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Glycolipid-anchored acetylcholinesterases from rabbit lymphocytes and erythrocytes differ in their sensitivity to phosphatidylinositol-specific phospholipase C

Patricia Richier, Martine Arpagaus and Jean-Pierre Toutant

Différenciation cellulaire et Croissance, Centre INRA de Montpellier, Montpellier (France)

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The type of membrane association of acetylcholinesterase (AChE, EC 3.1.1.7) was studied in rabbit lymphocytes and erythrocytes. In both cases, the unique AChE molecular form was an amphiphilic dimer (referred to as G2a) anchored in the membrane by a glycosylphosphatidylinositol. In lymphocytes, G2a AChE was directly converted into its hydrophilic G2h counterpart by a treatment with *Bacillus thuringiensis* phosphatidylinositolinositol-phospholipase C (PI-PLC, EC 3.1.4.10). In erythrocytes, AChE was resistant to PI-PLC but was rendered sensitive by a prior deacylation with alkaline hydroxylamine. This observation suggests that, as previousiy reported for human erythrocyte AChE, an acylation of the inositol ring in the glycolipid anchor of rabbit erythrocyte AChE (that does not occur in lymphocytes) prevents the cleavage.

Introduction

Mammalian erythrocytes possess a membrane-bound, externally-exposed, acetylcholinesterase (AChE) of unknown function. The only AChE molecular form found in erythrocyte membranes is an amphiphilic globular dimer [1,2] that is referred to as G_2a [3,4] in an extension of the nomenclature of Massoulié and Bon [5]. G_2a AChE was shown to be released by phosphatidylinositol-specific phospholipase C (PI-PLC) from porcine, bovine and rat erythrocytes but not from human or murine red blood cells [6,7]. Both PI-PLC-resistant and -sensitive species are, however, anchored in the membrane by a glycosylphosphatidylinositol (GPI) moiety [8]. Resistance to PI-PLC of human erythrocyte AChE results from the presence of an additional acyl chain on the inositol ring in the glycolipid anchor [9,10] that prevents the formation of a cyclic *myo*-inositol 1:2-monophosphate intermediate and thus the PI-PLC cleavage [11]. Deacylation of human and mouse erythrocyte AChEs by alkaline hydroxylamine treatment renders the enzyme sensitive to further cleavage by PI-PLC [4,12].

AChE is also present in a number of other blood cells and elements, particularly T lymphocytes [13] and platelets [14,15,16]. A major amphiphilic, membrane-bound G_2 form was identified in human and bovine lymphocytes [17,18], but it was not reported whether this enzyme was (i) glycolipid-anchored and (ii) sensitive or resistant to PI-PLC. The latter point is important in relation to the potential role of enzymatic cleavage fragments of glycolipid anchors in cellular transduction [19] and particularly in T-cell activation [20].

In this report, we studied the PI-PLC-resistance/sensitivity of AChE in rabbit erythrocytes and lymphocytes.

Materials and Methods

Preparation of blood cell membranes

Peripheral venous blood was collected in heparinized flasks. Separation of lymphocytes and erythrocytes was performed by centrifugations on Ficoll-sodium diatrizoate solution (Ficoll Paque, Pharmacia) according to the commercial directions for use: two successive centrifugations were performed in order to remove all erythrocytes (bottom of the tube) from

Correspondence to: J.-P. Toutant, Différenciation cellulaire et Croissance, INRA, 2, place Viala, 34060 Montpellier Cedex 02, France.
Abbreviations: AChE, acetylcholinesterase; DAF, decay accelerating factor; G_2a, G_2h, amphiphilic and hydrophilic dimers of AChE; GPI, glycosylphosphatidylinositol; PI-PLC, phosphatidylinositol-specific phospholipase C.

lymphocytes (interface plasma-ficoll). Platelets and plasma were eliminated from the lymphocytes by a washing step with a balanced salt solution ('AB' solution, Pharmacia). Lymphocytes obtained in these conditions were free of plasma (as judged by the absence of plasma cholinesterases in non-denaturing electrophoresis) and of platelets (centrifugation of lymphocytes prepared as above on a 5–20% sucrose gradient indicated no contamination by low-density particles).

