} increased labelling by CT, but decreased labelling by IAP. In addition, Mg^{2+} potentiated the effects of the guanine nucleotides, increasing the inhibitory effects of GTP[S] and the activatory effects of GDP[S] or GTP. Preincubating liver membranes at 30 °C in the presence of 10 mM- $MgCl_2$ inhibited labelling by IAP irreversibly.
3. Pretreatment of liver membranes with 4.95 mM-*N*-ethylmaleimide decreased labelling by CT by ~ 15%, but almost completely blocked labelling by IAP.
4. These results suggest that the undissociated, GDP-bound, conformation of N_i , the inhibitory GTP-binding protein of adenylate cyclase, is the preferred substrate for ADP-ribosylation by IAP. This conformation, which is prevalent in native membranes, is sensitive to temperature, Mg^{2+} ions and alkylating agents such as *N*-ethylmaleimide. At 30 °C, Mg^{2+} may cause dissociation and denaturation of N_i in native membranes.

INTRODUCTION

There are two distinct guanine-nucleotide-binding proteins which mediate hormone and neurotransmitter action on adenylate cyclase, N_s (or G_s), which mediates activation, and N_i (or G_i), which mediates inhibition. N_s and N_i are multisubunit proteins, which appear to dissociate upon activation into a GTP-binding moiety, designated $N\alpha$ (42–52 kDa from N_s and 41 kDa from N_i), and a smaller regulatory unit, $N\beta\gamma$ (35 and 5 kDa, from both N_s and N_i) (reviewed by Gilman, 1984). Cholera toxin (CT) stabilizes N_s in an active conformation by catalysing ADP-ribosylation of $N_s\alpha$. This reaction requires GTP or its non-hydrolysable analogues (Cassel & Pfeuffer, 1978; Gill & Meren, 1978; Doberska *et al.*, 1980). Similarly, *Bordetella pertussis* toxin (or islet-activatory protein, IAP) catalyses ADP-ribosylation of $N_i\alpha$ (Katada & Ui, 1982), but this modification blocks the activation of N_i by GTP and non-hydrolysable analogues of GTP. Consistent with this action, GTP[S] has been reported to block IAP labelling of $N_i\alpha$ (Katada *et al.*, 1984; Malbon *et al.*, 1984). However, labelling by IAP occurs in both the presence and the absence of GTP (Katada & Ui, 1982; Bokoch *et al.*, 1983; Hildebrandt *et al.*, 1983; Sekura *et al.*, 1983; Tsai *et al.*, 1984). Thus it is not clear whether IAP blocks inhibition by N_i by modifying its 'inactive' or 'active' conformations.

The conformation of N_i is influenced not only by

guanine nucleotides but also by Mg^{2+} . Pure N_i , which is activated by GTP[S], binds the nucleotide essentially irreversibly in the presence of Mg^{2+} (Bokoch *et al.*, 1984), and Codina *et al.* (1983) have shown that N_i dissociates into subunits in the presence of guanine nucleotides and Mg^{2+} . However, a systematic study of the effects of Mg^{2+} on ADP-ribosylation by IAP has not been documented.

In this study, we show that N_i in membranes can be maximally ADP-ribosylated by IAP if it is in the GDP-bound form, whereas GTP[S], Mg^{2+} , preincubation in the presence of Mg^{2+} at 30 °C and covalent modification by *N*-ethylmaleimide all tend to block labelling by IAP. The results are discussed in terms of the 'inactive-undissociated' and 'active-dissociated' conformations of N_i within the plasma membrane.

EXPERIMENTAL

Materials

GTP[S], p[NH]ppG, GDP[S], phosphocreatine, creatine kinase and ATP were from Boehringer. Cyclic AMP was from Sigma. CT was from Sigma or from Calbiochem. IAP was supplied by Dr. L. Irons, Public Health Laboratory Service, Porton Down, Salisbury, Wilts., U.K. [α - ^{32}P]ATP was synthesized from [^{32}P]P_i as described by Martin & Voorheis (1977), or obtained from Amersham. [^{32}P]P_i was also from Amersham.

