Biochem. J. (1987) 244, 117–121 (Printed in Great Britain)

Proteolysis and deglycosylation of human C1̄ inhibitor

Effect on functional properties

Angeline REBOUL, Marie-Hélène PRANDINI* and Maurice G. COLOMB†
Laboratoire d'Immunochimie–CEN-G, INSERM U238, unité alliée CNRS, USTMG, Grenoble, France

The effects of proteolysis and deglycosylation on C1̄ inhibitor (C1̄Inh) were tested with respect to both its ability to form complexes with C1̄s and its capacity to block C1 autoactivation. Limited proteolysis of C1̄Inh by *Staphylococcus aureus* V8 proteinase, proline-specific endopeptidase or elastase generated a major high-M_r (~ 86000) fragment. In contrast with the fragment produced by elastase, which was inactive, the fragments resulting from V8 proteinase and proline-specific endopeptidase treatment retained activity. Deglycosylation with *N*-glycanase or *O*-glycanase, or both, had no major effect on the functional activity of C1̄Inh.

INTRODUCTION

C1̄ inhibitor (C1̄Inh) is a highly glycosylated plasma protein (Pensky & Schwick, 1969; Haupt *et al.*, 1970; Harrison, 1983; Bock *et al.*, 1986). It is the only known plasma inhibitor of the C1̄ serine proteinases C1̄r and C1̄s (Sim *et al.*, 1979), but it also inactivates several other enzymes of plasma, such as kallikrein (Gigli *et al.*, 1970), factors XIa and XIIa (Forbes *et al.*, 1970) and plasmin (Harpel & Cooper, 1975). More recently C1̄Inh has also been shown to control the activation of C1 (Ziccardi, 1982). C1̄Inh deficiency is associated with the pathological condition hereditary angioneurotic oedema ('HANE'), which is caused by uncontrolled complement activation (Donaldson & Evans, 1963).

Biosynthesis of C1̄Inh *in vitro* has been studied in our laboratory with monocytes (Bensa *et al.*, 1983; Reboul *et al.*, 1985) or Hep G_2 cells (Prandini *et al.*, 1986). As these cells also synthesize C1r and C1s, C1̄Inh is likely to control their state of activation and their activity. We have shown that C1̄Inh undergoes maturation, which is expressed as a progressive glycosylation of the polypeptide backbone during biosynthesis; even 'early' underglycosylated forms can interact with C1̄s. C1̄Inh is known to react with C1r and C1s in two different ways: inhibition of the activated proteinases involves apparently covalent complexes of the proteinases with C1̄Inh, whereas control of the activation of the proenzyme C1r involves non-covalent interactions. As regards the interactions between C1̄Inh and C1 that are involved in the control of C1 activation, the importance of the backbone-attached sugars is not well-defined. Thus, in order to understand better the role of C1̄Inh glycosylation, we studied the influence of glycanase treatment on C1̄Inh activity.

Observations of electron micrographs of C1̄Inh revealed a highly elongated molecule consisting of a globular and a rod-like domain (Odermatt *et al.*, 1981). Neutron scattering has confirmed the very elongated structure of C1̄Inh (Perkins, 1985). To seek the functional domains inside this structure we studied the effect of limited proteolysis of C1̄Inh on its properties; the dual inhibitory role of C1̄Inh in both C1 autoactivation and C1̄ activity was taken into account in order to estimate its functional activity. Interpretation of our data may be aided by recent results on C1̄Inh sequence. Indeed, a nearly complete C1̄Inh amino acid sequence has been published by Bock *et al.* (1986). Partial and complete cDNA clones of human C1̄Inh have recently been isolated and sequenced and the C1̄Inh gene mapped to human chromosome 11 (Tosi *et al.*, 1986; Bock *et al.*, 1986; Davis *et al.*, 1986).

MATERIALS AND METHODS

Materials

Elastase (EC 3.4.21.11) from bovine pancreas (70 units/mg) and thermolysin were purchased from Sigma. Endoproteinase Glu-C (EC 3.4.21.19) from *Staphylococcus aureus* V8 (20 units/mg) was from Boehringer. Proline-specific endopeptidase (EC 3.4.21.26) from *Flavobacterium meningosepticum* (35 units/mg) was obtained from Seikagaku Kogyo, Tokyo, Japan. Neuraminidase (EC 3.2.1.18) was from Sigma; *N*-glycanase (peptide *N*-glycosidase F) and *O*-glycanase (endo-α-*N*-acetylgalactosaminidase) were from Genzyme. $Na^{125}I$ was purchased from Oris, Saclay, France. Molecular-mass markers were obtained from Sigma.