Membranes were prepared from lymphocytes and erythrocytes. Washed pellets were incubated with 10–20 volumes distilled water for 1 h at 4°C and spun for 15 min at $10000 \times g$. Pelleted membranes were extracted in 5 volumes of Low Salt Triton (LST) buffer for 15 min at 4°C with intermittent stirring. LST buffer contained 1% Triton X-100, 10 mM Tris-HCl (pH 7.4), 1 mM EGTA, 5 mM EDTA, and proteinase inhibitors (Boehringer Mannheim): aprotinin (2 μg/ml), leupeptin (0.5 μg/ml), and pepstatin (0.7 μg/ml). After centrifugation for 15 min at $10000 \times g$ AChE was recovered in the supernatant (Detergent extract). Occasionally intact lymphocytes were extracted directly in LST buffer and AChE recovered in the supernatant as above.

Preparation of spleen cells

Rabbit spleens were dilacerated in cold PBS (140 mM NaCl, 50 mM KCl, 5 mM $MgCl_2$ in 10 mM phosphate buffer, pH 7.0). Cells were filtered on a nylon cheese cloth and the resulting suspension was centrifuged at $2000 \times g$ for 5 min. Spleen lymphocytes were collected at the top of the pellet.

Treatments of AChE samples with enzymes and hydroxylamine

Purified phosphatidylinositol-specific phospholipase C from *Bacillus thuringiensis* (PI-PLC, EC 3.1.4.10) was purchased from Boehringer. Sialidase from *Clostridium perfringens* (EC 3.2.1.18) was from Sigma (Type VIII). Hydroxylamine monohydrochloride, acetylthiocholine, butyrylthiocholine, eserine, triethylamine and edrophonium chloride were from Sigma.

AChE in solution was digested for 1 h at 37°C with PI-PLC diluted to 5 μg/ml. 20 μl of AChE sample were incubated with 1 μl of neuraminidase (10 U/ml) for 1 h at 37°C. Where necessary, deacylation with hydroxylamine was achieved by mixing a sample of detergent extract of membranes with hydroxylamine (to 1.0 M), triethylamine (to 0.2 M) and edrophonium chloride (to 1 mM). The pH was adjusted to 10.8 with 10 M NaOH and the incubation was performed for 2 h at 37°C. Edrophonium chloride was included in order to protect the catalytic site from inactivation during alkaline treatment. Hydroxylamine-treated samples were dialyzed against 0.1% Triton X-100, 0.1 M Tris-HCl (pH 7.0) for 1 h at 4°C, concentrated to the initial activity in a Speed-Vac and digested with PI-PLC as above.

AChE and protein assays

The AChE activity of extracts and of gradient fractions was assayed according to the spectrophotometric method of Ellman et al. [21]. In our conditions (412 nm, 1 ml Ellman medium, 1 cm path length, 20°C), one optical density unit (1 OD) corresponds to the hydrolysis of 75 nmol of acetylthiocholine. Protein content of samples was determined by the bicinchoninic acid method following the manufacturer's instructions for use (Pierce, Rockford, IL).

Nondenaturing polyacrylamide gel electrophoresis

Electrophoreses were performed as described previously [22]. Gels and running buffers contained 50 mM Tris-glycine (pH 8.9) and 0.5% Triton X-100. Samples (15 μl) were run for 3–4 h at 10 V/cm. Gels were rinsed in distilled water and stained for AChE activity

TABLE I

AChE activities recovered in preparations of human and rabbit erythrocytes and lymphocytes

Values in this table are from one representative experiment on rabbit and human blood.

	Erythrocytes [a]			Lymphocytes [a]			R [c]
	AChE activity [a] (OD/min per ml)	Protein content [a] (mg/ml)	AChE activity per cell [b] (OD/min per cell)	AChE activity [a] (OD/min per ml)	Protein content [a] (mg/ml)	AChE activity per cell [b] (OD/min per cell)	
Human	22.4	1.1	$0.55 \cdot 10^{-8}$	0.04	6.6	$0.4 \cdot 10^{-9}$	0.7
Rabbit	2.4	0.7	$0.56 \cdot 10^{-9}$	37.8	6.5	$0.27 \cdot 10^{-6}$	465

[a] Erythrocytes and lymphocytes were recovered after centrifugation of 10 ml of heparinized blood on a Ficoll-Paque solution. All lymphocytes were collected and extracted in 250 μl of LST buffer. Plasma membranes (ghosts) from 200 μl of red cell pellet were extracted in 500 μl of LST buffer. AChE activity and protein content refer to these extracts. Total volumes of erythrocyte pellets from 10 ml of blood were 5.5 ml for rabbit and human.