Abbreviations used: CT, cholera toxin; IAP, *Bordetella pertussis* toxin (islet-activating protein); GTP[S], guanosine 5'-[γ -thio]triphosphate; GDP[S], guanosine 5'-[β -thio]diphosphate; p[NH]ppG, guanosine 5'-[β , γ -imido]triphosphate; A-GTP, guanosine 5'-triphospho-5'-(4-azidoanilide); N_i , inhibitory GTP-binding protein of adenylate cyclase; N_s , stimulatory GTP-binding protein of adenylate cyclase.

* To whom reprint requests should be addressed.

Fig. 1. ADP-ribosylation of brain membrane proteins by pertussis (IAP) and cholera (CT) toxins

The method of labelling is described in the Experimental section. (a) Membranes (50 $\mu\text{g}/100\ \mu\text{l}$) were preincubated for 5 min at 37 °C with 100 μM -GTP[S] and 6 mM- MgCl_2 and washed before labelling in the usual reaction mixture. The labelling reaction mixture contained 100 μM -GTP (lanes 1–4); 6 mM-phosphocreatine and 5 units of creatine kinase (CK) were added to the reaction mixture shown in lane 4. (b) Lanes 1–5, IAP; lanes 6–10, CT; lane 11, no toxin. The guanine nucleotide concentrations were: GTP 100 μM , GDP[S] 100 μM , GTP[S] 10 μM ; forskolin (Fskln) was 10 μM . (c) Lanes 1–6, IAP; lanes 7–12, CT; MgCl_2 2 mM, GTP[S] 200 μM , GDP[S] 200 μM . (d) Coomassie Blue staining of lanes 3 and 4 (Fig. 1a) and M_r markers.

$[\alpha\text{-}^{32}\text{P}]\text{NAD}$ was synthesized from $[\alpha\text{-}^{32}\text{P}]\text{ATP}$ as described by Cassel & Pfeuffer (1978). A-GTP was synthesized as described by Wong & Martin (1985).

Methods

Membrane preparation. A synaptosomal membrane fraction was prepared by the method of Jones & Matus (1974), by using frontal cortices from 130–150 g male rats. Membranes were washed in 50 mM-Tris/HCl, pH 7.4, and stored in liquid N_2 until used. Rat liver membranes were prepared as described by Pilgis *et al.* (1974).

Labelling with $[\alpha\text{-}^{32}\text{P}]\text{NAD}^+$ and toxins. IAP was preactivated by incubating portions (4 μg in 25 μl) in 50 mM-dithiothreitol for 1 h at 25 °C. CT was preactivated for 10 min at 37 °C in 20 mM-dithiothreitol. Activated toxins were used immediately. The labelling mixture contained, in 100 μl , 100 mM-potassium phosphate

buffer, pH 7.0, 10 mM-thymidine, 1 mM-ATP, 50–75 μg of membrane proteins and 0.1–0.5 μM - $[\alpha\text{-}^{32}\text{P}]\text{NAD}^+$ (1×10^6 – 5×10^6 c.p.m.), which was added last to start the reaction. After 20 min (liver) or 45 min (brain) at 30 °C, a stopping solution of 1 ml of ice-cold 50 mM-potassium phosphate buffer containing 0.1 mM-phenylmethane-sulphonyl fluoride was added, and membranes were washed once by centrifugation (15000 g , 10 min). Pellets were solubilized in 60 μl of solubilizing buffer, and proteins were separated by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Laemmli, 1970), in 10% acrylamide. Autoradiography of dried gels with preflashed Fuji RXNIF film was at $-80\ ^\circ\text{C}$.

RESULTS

ADP-ribosylation of brain and liver membrane proteins by CT and IAP with $[\alpha\text{-}^{32}\text{P}]\text{NAD}^+$ is shown in Figs. 1 and 2. Fig. 1(a) shows the labelling of brain

Fig. 2. ADP-ribosylation of liver membrane proteins by IAP and CT

Lanes a–j: liver membranes (50 μ g) were incubated with 1 mM-EDTA, 50 μ M-GTP[S] or 50 μ M-GTP, together with the ADP-ribosylation reaction medium as described in the Experimental section, with or without 10 mM-MgCl₂, in the presence of CT and IAP as indicated. Lanes 1–5: IAP labelling. The guanine nucleotide concentrations were GTP 100 μ M, GDP[S] 100 μ M, GTP[S] 10 μ M, forskolin (Fskln) 10 μ M, in the presence of 1 mM-EDTA. Lanes 6–10: the guanine nucleotide concentration used was 500 μ M. The labelling reaction was carried out in the dark in the presence of 1 mM-EDTA.

