Glycosylphosphatidylinositol is involved in the membrane attachment of proteins in granules of chromaffin cells

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Incubation at 37 °C or treatment of granule membranes of chromaffin cells with *Staphylococcus aureus* phosphatidylinositol-specific phospholipase C converted from an amphiphilic to a hydrophilic form two proteins with molecular masses of 82 and 68 kDa respectively. Their release is time- and enzyme-concentration-dependent. We showed that they were immunoreactive with an anti-(cross-reacting determinant) antibody known to be revealed only after removal of a diacylglycerol anchor. Furthermore, the action of HNO₂ suggests the presence of a non-acetylated glucosamine residue in the determinant. This is one of the first reports suggesting that a glycosylphosphatidylinositol anchor might exist in membranes other than the plasma membrane. We showed that the 68 kDa protein is probably not the subunit of dopamine (3,4-dihydroxyphenethylamine) β -hydroxylase, an enzyme present in granules in both soluble and membrane-associated forms.

INTRODUCTION

Among the various mechanisms of membrane protein anchorage known, the most unusual described to date is that involving glycosylphosphatidylinositol (GPI). The structure of this anchor exhibits three regions, an inositol phosphate linked to diacylglycerol responsible for the attachment, a linkage between a glycan and the inositol phosphate via a glycosidic bond with a non-acetylated glucosamine, and finally an ethanolamine that is amide-linked to the C-terminal amino acid [1]. The carbohydrate moiety of this GPI anchor can be revealed by an anti-CRD (cross-reacting determinant) antibody [2] when the diacylglycerol is removed [3–6]. Treatment with bacterial PI-PLC from *Staphylococcus aureus* or *Bacillus thuringiensis* [1] cleaves the diacylglycerol from the anchor and releases hydrophilic forms of the membrane-attached proteins [1,7,8]. Another peculiarity of that structure is the presence of a glucosamine residue with a free amino group [9–12]. A growing number of proteins from different organisms and with a great variety of functions have been reported as exhibiting these structural features (for a review, see [1]), this diversity being in sharp contrast with the fact that they are all located on the cell surface. One might wonder whether this type of attachment might also be used by proteins of intracellular compartments. To examine that issue we used membranes of chromaffin granules from bovine adrenal medulla. We report here that two proteins of these secretory organelles exhibit properties characteristic of such a structure.

MATERIALS AND METHODS

Biological materials

The plasma membranes or granule membranes from bovine adrenal medulla were prepared as described by

Aunis & Perrin [13]. The PI-PLC (EC 3.1.4.10) from *Staphylococcus aureus*, purified as described by Low & Finean [14], was a gift of Dr. J. Barbet and Dr. M. Pierres (Centre d'Immunologie INSERM-CNRS, Marseille, France). Anti-CRD, anti-NSP4 antibodies and anti-DBH were prepared as described by Capdeville *et al.* [3], Rougon *et al.* [17] and Aunis *et al.* [18].

Membrane treatment

Samples of membranes (10 mg/ml) were incubated at 37 °C in Tris/HCl buffer (100 mM, pH 7.4), in the presence of proteinase inhibitors [aprotinin (20 units/ml), 10 μ M-pepstatin, 80 μ M-leupeptin and α_2 -macroglobulin (10 μ g/ml)] and with or without PI-PLC, DTT, HgCl₂ or ZnCl₂ as indicated in the Figure legends. After incubation, samples were cooled on ice and centrifuged at 100000 *g* for 1 h or subjected to phase separation with Triton X-114/NaCl [15,16].