[b] For estimation of AChE activity per cell the following numbers were used. Erythrocytes: $5.4 \cdot 10^6$ cells/μl of blood in human male, $5.7 \cdot 10^6$ cells/μl for rabbit. Lymphocytes: $2.5 \cdot 10^3$ cells/μl for human and $3.5 \cdot 10^3$ cells/μl for rabbit [40].

[c] *R* is the ratio between AChE activity per cell in lymphocytes and erythrocytes.

according to Ref. 23 with acetylthiocholine (Sigma) as a substrate. The specificity of AChE detection was checked (1) in the presence of 10^{-4} M eserine and (2) with butyrylthiocholine iodide as substrate without inhibitor.

Sucrose gradient centrifugation

Sedimentation analyses were performed in 11 ml, 5–20% sucrose gradients in LST or LS buffers (LS = LST buffer without detergent). Samples of 250 μl of detergent extracts were loaded and centrifuged for 18 h at 40000 rpm (200000 $\times g$) at 4°C in a SW41 rotor. 40 fractions were collected from the bottom of each gradient and assayed for AChE activity at pH 7. Alkaline phosphatase from calf intestine ($s_{20,w} = 6.1$) and β-galactosidase from *Escherichia coli* ($s_{20,w} = 16$) were used as internal markers of migration.