membrane proteins by CT or IAP in the presence of GTP. In order to increase labelling by CT, membranes were preincubated with GTP[S] and MgCl₂ and washed before the labelling. Otherwise, labelling of brain membranes by CT was weak relative to that observed in liver, comparable with labelling of rabbit liver membranes reported by Bokoch *et al.* (1983). The addition of a regenerating system (phosphocreatine and creatine kinase) did not enhance the labelling by CT and IAP. However, creatine kinase itself, a 43 kDa protein, was labelled. This identification was confirmed by the Coomassie-Blue-staining pattern of lanes 3 and 4 as shown in Fig. 1(d).

The effects of guanine nucleotide analogues or forskolin are shown in Fig. 1(b). Labelling by CT (of multiple polypeptides) was entirely dependent on the addition of GTP (lane 9) or GTP[S] (lane 7), with little labelling occurring in the presence of ATP alone (lane 10) or ATP and GDP[S] (lane 8). The catalytic unit effector forskolin (Neer & Salter, 1981) had no effect (lane 6). In contrast, IAP labelling was detected in the absence of added guanine nucleotides (lane 5). However, labelling was markedly enhanced by GTP (lane 4) and GDP[S] (lane 3) and blocked by GTP[S] (lane 2). Forskolin also elevated labelling to the same extent as GTP (lane 2). Fig. 1(c) shows that 2 mM-Mg²⁺ slightly enhanced the labelling by CT in the presence of GTP[S], but decreased the labelling by IAP in the presence and absence of GDP[S]. Fig. 1(c) also shows that IAP labelled two polypeptides in brain membranes (~39 kDa and 41 kDa). The appearance of two bands was confirmed by densitometric analysis of lane 2 (Fig. 1b) and Fig. 1(c), as well as five additional experiments (results not shown).

The two polypeptides are similar in size to those observed by Sternweis & Robishaw (1984) and Neer *et al.* (1984) in bovine brain.

Similar results were obtained with liver membranes (Fig. 2, lanes 1–10). Labelling by CT was observed only in the presence of GTP[S], GTP or A-GTP (lanes 6–10). A-GTP activates liver membrane adenylate cyclase in the dark and inactivates N_s function after u.v. irradiation (Wong & Martin, 1983, 1985). IAP labelled only one polypeptide in liver membranes, of ~41 kDa. The intensity of IAP labelling as a function of guanine nucleotides (lanes 1–5) followed the same order as shown for brain membranes: GDP[S] > GTP > no additions = forskolin > GTP[S]. In additional experiments, p[NH]-ppG and GTP[S] decreased IAP labelling to the same extent in both membrane systems, whereas ATP[S] had no effect. The effects of Mg²⁺ are shown in Fig. 2, lanes (a)–(j). In addition to its enhancing the labelling of N_s by CT, Mg²⁺ stimulated the labelling of an additional polypeptide, of 68 kDa (lanes a–d). The labelling of this polypeptide was further enhanced by GTP[S] and GTP. Both Mg²⁺ and GTP[S] decreased ADP-ribosylation by IAP (lanes e and i), and when both were present no significant labelling was observed (lane g). Similar results were also observed in human platelet membranes (results not shown).

A quantitative analysis of the results shown in Figs. 1 and 2 as well as those derived from other experiments is given in Table 1. Mg²⁺ affects the labelling of N_i by IAP through two separate actions. On the one hand, it blocks labelling through direct action on N_i (columns 1–3). This inhibitory effect is enhanced by GTP[S] (over 90% inhibition in its presence, compared with 70% inhibition

Table 1. Dual actions of Mg^{2+} on ADP-ribosylation by IAP in the presence and absence of guanine nucleotides

Autoradiograms shown in Figs. 1, 2 and additional autoradiograms were scanned on a Joyce-Loebl microdensitometer. The intensity of labelling of the 41 kDa protein (liver) or the 39–41 kDa doublet (brain) was calculated by deriving peak area.