Triton X-114 procedure

Triton X-114 was precondensed as described by Bordier [15]. A portion (100 μ l) of 4% Triton X-114/0.3 M-NaCl was added to 100 μ l samples at 0 °C. The solubilized membranes were placed on ice for 6 min and then layered over a 0.25 M-sucrose cushion in 25 mM-Tris/HCl (pH 7.4)/0.15 M-NaCl (300 μ l) containing 0.06% Triton X-114, for 6 min at 30 °C. They were then centrifuged at 2500 *g* for 5 min in a swing-out rotor at 30 °C. The detergent-rich phase was collected under the cushion of sucrose and the aqueous phase above. Where indicated in the text and Figures, the Triton X-114 procedure of Pryde & Phillips [16] was used, giving a phospholipid-rich phase, a detergent-rich phase and an aqueous phase; each phase was washed as specified [16]. The different phases were precipitated by acetone. This

Abbreviations used: GPI, glycosylphosphatidylinositol; PI-PLC, phosphatidylinositol-specific phospholipase C; CRD, cross-reacting determinant; DBH, dopamine (3,4-dihydroxyphenethylamine) β -hydroxylase; DTT, dithiothreitol; PBS, phosphate-buffered saline (10 mM-phosphate/0.15 M-NaCl, pH 7.2); anti-NSP4, a monoclonal antibody known to be specific for a family of plasma-membrane glycosylated polypeptides.

facilitated concentration of the sample as well as removal of excess Triton X-114.

Electrophoresis and immunoblotting

Protein precipitates were dried and resuspended in non-reducing SDS sample buffer. Samples were electrophoresed on 6.6–15%-(w/v)-polyacrylamide gradient gels or 10% gels under non-reducing conditions and then transferred on to nitrocellulose sheets by electrophoretic transfer (7 h at 40 V). Sheets were first stained with Ponceau Red to reveal proteins, then incubated with 2% (w/v) haemoglobin/PBS/NaN₃ to block non-specific binding sites and allowed to react with the antibodies diluted in the same buffer for 12 h at 4 °C. Anti-CRD antibody was used at a dilution of 1:200 and anti-DBH at a dilution of 1:2000. For anti-NSP4, hybridoma culture supernatant was used at a dilution of 1:3. Washes were done with PBS/NaN₃. Radioiodinated Protein A or rabbit anti-rat antibodies (10⁶ c.p.m./ml) were used to detect bound antibodies, and the dried blots were exposed to Fuji X-ray films. The autoradiograms were quantified by scanning densitometry.

HNO₂ treatment

For HNO₂ treatment, the samples of granule membranes were incubated as described above, and the aqueous phase separated by Triton X-114/NaCl was acetone-precipitated and then suspended in 0.25 M-sodium acetate (pH 4.0) with 0.25 M-NaCl (control) or freshly prepared 0.25 M-NaNO₂. Incubation was carried out for 3 h at 20 °C, terminated by neutralizing with NH₄OH, and proteins were precipitated with acetone and resuspended in non-reducing SDS sample buffer before electrophoresis. The nitrocellulose sheet was incubated for 16 h in PBS/NaN₃ at 37 °C before the reaction with antibodies to allow renaturation of antigenic sites [19].

Measurement of protein

Protein concentration was determined as described by Markwell *et al.* [20], with bovine serum albumin as a standard.

RESULTS AND DISCUSSION

Numerous reports have described plasma-membrane proteins anchored in the lipid bilayer via a C-terminal glycolipid moiety containing covalently attached PI [1]; however, little is known about the existence of intracellular GPI-linked membrane proteins. We searched for such a species in purified granule membranes from bovine adrenal chromaffin cells.

Immunoreactivity of plasma and granule membranes of chromaffin cells with anti-NSP4 antibody

To ascertain that our preparation of chromaffin-granule membranes was not contaminated with plasma membranes, we used a monoclonal antibody (anti-NSP4) [17] known to be specific for a family of plasma membrane glycosylated polypeptides. Fig. 1 shows the results of an immunoblot experiment on plasma and granule membrane fractions respectively. The same quantity of protein was used for each fraction, and the immunoreactivity was only detected in plasma membranes. We estimated the contamination of granule

Fig. 1. Immunoreactivity of plasma and granule membranes of chromaffin cells with anti-NSP4 antibody

Plasma (lane 1) or granule membrane (lane 2) proteins (200 µg) were analysed by SDS/10%-(w/v)-polyacrylamide-gel electrophoresis and immunoblotting was performed as described in the Materials and methods section. The immunoblot was probed with anti-NSP4 antibody. Positions of standard molecular-mass markers are indicated by arrows. Note the absence of immunoreactivity in the granule membrane fraction.

membranes with plasma membranes to be less than 5%.