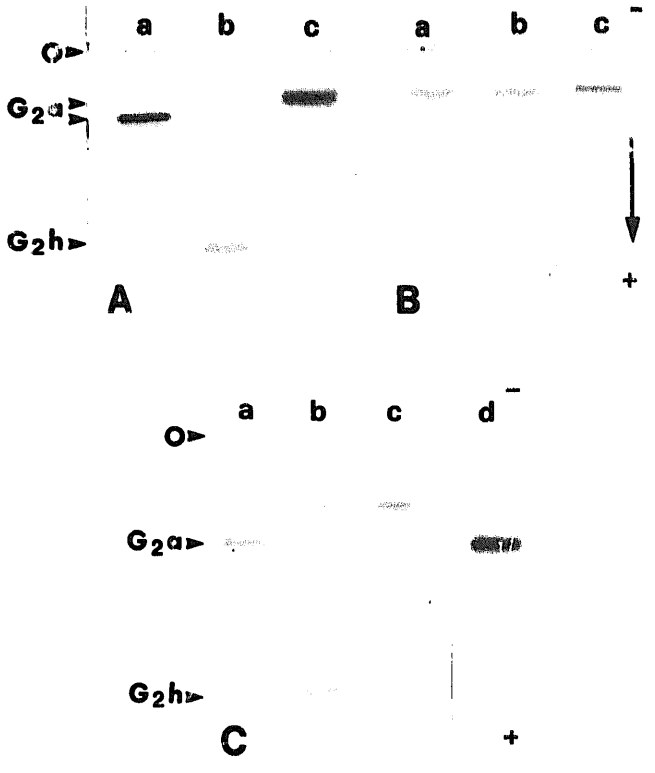

Fig. 1. ND-PAGE analysis of AChE from membranes of rabbit lymphocytes (A), erythrocytes (B) and spleen cells (C). Lymphocytes and erythrocytes were separated on Ficoll-Paque. Spleen cells were prepared as described in Methods. AChE was extracted using LST buffer. Gel (7% polyacrylamide) and running buffer contained 0.5% Triton X-100. Electrophoresis was for 3 h at 10 V/cm. AChE activity was detected by the histochemical method of Karnosky and Roots [23]. Lanes a: control samples; b: PI-PLC-treated; c: sialidase-treated. Lane d in C shows for comparison the migration of AChE from peripheral lymphocytes. O: origin of migration; G_2a: amphiphilic dimers; G_2h: hydrophilic dimers. Decreased migration after sialidase treatment is due to the release of negative charges after sialic acids cleavage [30]. The shift $G_2a \rightarrow G_2h$ results essentially from the loss of Triton X-100 micelles bound to the released hydrophobic domains [22]. AChE is sensitive to PI-PLC in rabbit lymphocytes (peripheral or from spleen) but resistant in erythrocytes. AChE in rabbit erythrocytes is less affected by sialidase than lymphocyte AChE: thus lymphocyte AChE is retarded after treatment by sialidase and migrates approximately like erythrocyte AChE.

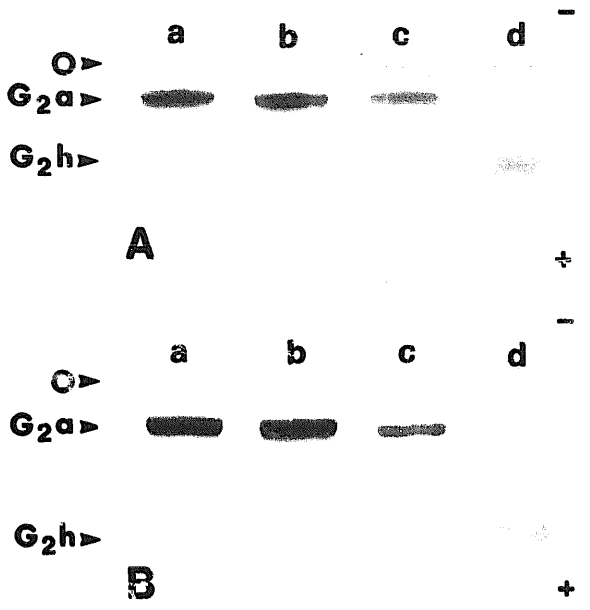

Fig. 2. Effect of alkaline hydroxylamine on PI-PLC susceptibility of AChE G_2a forms. AChEs from rabbit (A) and human (B) erythrocytes (lanes a: control samples) were treated with PI-PLC (lanes b) or by hydroxylamine at pH 10.8 (2 h at 37°C). After dialysis and concentration (see Methods) samples were incubated with PI-PLC (lanes d) or equivalent volume of LST buffer (lanes c). Total conversion into G_2h form was observed in lanes d and no effect in lanes b indicating that a prior deacylation is required for the cleavage of the glycolipid anchor. Note that a small extent of conversion was observed for rabbit AChE (but not for human AChE) after treatment by hydroxylamine alone (lanes c in A and B).

Results

We reported earlier that human red blood cells collected after sedimentation of heparinized blood was a convenient source of amphiphilic dimers of AChE (G_2a) which migrated as a single band in non-denaturing electrophoresis [4]. We observed that rabbit blood cells, prepared in the same conditions, repeatedly showed two bands in non-denaturing electrophoresis. These bands both corresponded to amphiphilic dimers [24,25] which sedimented at 5.8 S in sucrose gradient containing Triton X-100 but aggregated in the absence of detergent in the gradient (not shown). We tested whether the two components of rabbit AChE could originate from distinct blood cell types.

We isolated rabbit blood lymphocytes by two successive centrifugations on Ficoll-Paque and extensive washing. Erythrocytes were also collected from the bottom of the tube in the first centrifugation. Fig. 1(A and B) shows the properties of AChE in both cell types. Lymphocytes contained a G_2a form migrating

faster than that of erythrocytes (Fig. 1A and B, lanes a). In white cells, this form was sensitive to PI-PLC (complete conversion to G_2h, lane b), and to sialidase (lane c), but resistant to both treatments in red cells (Fig. 1B, lanes b and c). We noted that AChE activity present in rabbit lymphocytes was exceptionally high: the estimated AChE activities per cell were approx. $0.3 \cdot 10^{-6}$ OD/min per cell for lymphocytes and $0.6 \cdot 10^{-9}$ OD/min per cell for erythrocytes (ratio: 500, see Table I). Thus the two AChE bands observed in the crude preparation of rabbit blood cell membranes were due to the presence of (a few) white cells (fast-migrating band of AChE) among collected erythrocytes (slow-migrating AChE band). This contamination likely took place also during the preparation of human blood cells that was performed in the same conditions. It did not result in a second band because of the low AChE activity present in human lymphocytes ($0.4 \cdot 10^{-9}$ OD/min per cell versus $0.55 \cdot 10^{-8}$ OD/min per cell in erythrocytes, ratio 0.7, Table I).