Methods

The purification of C1̄Inh from fresh plasma was performed by the method of Reboul *et al.* (1977), slightly modified by Prandini *et al.* (1986). C1q and C1̄s were purified from recalcified plasma as described by Arlaud *et al.* (1979).

Purified C1̄Inh, C1̄s, C1q and $C1r_2$–$C1s_2$ were estimated from their absorbances at 280 nm by using

Abbreviations used: iPr_2P-F, di-isopropyl phosphorofluoridate; NPGB, *p*-nitrophenyl-*p'*-guanidinobenzoate; the nomenclature of the complement components and subcomponents is that recommended by the World Health Organisation (1968), the activation of a component being indicated by an overbar.

* Present address: INSERM U.217, DRF/HEM, CEN-Grenoble, France.

† To whom correspondence and requests for reprints should be sent at the following postal address: DRF/ICH, CEN-G, 85 X, 38041 Grenoble Cédex, France.

$A_{1cm}^{1\%}$ values of 4.5 (Haupt *et al.*, 1970), 9.5 (Sim *et al.*, 1977), 6.8 (Reid *et al.*, 1972) and 10.6 (Sim *et al.*, 1977) respectively.

SDS/polyacrylamide-gel electrophoresis

Samples for electrophoresis were diluted with an equal volume of 0.126 M-Tris/HCl buffer, pH 7.0, containing 4% SDS, 20% (v/v) glycerol and 5% (v/v) 2-mercaptoethanol and boiled for 5 min.

SDS/polyacrylamide-gel electrophoresis was performed as described by Laemmli (1970), with 10 or 8.5%-acrylamide slab gels. After electrophoresis the gels were stained with Coomassie Brilliant Blue R250, dried and, if necessary, autoradiographed with Kodak X-Omat AR film at −70 °C. Standards for M_r determination, included in each gel, were myosin (M_r 205000), β-galactosidase (M_r 116000), phosphorylase *b* (M_r 97400), bovine albumin (M_r 66000), ovalbumin (M_r 45000) and carbonic anhydrase (M_r 29000).

Endopeptidase treatment of C1Inh

Proteolysis by elastase or proline-specific endopeptidase was performed in 145 mM-NaCl/5 mM-triethanolamine, pH 7.4; for V8 proteinase treatment, C1Inh was dialysed against 0.1 M-NH_4HCO_3. Proteolyses were conducted at 37 °C, with incubation times and C1Inh/proteinase ratios (w/w) of 1 h and 100:1 (elastase), 4 h and 50:1 (V8 proteinase), and 20 h and 3:1 (proline-specific endopeptidase). The reactions were terminated by treating the samples for electrophoresis as described above or by addition of 5 mM-iPr_2P-F for 30 min at 37 °C, followed by extensive dialysis against the appropriate buffer.

Deglycosylation of C1Inh

Neuraminidase and glycanases were used in sequence. C1Inh (1.5 mg/ml), dialysed against 20 mM-Tris/maleate buffer, pH 7.0, was incubated with neuraminidase (0.5 units/mg of C1Inh) for 1 h at 37 °C. Glycanase treatments were then performed by adding *O*-glycanase (50 munits/mg of C1Inh) or/and *N*-glycanase (5 units/mg of C1Inh) and continuing the incubation for $4\frac{1}{2}$ h at 37 °C. When *O*-glycanase was used, 1 mM-calcium acetate and 10 mM-D-galactono-γ-lactone were included in the medium.

Reactivity with C1s

C1Inh was incubated with 1–2-fold molar excesses of C1s for 30 min at 37 °C in 145 mM-NaCl/5 mM-triethanolamine buffer, pH 7.4. After incubation, formation of complexes between C1Inh and C1s was analysed by SDS/polyacrylamide-gel electrophoresis under reducing conditions.