Endogenous PI-PLC activity in chromaffin-granule membranes

Literature data state that anti-CRD antisera, recognizing the carbohydrate moiety of the cleaved GPI anchor [2], react with numerous GPI-anchored proteins [3–6]. We used such an antiserum as a probe for candidate proteins having a GPI anchor in the granule membranes.

Fig. 2 shows electrophoresis of plasma-membrane fractions and their immunoreactivity with anti-CRD antiserum under various conditions. Membranes suspended in Tris/HCl buffer were centrifuged at 100 000 g to yield insoluble (P) and solubilized proteins (S). In a first set of experiments (A), considered as zero-time controls, the P and S fractions were boiled in non-reducing SDS sample buffer immediately after centrifugation at 4 °C. In a second set (B), membrane samples

Fig. 2. Immunoreactivity of plasma membranes with anti-CRD antibody

Plasma membranes were (a) centrifuged at 100 000 *g* for 1 h at 4 °C, (b) incubated for 1 h at 37 °C or (c) incubated with PI-PLC (100 units/ml) for 1 h at 37 °C before centrifugation. Soluble (S) and membrane-bound (P) proteins were analysed for their immunoreactivity with anti-CRD antibody as described in the Materials and methods section. Protein quantities comparable with those in Fig. 1 were used.

were incubated for 1 h at 37 °C before centrifugation or, in a third set (C), with 100 units of *S. aureus* PI-PLC/ml. Incubation at 37 °C as well as treatment with exogenous enzyme leads to the appearance in the soluble fractions of proteins migrating at 205, 53 and 33 kDa molecular mass. These species are well-represented plasma-membrane proteins, since they could clearly be seen when nitrocellulose sheets were stained with Ponceau Red (results not shown). All these species are revealed with anti-CRD antiserum (Fig. 2c). Besides those, the antiserum also reacted with 92, 45 and 17.5 kDa proteins. Immunoreactivity was detected in the soluble fraction only after incubation at 37 °C (Fig. 2b) and with more intensity after PI-PLC addition (compare S from Figs. 2a, 2b or 2c).

When membrane granule fractions were submitted to the same treatments (Figs. 3a and b), no immunoreactivity could be seen either in the pellet or soluble fractions of membranes centrifuged at 4 °C; by contrast, two major bands were revealed at 82 and 68 kDa in the soluble fraction after incubation at 37 °C (Fig. 3b). These bands could not be detected by Ponceau Red staining of proteins, indicating they are not major species of the membrane granules. Pellet-fraction immunoreactivity was very weak in comparison with that of soluble fractions. This would indicate that these proteins were anchored via GPI.

The CRD determinant is known to be cryptic in the membrane form and to be revealed only when proteins are converted into a soluble form by the action of a PI-PLC [3–6]. To ascertain further that the observed protein release could be attributed to the action of an endogenous

Fig. 3. Endogenous PI-PLC activity in chromaffin-granule membranes

Granule membranes were (a) centrifuged at 100 000 *g* for 1 h at 4 °C, (b) incubated for 1 h at 37 °C or (c) incubated for 1 h at 37 °C in the presence of 25 mM-DTT before centrifugation. Soluble (S) and membrane-bound (P) proteins were analysed for their immunoreactivity with anti-CRD antibody as in Fig. 2. Protein quantities comparable with those in Fig. 1 were used. Note that no positivity could be detected in the absence of incubation at 37 °C.