Guanine nucleotide	[Mg^{2+}]...	Ratio of labelling intensities						
		$\frac{+Mg^{2+}}{-Mg^{2+}}$			$\frac{\text{Guanine nucleotide}}{\text{Buffer}}$			
		Liver		Brain	Liver		Brain	
		10 mM (1)	10 mM (2)	2 mM (3)	0 (4)	10 mM (5)	0 (6)	10 mM (7)
None		0.28–0.31	0.33	0.68				
GDP[S]		0.52	0.65	0.63	1.6	2.9	1.7	3.3
GTP		0.4–0.56	—	—	1.17–1.47	1.87–2.3	—	—
GTP[S]		0.10–0.11	> 0.05	0.60	0.51–0.83	0.19–0.33	0.25	0

Fig. 3. Effects of preincubation with Mg^{2+} at 30 °C on ADP-ribosylation by IAP

(a) Liver membranes (60 μ g) were incubated in the presence of 2.5 mM-dithiothreitol, 2 mM-EDTA, 37.5 mM-Tris/HCl, pH 7.4, at 0 °C or 30 °C, with or without GDP[S] (100 μ M) and $MgCl_2$ (10 mM) as indicated. After 20 min, the suspension was cooled to 0 °C for 15 min. The membrane was then IAP-labelled without any added guanine nucleotide. The band labelled is the 41 kDa polypeptide. (b) Liver membranes (0.75 mg/ml) were preincubated at 30 °C as described in (a) with or without 12.5 mg of bovine serum albumin/ml and 10 mM- $MgCl_2$ as indicated. After 20 min, albumin was added to the appropriate incubations (lanes 2, 4, 6) such that all incubations contained the same amount of albumin before the IAP labelling. For lanes 5 and 6, excess EDTA was added such that there was 1.2 mM-EDTA in excess of the Mg^{2+} concentration. The suspension was incubated for a further 10 min at 30 °C. After cooling the incubation mixture on ice for 15 min, labelling by IAP was performed as described in the Experimental section, except that 100 mM-Tris/HCl, pH 8.0, was used instead of potassium phosphate buffer. GTP (100 μ M) was present in each incubation. Abbreviation: d.f., dye front.

in its absence) and is partially relieved by GDP[S] or GTP [50% (liver) or 36% (brain), compared with 70% inhibition in the absence of these two guanine nucleotides]. On the other hand, Mg^{2+} promotes the respective actions of the guanine nucleotides (columns 4–7). Thus GDP[S] increases labelling about 3-fold in both systems

in the presence of 10 mM- Mg^{2+} , compared with ~ 1.7-fold in the absence of Mg^{2+} , whereas GTP enhances labelling (liver) from ~ 1.2- to ~ 2-fold. Inhibition by GTP[S] is elevated from ~ 33% (no Mg^{2+}) to ~ 80% (10 mM- Mg^{2+}) in liver and from 75% to 100% in brain. Generally, the effects of guanine nucleotides are more pronounced in

Fig. 4. Effects of *N*-ethylmaleimide on ADP-ribosylation by CT or IAP

Liver membranes (0.5 mg/ml) were incubated with 4.95 mM-*N*-ethylmaleimide (NEM; lanes 1, 3) at 4 °C for 5 min in the presence of 20 mM-Tris/HCl, pH 7.4. At the end of incubation, 5.5 mM-dithiothreitol was added to stop the reaction. Controls (lanes 2, 4) contain the same amounts of dithiothreitol and NEM, which were pre-mixed before their addition. After extensive washing with toxin-labelling buffer, labelling was performed in the presence of 1 mM-GTP, with CT or IAP as indicated.

brain than in liver membranes in the absence of Mg^{2+} , whereas the effects of Mg^{2+} are more pronounced in liver membranes.

Fig. 3(a) shows the effect of pretreating liver membranes with Mg^{2+} at 30 °C on labelling by IAP. Incubation at 30 °C in the absence of Mg^{2+} (in the presence of EDTA) had no effect on the extent of either basal or GDP[S] labelling (lanes 1, 2, 5, 6). However, preincubation with Mg^{2+} at 30 °C blocked basal labelling by IAP (lane 3) as well as labelling in the presence of GDP[S] (lane 7). At 0 °C, Mg^{2+} decreased basal labelling (lane 4) and GDP[S] enhanced the labelling under parallel conditions (lane 8). EDTA did not reverse the effects of preincubation with Mg^{2+} at 30 °C (Fig. 3b, lane 6), and bovine serum albumin added during the preincubation did not protect against the subsequent decrease in labelling (lanes 3 and 5). Labelling by CT was not significantly affected under similar conditions (results not shown).