Fig. 1(C) shows that the AChE G_2a forms present in rabbit spleen cells (majority of lymphocytes) had similar properties as in peripheral lymphocytes: same migration and complete sensitivity to PI-PLC and sialidase.

Effect of alkaline hydroxylamine on PI-PLC resistance in erythrocyte AChE

As mentioned previously, treatment with hydroxylamine at alkaline pH (see Methods) cleaves O-ester bonds and removes the additional acyl chain that prevents the action of PI-PLC in GPI-anchored proteins resistant to this enzyme [4]. This renders the initially-resistant molecule sensitive to PI-PLC cleavage. Fig. 2(A) shows that the G_2a form of AChE in rabbit erythrocytes which was resistant to PI-PLC became completely sensitive after deacylation with hydroxylamine (lane d). Note that a small extent of conversion into G_2h form was observed after hydroxylamine alone (lane c). A total conversion of G_2a into G_2h was observed for human erythrocyte AChE after hydroxylamine and PI-PLC (Fig. 2B, lane d) but no conversion was observed in this case after hydroxylamine alone (lane c).

Discussion

AChE molecular forms present in rabbit lymphocytes and erythrocytes were characterized. In both cases we found dimers anchored to the membrane through glycolipid anchors. However, these forms differed in the resistance (erythrocyte AChE) or sensitivity (lymphocyte AChE) of the glycolipid domain to cleavage by PI-PLC. This observation should be related to that of Bon and her colleagues [26] who reported only a partial conversion of AChE extracted from a crude extract of rabbit blood cells by PI-PLC. This extract likely contained a few contaminant lymphocytes (PI-PLC-sensitive AChE) among erythrocytes (PI-PLC-resistant AChE).

Structural basis for the PI-PLC resistance / sensitivity

A prior treatment by alkaline hydroxylamine rendered the rabbit erythrocyte AChE sensitive to a further digestion with PI-PLC as previously reported for human and murine erythrocytes [4,12]. Thus it is reasonable to conclude that for rabbit erythrocyte AChE too, acylation of inositol ring in the glycolipid anchor is responsible for the resistance to PI-PLC [9,10].

It is interesting to note that a small amount of amphiphilic dimers (G_2a form) in rabbit erythrocyte AChE was converted into G_2h by alkaline hydroxylamine alone (Fig. 2A, lane c) whereas such a conversion did not occur for the human enzyme treated in the same conditions (Fig. 2B and Ref. 4). Structural analyses have shown that the glycolipid anchors of human and bovine erythrocyte AChEs possessed a terminal alkylacylglycerol [9,10,27]. Thus, deacylation by alkaline hydroxylamine cleaves off two acyl chains (one on the inositol ring and one at the *sn*-2 position of glycerol), but the alkyl chain at the *sn*-1 position is unaffected by the treatment. This hydrophobic chain is sufficient to retain the detergent micelle and to maintain a slow migration of the whole molecule in non-denaturing electrophoresis [4]. At variance the conversion of some molecules in rabbit erythrocyte AChE by alkaline hydroxylamine alone indicates that these forms might possess a terminal diacylglycerol besides a majority possessing an alkylacylglycerol. This observation is of interest: diacylglycerol species have been demonstrated in glycolipid anchors of *Trypanosoma* variant surface glycoproteins (see Ref. 28 for a review) as well as in *Torpedo* AChE [29] but structural heterogeneity in the glycolipid domain of AChE originating *from a single cell type* has been so far limited to the composition of hydrophobic chains [9,10].