PI-PLC and not to the action of proteinases, we tested the actions of DTT and Zn^{2+} or Hg^{2+} , which, respectively, are known to increase or inhibit the action of PI-PLC from *Trypanosoma brucei* [21–23], *Paramecium* [24], bacteria [14,26] or liver plasma membranes [25]. The endogenous activity exhibited by chromaffin-granule membrane fractions is sensitive to such agents; for example, 25 mM-DTT (Fig. 3c) increased the intensity of the anti-CRD-reacting proteins by a factor of 1.5. By contrast, ZnSO_4 (1 mM) or HgCl_2 (0.1 mM) inhibited the release by a factor of 2 (results not shown). The 82 and 68 kDa proteins are specifically located in the granules, since we showed that they were never detected in plasma membranes (cf. Figs. 2 and 3).

It is unlikely that the PI-PLC activity we detected corresponds to that reported by Creutz *et al.* [27], which was associated with chromaffin-granule-binding proteins (chromobindins) and was detected by using PI as a substrate. The eukaryotic PI-PLC reported thus far that cleave GPI-linked proteins are described as 'glycan-specific' and are not able to hydrolyse PI [21,25]. Thus the endogenous PI-PLC described in that study is presumably localized in the granule membrane, in accordance with what has been reported for other glycan specific PI-PLC [25,28].

Release of 82 and 68 kDa proteins from chromaffin-granule membranes by *S. aureus* PI-PLC

Additional evidence of GPI tail cleavage is presented in Table 1 with the use of an exogenous PI-PLC prepared from *S. aureus*. The concentration- and time-dependence of the liberation of the 68 and 82 kDa proteins was

Table 1. Release of 82 and 68 kDa proteins from chromaffin-granule membranes by *S. aureus* PI-PLC

Granule membranes were incubated for 1 h at 37 °C with various concentrations of PI-PLC or for various times with PI-PLC (100 units/ml). Protein separation and immunoblots with anti-CRD antiserum were conducted as described in the Materials and methods section. Immunoreactivities of 82 and 68 kDa proteins were estimated by scanning densitometry of the immunoblots. Results are comparable, since experiments were run in parallel and electrophoresis was carried out on the same gel. The results shown are from a single experiment, but are typical of those obtained in three other similar experiments.

Band	Anti-CRD immunoreactivity [density (% of control)]				Time in the presence of PI-PLC (100 units/ml) (min):			
	[PI-PLC] (units/ml):							
	0	10	50	100	0	15	30	60
82 kDa	100	157	168	186	100	110	140	180
68 kDa	100	137	155	176	100	103	115	165

Fig. 4. Phase separation of the soluble and the membrane forms of the incubated granule-membrane proteins by the Triton X-114 procedure

Separation was (a) as described by Pryde & Phillips [16], or (b) as described by Bordier [15], without washing of the phases: phospholipid-rich phase (lane 1), detergent-rich phase (lanes 2 and 2') and aqueous phase (lanes 3 and 3'). Incubation, phase separation and immunoblots were performed as described in the Materials and methods section. Immunoblots were probed with anti-CRD antiserum.

measured as the appearance of anti-CRD immunoreactivity in the soluble fraction after the action of the enzyme. The addition of *S. aureus* PI-PLC did not lead to the detection of any other major proteins in this fraction (results not shown).

Phase separation of soluble and membrane forms of the incubated granule membrane proteins by the Triton X-114 procedure