Fig. 4 shows the effects of 4.95 mM-*N*-ethylmaleimide, a potent alkylating reagent, on ADP-ribosylation of liver membranes by the two toxins. It decreased labelling by CT by ~15% (lanes 1 and 2), whereas labelling by IAP was nearly abolished (lanes 3 and 4). The 20 kDa protein labelled by CT is one of the toxin subunits. Similar results were obtained in brain membranes.

DISCUSSION

ADP-ribosylation by CT

GTP, GTP[S] or p[NH]ppG are essential for labelling of brain and liver membrane proteins by CT. These results are thus consistent with all previous demonstrations of CT-dependent labelling, except that by Kahn & Gilman (1984), who found that GTP[S], but not fluoride, decreased the extent but enhanced the rate of labelling of purified N_s . A-GTP supported labelling of liver membrane proteins by CT almost as well as GTP (Fig. 2), as might be expected, since the photoreactive analogue stimulates adenylate cyclase activity as well as does GTP (Wong & Martin, 1983). This suggests that the bulky group attached to the terminal phosphate of A-GTP did not significantly affect the conformation of the proteins labelled by CT.

Both systems contain numerous substrates for CT-catalysed ADP-ribosylation. In brain membranes, the most abundant label is found in a 52 kDa rather than a 42 kDa polypeptide, corroborating the report by Neer *et al.* (1983) that brain N_s consists mainly of a 52 kDa rather than the 42–47 kDa subunit of N_s . Our results are thus similar to those reported by Berthillier *et al.* (1982), including the finding that creatine kinase is labelled in the presence of GTP and brain membranes.

CT also catalysed the ADP-ribosylation of more than one protein in rat liver membranes (see also Heyworth *et al.*, 1985). The Mg^{2+} -dependent labelling of a 68 kDa polypeptide by CT was not observed by Shimada & Kimura (1983), although the incubation buffer used in the present study was essentially the same. The difference may be due to the way in which the membranes are prepared. The function of this protein is unknown.

The extent of labelling of the 42 and 47 kDa proteins in liver, and of the 52 kDa protein in brain, was only slightly enhanced in the presence of Mg^{2+} , indicating that the binding of guanine nucleotides to a site involved in regulation of ADP-ribosylation by CT occurs in the absence of Mg^{2+} , as demonstrated by Gill & Meren (1983). Although Mg^{2+} has been shown to be essential for the irreversible binding of GTP[S] to N_s (Northup *et al.*, 1982), Sternweis & Robishaw (1984) have also observed reversible binding of GTP[S] to a GTP-binding protein in the absence of Mg^{2+} .

ADP-ribosylation by IAP

Labelling by IAP has been researched less extensively. It appears that liver membranes contain one labelled polypeptide substrate, compared with two labelled polypeptides in rat brain membranes. It has been reported by Malbon *et al.* (1984) that two polypeptides are also labelled in fat-cell membranes. Katada & Ui (1982), however, showed that trypsin causes the degradation of the 41 kDa form of ADP-ribosylated N_s to a 40 kDa form of the protein in C6 glioma cells. This transition, which occurs after labelling in the presence of GTP, is promoted by p[NH]ppG and prevented by GDP[S]. If the same phenomenon were to account for the two polypeptides appearing in brain membranes, one would expect that both bands would be labelled to a different extent, depending on whether GDP[S] or GTP[S] was present. Although the 41 kDa band was 75–100% as intense as the 39 kDa band in different experiments, the intensity of labelling of both bands increased with GTP or GDP[S] and decreased with GTP[S] to the same extent.

Thus we consider that tryptic degradation is not the cause for the appearance of two substrates in brain membranes. Sternweis & Robishaw (1984) have shown that two polypeptides from bovine brain are labelled by IAP, whereas Neer *et al.* (1984) have demonstrated labelling of three polypeptides from bovine brain which appear to have different functions. It remains to be seen whether their labelling is blocked by GTP[S] and enhanced by GDP[S].