Preparation of proenzymic $C1r_2$–$C1s_2$

$C1r_2$–$C1s_2$ was prepared from serum by the method of Arlaud *et al.* (1980), with modifications taking into account the dissociating effect of diamines on C1 (Villiers *et al.*, 1984). All manipulations were performed as close to 0 °C as possible. Human serum (800 ml), thawed and centrifuged from 15 min at 20000 *g*, was adjusted to pH 7.0 before incubation with 1 mM-NPGB and 1 mM-iPr_2P-F for 25 min. Then 80 mg of insoluble IgG–ovalbumin immune complexes [prepared at equivalence as described by Arlaud *et al.* (1979)] was then added in the serum and the suspension incubated for 25 min. The immune-complex-bound C1 was separated by centrifugation for 8 min at 7000 *g* and the aggregates were washed three times with 160 ml of 120 mM-NaCl/5 mM-$CaCl_2$/20 mM-Tris/HCl, pH 7.0, containing 1 mM-iPr_2P-F and 1 mM-NPGB.

The aggregates were extracted with 40 ml of 120 mM-NaCl/5 mM-$CaCl_2$/20 mM-Tris/HCl, pH 7.0, containing 20 mM-1,3-diaminopropane, 1 mM-iPr_2P-F and 1 mM-NPGB. The extracted $C1r_2$–$C1s_2$ appeared highly contaminated with albumin and was thus purified by chromatography on a column of Sepharose 4B coupled to anti-(human albumin) IgG. The column was equilibrated in 120 mM-NaCl/5 mM-$CaCl_2$/20 mM-Tris/HCl, pH 7.0, containing 1 mM-iPr_2P-F and 1 mM-NPGB. The perfusate, containing $C1r_2$–$C1s_2$, was incubated with 5 mM-iPr_2P-F for 30 min, concentrated by ultrafiltration on a PM10 membrane (Amicon) to about 1–2 mg/ml, extensively dialysed against 120 mM-NaCl/5 mM-$CaCl_2$/20 mM-Tris/HCl, pH 7.0, and stored at −80 °C before use.

$C1r_2$–$C1s_2$ was labelled with ^{125}I, at 0 °C in the presence of 5 mM-iPr_2P-F, by the iodogen method of Fraker & Speck (1978).

Reconstitution and autoactivation of C1

C1 was reconstituted with stoichiometric amounts of C1q and ^{125}I-labelled $C1r_2$–$C1s_2$ (C1q, 0.28 mg/ml; $C1r_2$–$C1s_2$, 0.24 mg/ml; final concns.), in the presence of 5 mM-$CaCl_2$. The activation was assessed after incubation for 1 h at 37 °C followed by SDS/polyacrylamide-gel electrophoresis and autoradiography. Autoradiograms were scanned with a Vernon photometer. The activation, leading to the proteolytic cleavage of single polypeptide chains into two disulphide-bound chains, was estimated as the percentage of radioactivity transferred from the single band of proenzymic C1r and C1s to the A- and B-chains of the activated enzymes.

RESULTS

Limited proteolysis of C1Inh

Endopeptidases with different specificities were used. *Staph. aureus* V8 proteinase, when the hydrolysis is performed in NH_4HCO_3, cleaves on the *C*-terminal side of glutamic acid residues (Houmard & Drapeau, 1972), proline-specific endopeptidase is a post-proline-cleaving enzyme (Yoshimoto *et al.*, 1980), and pancreatic elastase, which has a broader specificity, hydrolyses peptide bonds on the *C*-terminal side of amino acids bearing uncharged non-aromatic side chains, with a preference for alanine (Shotton, 1970). These enzymes are sensitive to iPr_2P-F and, furthermore, C1Inh shows no inhibitory effect towards these proteinases.

For each endopeptidase, under the conditions used, the electrophoretic pattern of proteolysed C1Inh shows a major band, often appearing as a doublet (Fig. 1, lanes 2, 3 and 4). The M_r values of the fragments are very similar (~ 86000), but only V8 proteinase and proline-specific endopeptidase generated fragments still able to form apparently covalent complexes with C1s B-chain (Fig. 1, lanes 5 and 6). Elastase treatment suppressed the capacity of C1Inh to block C1s (Fig. 1, lane 7). Limited proteolysis by thermolysin also generated a fragment of M_r ~ 86000 unreactive with C1s (results not shown).