According to results from the literature, it appears that GPI-anchored proteins share common biochemical properties. In particular, the membrane and soluble form for a given protein distribute differently in Triton X-114 phases [15,16]. We have applied this separation technique to chromaffin-granule membranes before and after incubation at 37 °C, or after addition of *S. aureus* PI-PLC. Fig. 4 reports the results when anti-CRD immunoreactivity was monitored in the different phases. No immunoreactivity could be detected in the phospholipid-rich phase or detergent-rich phase which contained amphiphilic membrane proteins [16] (lanes 1, 2 and 2'). Binding to proteins was seen in the aqueous phase containing the hydrophilic forms of 82 and 68 kDa obtained after cleavage (lane 3 and 3'). This result is in good agreement with what was expected for GPI-anchored proteins and the pattern of reactivity of anti-CRD antibodies, which detect such proteins only when cleaved. It should be noted that the Triton X-114 method considerably improved the separation procedure over centrifugation, in which some soluble form remained associated with the pellet (see, e.g., Fig. 3, in which some immunoreactivity was seen in pellets after incubation at 37 °C). For routine experiments we set up a simplified method, using Triton X-114, that gave satisfactory results when washing of the phases was omitted (Fig. 4b).

Effect of HNO₂ on the anti-CRD immunoreactivity of the 82 and 68 kDa proteins

The precise structure of GPI anchors has been reported only for two GPI-anchored proteins, namely variant surface glycoprotein [29] and Thy-1 antigen [30]. However, Low *et al.* [9] suggest that they share common structural features and that the PI group is linked to a non-*N*-acetylated glucosamine residue. This particular feature allows the cleavage of the glycosidic bond with inositol upon deamination with HNO₂ [10–12]. The resulting modifications of the structure of the glycosidic moiety (deamination of glucosamine and cleavage of the inositol–glucosamine bond) are expected to affect the immunoreactivity of anti-CRD antiserum. When the aqueous phase containing the 82 and 68 kDa proteins was incubated in the presence of HNO₂ the reactivity with the anti-CRD antibody was inhibited (~45%), as reported in Fig. 5. Because it is known that acid treatment denatures proteins and consequently reduces their solubility, control and experimental samples were treated under acid conditions, then neutralized, and the protein concentrations compared for both samples before electrophoresis. The inhibition was not complete, but this is not surprising, since the cleavage of the glycosidic linkage in glucosamine-containing glycosides is not quantitative [12,31,32].

DBH is not released from membranes by PI-PLC

Two enzymes present in chromaffin granules are described both as membrane-bound and water-soluble: acetylcholinesterase [33] and DBH [34]. We investigated whether the 68 kDa protein released by PI-PLC and immunoreactive with anti-CRD antiserum could in fact correspond to DBH. Under reducing conditions this enzyme has a subunit molecular mass of about 69 to 75 kDa [35].

Fig. 6(a) shows the molecular species revealed by an anti-DBH antiserum by immunoblotting after electro-

Fig. 5. Effect of HNO_2 on the anti-CRD immunoreactivity of the 82 and 68 kDa proteins

Incubation, aqueous-phase separation, HNO_2 treatment, immunoblot and scanning densitometry were performed as described in the Materials and methods section. Continuous and broken lines indicate immunoreactivity of 82 and 68 kDa proteins of control and after NaNO_2 treatment respectively.

phoresis performed under either non-reducing (lanes 1 and 2) or reducing conditions (lanes 3 and 4). In agreement with the literature, a 68–75 kDa species was the major form detected under the latter conditions [35]. After Triton X-114 phase partitioning of the granule membranes, immunoreactivity could be detected both in detergent-rich (lanes 1 and 3) and aqueous phases (lanes 2 and 4). This observation is consistent with that of Pryde & Phillips [16] when they tested the activity of the enzyme in different fractions after such treatment. When anti-CRD immunoreactivity was looked for in the aqueous phase under the same experimental conditions (lanes 5 and 6), a 68 kDa band was detected that co-migrated with the DBH subunit observed under reducing conditions, as expected. However, because the intensity of the 68 kDa CRD-immunoreactive band does not increase under reducing conditions (cf. lanes 5 and 6) it is unlikely that it corresponds to DBH. Furthermore, under non-reducing conditions, anti-CRD antiserum does not reveal a band with high molecular mass corresponding to non-reduced DBH (dimeric form). To further ascertain that DBH is not anchored in the membranes by a GPI, we verified that it could not be released as a soluble form by treatment with *S. aureus* PI-PLC (Fig. 5b). When DBH immunoreactivity was compared in membranes not treated (lanes 1 and 2) or treated (lanes 3 and 4) with PI-PLC, no increase of immunoreactivity could be seen in the water-soluble fraction after Triton X-114 partitioning. This was observed even when a large excess of enzyme was used.