The conformation of N_i

Our data suggest that the GDP-bound, non-dissociated, conformation of native N_i is the substrate preferred by IAP. Thus, in contrast with the results obtained with CT, labelling was decreased by all the non-hydrolysable analogues of GTP that are known to dissociate the $\alpha\beta$ subunit complex of N_i (Codina *et al.*, 1983) and enhanced by the non-phosphorylatable analogue of GDP, GDP[S]. Similarly, Tsai *et al.* (1984) have shown that GDP[S] enhances labelling by IAP of the α subunit of the purified N_i in the presence of the β subunit. However, GTP[S] and p[NH]ppG did not appear to decrease the 'basal' labelling by IAP, perhaps because N_i was not able to dissociate in their purified system. In addition, Tsai *et al.* (1984) and Neer *et al.* (1984) have demonstrated that N_i must be undissociated for effective labelling by IAP. The data of Van Dop *et al.* (1984) suggest that the 'GDP-form' of transducin, the GTP-binding protein in retinal rod outer segments, is also the preferred conformation for its ribosylation by IAP, although GDP[S] was not added to test this directly.

The effect of Mg^{2+}

The hypothesis that the 'GDP-form' of N_i is the substrate for ADP-ribosylation by IAP is supported further by the experiments with Mg^{2+} . In our study Mg^{2+} had three actions: (a) a decreased labelling by IAP irrespective of the guanine nucleotide present, (b) potentiation of the respective functions of the guanine nucleotides (Figs. 1 and 2, Table 1) and (c) irreversible inhibition of labelling by IAP after pretreatment of membranes at 30 °C (Fig. 3), an effect that has not been reported previously. Preincubation at 0 °C in the presence of Mg^{2+} and incubation at 30 °C in the presence of EDTA had no effect, indicating that Mg^{2+} specifically induced thermal inactivation which could not be reversed by the subsequent addition of EDTA. The lack of an effect of bovine serum albumin indicates the absence of an active Mg^{2+} -dependent proteinase. Thus these data suggest that, on the one hand, Mg^{2+} enhances the binding of the guanine nucleotides and helps to stabilize the GDP- or GTP-forms of N_i . Therefore labelling is relatively intensified in the presence of GDP[S] and Mg^{2+} , but is further decreased in the presence of GTP[S] and Mg^{2+} . On the other hand, Mg^{2+} promotes dissociation of N_i to a form that cannot support labelling by IAP, thus explaining the overall decrease in labelling, potentiated by guanine nucleotides which promote dissociation. At elevated temperatures dissociation is enhanced by Mg^{2+} and is followed by irreversible denaturation of the dissociated subunit(s). This accounts for the lack of subsequent responses to GDP[S] and EDTA.

Supporting evidence comes from data published by three groups. Codina *et al.* (1984) have reported a Mg^{2+} - and temperature-dependent dissociation of purified human erythrocyte N_i . This was guanine-nucleotide-

dependent and reversible. However, Katada *et al.* (1984) have observed the release of β -subunit activity (e.g. dissociation) and denaturation of N_i after incubating cholate extracts from platelet membranes with Mg^{2+} at 30 °C in the absence of guanine nucleotides, and Deterre *et al.* (1984) have demonstrated that GDP-bound transducin dissociates in the presence of 10 mM- Mg^{2+} or $-Ca^{2+}$.

The role of GTP

GTP is the only nucleotide which supports labelling by both CT and IAP, in contrast with the action of GTP[S]. One possibility is that GTP is converted into GDP in the active site of N_i . This is supported by the fact that, in the presence of Mg^{2+} , GTPase is more active, hence the enhancement by GTP of labelling by IAP (Fig. 2, Table 1). Thus IAP may shift the equilibrium

to the left during the constant turnover of GTP and GDP in the active site, as this is the conformation preferred by IAP.

The fact that labelling by IAP also occurs in the absence of guanine nucleotides suggests that N_i exists in the GDP-bound, undissociated, conformation in native membranes. This may also account for the inhibition of cellular and membrane N_i function by IAP.

We thank Dr. L. Irons for providing the pertussis toxin. This work has been supported by a Shell scholarship (S.K.F.W.), the Medical Research Council (B.R.M.) and an Amersham Senior Research Fellowship from Christ's College (A.M.T.).