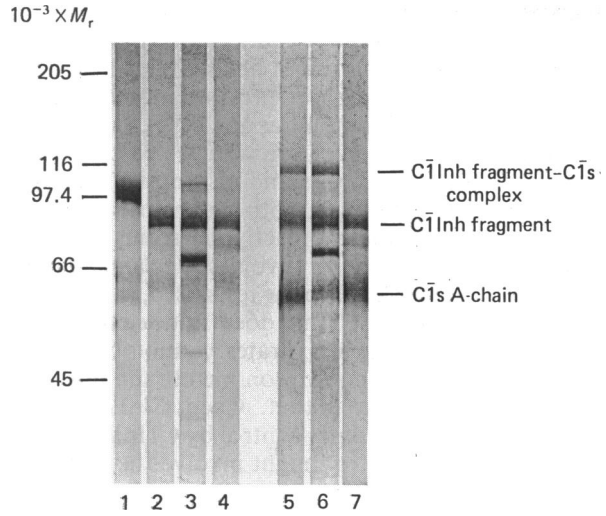

Fig. 1. Limited proteolysis of C1Inh: effect on reactivity with C1s

Proteolytic treatments, incubation with C1s and electrophoretic separation were as described in the Materials and methods section. Lane 1, reference C1Inh; lane 2, C1Inh proteolysed by V8 proteinase; lane 3, C1Inh proteolysed by proline-specific endopeptidase; lane 4, C1Inh proteolysed by elastase; lane 5, C1Inh proteolysed by V8 proteinase, then incubated with C1s; lane 6, C1Inh proteolysed by proline-specific endopeptidase, then incubated with C1s; lane 7, C1Inh proteolysed with elastase, then incubated with C1s. When C1Inh was proteolysed by proline-specific endopeptidase, a high C1Inh/proteinase ratio (3:1, w/w) was used, and the proteinase was thus visible on the gel (extra band on gels 3 and 6).

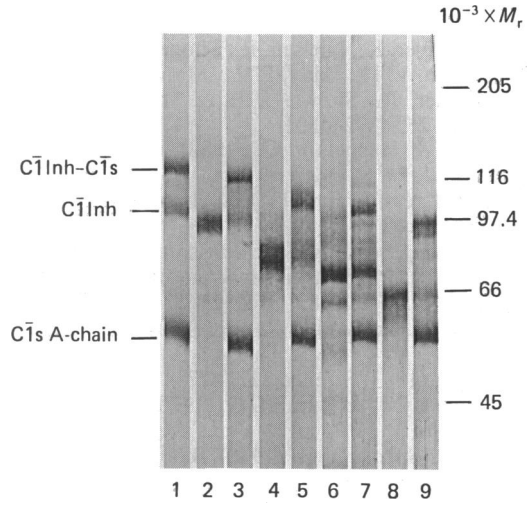

Fig. 2. Deglycosylation of C1Inh: effect on reactivity with C1s

Neuraminidase, *O*- and *N*-glycanase treatments, incubation with C1s and electrophoretic separation were as described in the Materials and methods section. Lane 1, reference C1Inh incubated with C1s; lanes 2 and 3, neuraminidase-treated C1Inh (lane 2) after incubation with C1s (lanes 3); lanes 4 and 5, desialylated C1Inh treated with *N*-glycanase (lane 4) after incubation with C1s (lane 5); lanes 6 and 7, desialylated C1Inh treated with *O*-glycanase (lane 6) after incubation with C1s (lane 7); lanes 8 and 9, desialylated C1Inh treated with *N*- and *O*-glycanase (lane 8) after incubation with C1s (lane 9).

Table 1. Efffect of proteolysis or deglycosylation on the ability of C1Inh to block C1 autoactivation

C1Inh, after treatment with proteinases or glycanases as described in the Materials and methods section was incubated with iPr_2P-F (5 mM final concn.) for 30 min at room temperature and dialysed against 120 mM-NaCl/5 mM-$CaCl_2$/20 mM-Tris/HCl, pH 7.0. C1, reconstituted as described in the Materials and methods section, from C1q and ^{124}I-labelled $C1r_2$–$C1s_2$, was incubated for 1 h at 37 °C in presence of the indicated proteins. C1 activation was measured, after SDS/polyacrylamide-gel electrophoresis under reducing conditions and autoradiography, by the percentage of radioactivity transferred from proenzymic C1r and C1s to the A- and B-chains of C1r and C1s.