At this stage it cannot be excluded that DBH has a glycolipid membrane anchor that is resistant to the action of PI-PLC, as has recently been reported for acetylcholinesterase from human erythrocytes [36]. Further chemical investigations would be needed to exclude this, although our data already show that it is rather unlikely.

Fig. 6. DBH immunoreactivity after PI-PLC treatment of membranes

(a) Granule membranes were incubated for 1 h at 37 °C and submitted to Triton X-114 phase separation and run on to a 10% polyacrylamide gel under non-reducing (lanes 1, 2 and 5) or reducing (lanes 3, 4 and 6) conditions. Detergent-rich phases (lanes 1 and 3) and aqueous phases (lanes 2 and 4) were allowed to react with anti-DBH antibody. The same aqueous phases were allowed to react with anti-CRD under non-reducing (lane 5) or reducing conditions (lane 6). The positions of standard molecular-mass markers are indicated by arrows. (b) Incubation of granule membranes at 37 °C for 1 h without (lanes 1 and 2) or with (lanes 3 and 4) *S. aureus* PI-PLC (125 units/ml). After partitioning in Triton X-114, proteins were separated on a 6% polyacrylamide gel under non-reducing conditions; anti-DBH immunoreactivity was probed in the detergent-rich phase (lanes 1 and 3) and the aqueous phase (lanes 2 and 4). The same quantities of membranes were used for the control and the experiment samples.

Conclusions

The mechanism by which the 82 and 68 kDa proteins from bovine chromaffin granules are attached to the membrane was investigated. The present results strongly suggest that they are anchored via a GPI tail, because: (i) they are released as soluble forms by treatment of the membranes by *S. aureus* PI-PLC in a dose- and time-dependent manner; (ii) they expressed a CRD determinant after solubilization; (iii) this determinant probably contains a non-acetylated glucosamine; and (iv) phase separation in Triton X-114 showed that PI-PLC had converted them from an amphiphilic membrane form into a water-soluble form. None of these two proteins appeared to be DBH, for which two forms (membrane-bound and water-soluble) have been reported.

We thank Mrs. P. Malapert for excellent technical assistance and Dr. M. Pierres and Dr. J. Barbet for the *S. aureus* PI-PLC. This work was supported by the CNRS (UA 202) and the Université d'Aix-Marseille I.