REFERENCES

- Berthillier, B., D'Alayer, J. & Monneron, A. (1982) *Biochem. Biophys. Res. Commun.* **109**, 297–304
- Bokoch, G. M., Katada, T., Northup, J. K., Hewlett, E. L. & Gilman, A. G. (1983) *J. Biol. Chem.* **258**, 2072–2075
- Bokoch, G. M., Katada, T., Northup, J. K., Ui, M. & Gilman, A. G. (1984) *J. Biol. Chem.* **259**, 3560–3567
- Cassel, D. & Pfeuffer, T. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2669–2673
- Codina, J., Hildebrandt, J. D., Iyengar, R., Birnbaumer, L., Sekura, R. D. & Manclark, C. R. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4276–4280
- Codina, J., Hildebrandt, J. D., Birnbaumer, L. & Sekura, R. D. (1984) *J. Biol. Chem.* **259**, 11408–11418
- Deterre, D., Bigay, J., Pfister, C. & Chabre, M. (1984) *FEBS Lett.* **178**, 228–232
- Doberska, C. A., Macpherson, A. J. S. & Martin, B. R. (1980) *Biochem. J.* **186**, 749–754
- Gill, D. M. & Meren, R. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3050–3054
- Gill, D. M. & Meren, R. (1983) *J. Biol. Chem.* **258**, 11908–11914
- Gilman, A. G. (1984) *Cell* **36**, 577–579
- Heyworth, C. M., Whetton, A. D., Wong, S. K. F., Martin, B. R. & Houslay, M. D. (1985) *Biochem. J.* **228**, 593–603
- Hildebrandt, J. D., Sekura, R. D., Iyengar, R., Manclark, C. R. & Birnbaumer, L. (1983) *Nature (London)* **302**, 706–709
- Jones, D. S. & Matus, A. J. (1974) *Biochim. Biophys. Acta* **356**, 276–287
- Kahn, R. A. & Gilman, A. G. (1984) *J. Biol. Chem.* **259**, 6235–6240
- Katada, T. & Ui, M. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 3129–3133
- Katada, T., Northup, J. K., Bokoch, G. M., Ui, M. & Gilman, A. G. (1984) *J. Biol. Chem.* **259**, 3575–3585
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685

- Malbon, C. C., Rapiejko, P. J. & Garcia-Sainz, J. A. (1984) *FEBS Lett.* **176**, 301–306
- Martin, B. R. & Voorheis, P. (1977) *Biochem. J.* **161**, 555–559
- Neer, E. J. & Salter, R. S. (1981) *J. Biol. Chem.* **256**, 12101–12107
- Neer, E. J., Wolf, L. G. & Brundage, L. (1983) *Adv. Cyclic Nucleotide Res.* **17A**, 7
- Neer, E. J., Lok, J. M. & Wolf, L. G. (1984) *J. Biol. Chem.* **259**, 14222–14229
- Northup, J. K., Smigel, M. D. & Gilman, A. G. (1982) *J. Biol. Chem.* **257**, 11416–11423
- Pilkis, S. J., Exton, J. H., Johnson, R. A. & Park, C. R. (1974) *Biochim. Biophys. Acta* **343**, 250–267
- Sekura, R. D., Fish, F., Manclark, C. R., Meade, B. & Zhang, Y. L. (1983), *J. Biol. Chem.* **258**, 14647–14651
- Shimada, N. & Kimura, N. (1983) *FEBS Lett.* **159**, 75–78
- Sternweis, P. C. & Robishaw, J. D. (1984) *J. Biol. Chem.* **259**, 13806–13813
- Tsai, S. C., Adamik, R., Kanaho, Y., Hewlett, E. L. & Moss, J. (1984) *J. Biol. Chem.* **259**, 15320–15323
- Van Dop, C., Yamanaka, G., Steinberg, F., Sekura, R. D., Manclark, C. R., Stryer, L. & Bourne, H. R. (1984) *J. Biol. Chem.* **259**, 23–26
- Wong, S. K. F. & Martin, B. R. (1983) *Biochem. J.* **216**, 753–759
- Wong, S. K. F. & Martin, B. R. (1985) *Biochem. J.* **231**, 39–46

Received 2 April 1985/19 June 1985; accepted 16 July 1985