Tissue-specific repartition of PI-PLC-sensitive and -resistant glycolipid anchors

Another interesting observation is that the structural difference in the glycolipid anchor that explains the sensitivity/resistance of AChE to PI-PLC occurs in erythrocytes and lymphocytes, two cell types of common origin. A similar difference was already observed for glycolipid-anchored AChE in two sublines of erythroid cell line (K562, see Ref. 30) and for the decay accelerating factor of complement (DAF) which possesses a glycolipid anchor resistant to PI-PLC in human erythrocytes [31] but sensitive in neutrophils [32] and lymphocytes [31]. Further structural studies of glycolipid-anchored DAF in erythrocytes and nucleated cells lead Medof and his collaborators to the

conclusion that a correlation existed (i) between the absence of nucleus in the cell and the acylation of the inositol ring in glycolipid anchors and conversely (ii) between the presence of nucleus and non-acylated inositols [33]. The present observations extend this apparent correlation to rabbit AChE, another glycolipid-anchored protein (sensitive in lymphocytes, resistant in erythrocytes) and this is also true for AChE dimers from mouse erythrocytes (PI-PLC-resistant [12]) and lymphocytes (PI-PLC-sensitive (Richier, P., unpublished results)). Unfortunately, the proposed correlations are irreconcilable with the total release by PI-PLC of surface AChE from a number of anucleated cells: pig, rat, bovine [7] horse and sheep [34] erythrocytes as well as rabbit platelets [14].

The tissue-specific distribution of PI-PLC-sensitive and -resistant forms of GPI-anchored proteins is thus yet unexplained. It was recently suggested that acylation of inositol in the glycolipid anchor might be an obligatory step in biosynthesis of GPI-anchored proteins in *Trypanosoma* [35,36] and mammalian cells [37,38]. Inositol acylation in the anchor might be *stable* in those cells that possess GPI-anchored proteins resistant to PI-PLC but *transient* (i.e., followed by a step of deacylation) in cells presenting PI-PLC-sensitive surface proteins. Thus the tissue specificity of PI-PLC sensitivity/resistance of GPI-anchored proteins might simply result from a deacylating activity present in a majority of cell types and absent in some instances without any relation to the nucleated status of the cell.

PI-PLC-sensitive anchors in lymphocytes

It is interesting to note the presence of glycolipid anchors susceptible of cleavage by PI-PLC at the lymphocyte surface. A number of T cell-activating antigens are GPI-anchored proteins [20]. How these proteins mediate lymphocyte activation is still obscure [19,20]. One way might result from the increased lateral mobility conferred by GPI-anchoring that favours interactions with the T cell receptor/CD3 complex, but an alternative pathway might involve cleavage products of the anchor as potential activation messengers [39]. These fragments might be generated by activation of endogenous PI-PLC or PI-PLD and modulate kinase C activity [19,20].

Rabbit lymphocytes appear as a favourable material to further investigate this question since the enzyme is readily cleaved by PI-PLC and is exceptionally abundant.