REFERENCES

- Low, M. G. (1987) *Biochem. J.* **244**, 1–13
- Bordier, C., Etges, R. J., Ward, J., Turner, M. J. & Cardoso de Almeida, M. L. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 5988–5991
- Capdeville, Y., Baltz, T., Deregnaucourt, C. & Keller, A. M. (1986) *Exp. Cell Res.* **167**, 75–86
- Stieger, A., Cardoso de Almeida, M. L., Blatter, M. C., Brodbeck, V. & Bordier, C. (1986) *FEBS Lett.* **199**, 182–186
- Davitz, M. A., Gurnett, A. M., Low, M. G., Turner, M. J. & Nussenzweig, V. (1987) *J. Immunol.* **138**, 520–523
- Ferguson, M. A. J. & Cross, G. A. M. (1984) *J. Biol. Chem.* **259**, 3011–3015
- Low, M. G. & Zilversmit, D. B. (1980) *Biochemistry* **19**, 3913–3918
- Hooper, N. M., Low, M. G. & Turner, A. J. (1987) *Biochem. J.* **244**, 465–469
- Low, M. G., Ferguson, M. A. J., Futerman, A. H. & Silman, I. (1986) *Trends Biochem. Sci.* **11**, 212–215
- Strang, A. M., William, J. M., Ferguson, M. A. J., Holder, A. A. & Allen, A. K. (1986) *Biochem. J.* **234**, 481–484
- Ferguson, M. A. J., Low, M. G. & Cross, G. A. M. (1985) *J. Biol. Chem.* **260**, 14547–14555
- Low, M. G., Futerman, A. H., Ackermann, K. E., Sherman, W. R. & Silman, I. (1987) *Biochem. J.* **241**, 615–619
- Aunis, D. & Perrin, D. (1984) *J. Neurochem.* **42**, 1558–1569
- Low, M. G. & Finean, J. B. (1977) *Biochem. J.* **162**, 235–240
- Bordier, C. (1981) *J. Biol. Chem.* **256**, 1604–1607
- Pryde, J. G. & Phillips, J. H. (1986) *Biochem. J.* **233**, 525–533
- Rougon, G., Hirsch, M. R., Hirn, M., Guenet, J.-L. & Goridis, C. (1983) *Neuroscience* **10**, 511–520
- Aunis, D., Hesketh, J. E. & Devilliers, G. (1980) *J. Neurocytol.* **9**, 255–274
- Birk, H. W. & Koepsell, H. (1987) *Anal. Biochem.* **164**, 12–22
- Markwell, M. A. K., Hass, S. M., Bieber, L. L. & Tolbert, N. E. (1978) *Anal. Biochem.* **87**, 206–210
- Bülow, R. & Overath, P. (1986) *J. Biol. Chem.* **261**, 11918–11923
- Fox, J. A., Duszenko, M., Ferguson, M. A. J., Low, M. G. & Cross, G. A. M. (1986) *J. Biol. Chem.* **261**, 15767–15771
- Hereld, D., Krakow, J. L., Bangs, J. D., Hart, G. W. & Englund, P. T. (1986) *J. Biol. Chem.* **261**, 13813–13819
- Capdeville, Y., Cardoso de Almeida, M. L. & Deregnaucourt, C. (1987) *Biochem. Biophys. Res. Commun.* **147**, 1219–1225
- Fox, J. A., Soliz, N. M. & Saltiel, A. R. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2663–2667
- Taguchi, R., Asahi, Y. & Ikezawa, H. (1980) *Biochim. Biophys. Acta* **619**, 48–57
- Creutz, C. E., Dowling, L. G., Kyger, E. M. & Franson, R. C. (1985) *J. Biol. Chem.* **260**, 7171–7173
- Grab, D. J., Webster, P., Ito, S., Fish, W. R., Verjee, Y. & Lonsdale-Eccles, J. D. (1987) *J. Cell Biol.* **105**, 737–746
- Ferguson, M. A. J., Homans, S. W., Dwek, R. A. & Rademacher, T. W. (1988) *Science* **239**, 753–759
- Homans, S. W., Ferguson, M. A. J., Dwek, R. A., Rademacher, T. W., Anand, R. & Williams, A. F. *Nature (London)* **333**, 269–272
- Lindberg, B., Lonngren, J. & Svensson, S. (1975) *Adv. Carbohydr. Chem. Biochem.* **31**, 185–240
- Williams, J. M. (1975) *Adv. Carbohydr. Chem. Biochem.* **31**, 9–79
- Burgun, C., De Munoz, D. M. & Aunis, D. (1985) *Biochim. Biophys. Acta* **839**, 219–227
- Aunis, D., Bouclier, M., Pescheloché, M. & Mandel, P. (1977) *J. Neurochem.* **29**, 439–447
- Joh, T. H. & Hwang, O. (1987) *Ann. N.Y. Acad. Sci.* **493**, 342–349
- Roberts, W. L., Kim, B. H. & Rosenberry, T. L. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 7817–7821

Received 23rd March 1988/8 June 1988; accepted 10 June 1988