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References

1 Ott, P., Lustig, A., Brodbeck, U. and Rosenbusch, J.P. (1982) FEBS Lett. 138, 187–189.
2 Rosenberry, T.L. and Scoggin, D.M. (1984) J. Biol. Chem. 259, 5643–5652.
3 Toutant, J.P., Arpagaus, M. and Fournier, D. (1988) J. Neurochem. 50, 209–218.
4 Toutant, J.P., Roberts, W.L., Murray, N.R. and Rosenberry, T.L. (1989) Eur. J. Biochem. 180, 503–508.
5 Massoulié, J. and Bon, S. (1982) Annu. Rev. Neurosci. 5, 57–106.
6 Low, M.G. and Finean, J.B. (1977) FEBS Lett. 82, 143–146.
7 Futerman, A.H., Low, A.G., Michelson, D.M. and Silman, I. (1985) J. Neurochem. 45, 1487–1494.
8 Roberts, W.L., Kim, B.H. and Rosenberry, T.L. (1987) Proc. Natl. Acad. Sci. USA 84, 7817–7821.
9 Roberts, W.L., Myher, J.J., Kuksis, A., Low, M.G. and Rosenberry, T.L. (1988) J. Biol Chem. 263, 18766–18784.
10 Roberts, W.L., Santikarn, S., Reinhold, V.R. and Rosenberry, T.L. (1988) J. Biol. Chem. 263, 18776–18784.
11 Ikezawa, H. (1986) J. Toxicol.-Toxin Rev. 5, 1–24.
12 Toutant, J.P., Krall, J.A., Richards, M.K. and Rosenberry, T.L. (1991) Cell Mol. Neurobiol. 11, 219–230.
13 Szelényi, J.G., Bartha, E. and Hollán, S.R. (1982) Br. J. Haematol. 50, 241–245.
14 Shukla, S.D. (1986) Life Sci 38, 751–755.
15 Koekebakker, M. and Barr, R.D. (1988) Am. J. Hematol. 28, 252–259.
16 Sánchez-Yagüe, J., Cabezas, J.A. and Llanillo, M. (1990) Blood 76, 737–744.
17 Bartha, E., Rakonczay, Z., Kása, P., Hollán, S. and Gyévai, A. (1987) Life Sci. 41, 1853–1860.
18 Méflah, K., Bernard, S. and Massoulié, J. (1984) Biochimie 66, 59–69.
19 Low, M.G. and Saltiel, A.R. (1988) Science 239, 268–275.
20 Robinson, P.J. (1991) Immunol. Today 12, 35–41.
21 Ellman, G.L., Courtney, K.D., Andres, V. and Featherstone, R.M. (1961) Biochem. Pharmacol. 7, 88–95.
22 Arpagaus, M. and Toutant, J.P. (1985) Neurochem. Int. 7, 793–804.
23 Karnovsky, M.J. and Roots, L. (1964) J. Histochem. Cytochem. 12, 219–222.
24 Ott, P. and Brodbeck, U. (1978) Eur. J. Biochem. 88, 119–125.
25 Bon, S. and Massoulié, J. (1980) Proc. Natl. Acad. Sci. USA 77, 4464–4468.
26 Bon, S., Rosenberry, T.L. and Massoulié, J. (1991) Cell Mol. Neurobiol. 11, 157–172.
27 Roberts, W.L., Myher, J.J. Kuksis, A. and Rosenberry, T.L. (1988) Biochem. Biophys. Res. Commun. 150, 271–277.
28 Ferguson, M.A.J. and Williams, A.F. (1988) Annu. Rev. Biochem. 57, 285–320.
29 Bütikofer, P., Kuypers, F.A., Shackleton, C., Brodbeck, U. and Stieger, S. (1990) J. Biol. Chem. 265, 18983–18987
30 Toutant, J.P., Richards, M.K., Krall, J.A. and Rosenberry, T.L. (1990) Eur. J. Biochem. 187, 31–38.
31 Walter, E.I., Roberts, W.L., Rosenberry, T.L., Ratnoff, W.D. and Medof, M.E (1990) J. Immunol. 144, 1030–1036.
32 Davitz, M.A., Low, M.G. and Nussenzweig, V. (1986) J. Exp. Med. 163, 1150–1161.
33 Walter, E.I., Ratnoff, W.D., Long, K.E., Kazura, J.W. and Medof, M.E. (1992) J. Biol. Chem. 267, 1245–1252.
34 Taguchi, R., Suzuki, K., Nakabayashi, T. and Ikezawa, H. (1984) J. Biochem. (Tokyo) 96, 437–446.

35 Mayor, S., Menon, A.K. and Cross, G.A.M. (1990) J. Biol. Chem. 265, 6174–6181.
36 Field, M.C., Menon, A.K. and Cross, G.A.M. (1991) J. Biol. Chem. 266, 8392–8400.
37 Puoti, A., Desponds, C., Fankhauser, C. and Conzelmann, A (1991) J. Biol. Chem. 266, 21051–21059.
38 Urakase, M., Kaminati, T., DeGasperi, R., Sugiyama, E., Chang, H.M., Warren, C.D. and Yeh, E.T.H. (1992) J. Biol. Chem. 267, 6459–6462.
39 Su, B., Waneck, G.L., Flavell, R.A. and Bothwell, A.L.M. (1991) J. Cell Biol. 112, 377–384.
40 Altman, P.L. and Dittmer, D.S. (1964) Biology Data Book, pp. 267–274. Fed. Am. Soc. Expl. Biol., Washington.