Addition to C1	C1 autoactivation (%)
None	89
C1Inh	1
Elastase fragment of C1Inh	90
V8-proteinase fragment of C1Inh	2
Proline-specific-endopeptidase fragment of C1Inh	2
Ovalbumin	92
N-Glycanase-treated C1Inh	3
O-Glycanase-treated C1Inh	0
O- and *N*-Glycanase-treated C1Inh	0

Deglycosylation of C1Inh

Neuraminidase treatment lowered the C1Inh M_r from ~ 98000 to ~ 93000 (Fig. 2, lanes 1 and 2). We tested the susceptibility of desialylated C1Inh to two types of endoglycosidases which hydrolyse the linkage between glycans and the protein backbone. *N*-Glycanase cleaves asparagine-*N*-linked carbohydrate chains (Plummer *et al.*, 1984) and *O*-glycanase serine- or threonine-*O*-linked chains (Umemoto *et al.*, 1977). When glycanases were applied to desialylated C1Inh, an increase in electrophoretic mobility was observed and heterogeneous lower-M_r products were detected (Fig. 2, lanes 4 and 6). The major form generated by *N*-glycanase, in the presence or absence of 5 mM-iPr_2P-F, had a lower mobility (M_r ~ 81000) than the main form generated by *O*-glycanase (M_r ~ 74000). When C1Inh was treated with both *N*- and *O*-glycanase, a major form, of M_r ~ 65000, appeared on the electrophoretogram (Fig. 2, lane 8). No significant variations in M_r were observed when the glycanase treatments were conducted (in 0.1% SDS/0.7% Nonidet P40-supplemented medium) with C1Inh denatured by boiling for 3 min in 0.1% SDS/20 mM-Tris/maleate buffer, pH 7.

We tested the reactivity towards C1s of the different forms of deglycosylated C1Inh. They all reacted with C1s, as illustrated in Fig. 2 (lanes 3, 5, 7 and 9) by bands in the electrophoretogram corresponding to complexes with the C1s B-chain.

Effect of deglycosylation and proteolysis of C1Inh on its ability to block C1 autoactivation. C1 was reconstituted from purified C1q and ^{125}I-labelled proenzyme $C1r_2$–$C1s_2$ in the presence of Ca^{2+}, and the percentage of

autoactivation was estimated after incubation for 1 h at 37 °C. Results are shown in Table 1. An 89% activation of isolated C1 occurred after 1 h at 37 °C, and stoichiometric amounts of C1Inh blocked the activation, in agreement with previous results (Ziccardi, 1982).

Deglycosylation of C1Inh by *N*-glycanase, *O*-glycanase, or both enzymes, did not suppress the capacity of the protein to block C1 autoactivation.

We tested the ability of the three types of C1Inh proteolytic fragments described above to inhibit C1 autoactivation. As indicated in Table 1, the fragments obtained by limited proteolysis of C1Inh with V8 proteinase or proline-specific endopeptidase were still able to block C1 autoactivation; by contrast, the fragment generated by elastase was inactive. Ovalbumin, which was also tested because it shares sequence homologies with *C*-terminal parts of proteins of the serpin family (Doolittle, 1983), had no effect on C1 autoactivation.

DISCUSSION

Three proteinases of different specificities, namely V8 proteinase, proline-specific endopeptidase and elastase, each cleave C1Inh, generating a major, high-M_r, peptide. Despite their nearly identical M_r values, fragments generated by V8 proteinase and proline-specific endopeptidase are active, whereas the fragment generated by elastase is inactive.

V8 proteinase and proline-specific endopeptidase both probably cleave C1Inh in its *N*-terminal portion, at a distance from the reactive site; in the 105 *N*-terminal residues, which account for the rod-like part of the inhibitor, three glutamic acid and 15 proline residues represent potential sites for cleavage by these two proteinases. In contrast, elastase inactivates C1Inh, in agreement with previous results from Brower & Harpel (1982) and Catanese & Kress (1984). The effect of elastase on native C1Inh seems to be in favour of a cleavage in the *C*-terminal portion of the molecule, which contains the reactive site. Further characterization of the proteolytic fragments should be possible on the basis of recent sequence data.

We have shown that C1Inh is sensitive to glycanases, which release *N*- or *O*-linked sugars, a finding in agreement with the presence of these two types of oligosaccharides in the molecule (Bock *et al.*, 1986). The M_r of the protein after deglycosylation by both types of glycanases ($\sim$ 65000) is comparable with that (64000) of a cell-free-translation product of C1Inh human liver mRNA (Tosi *et al.*, 1986). These values are clearly higher than values (53000) provided by Bock *et al.* (1986), either calculated from C1Inh cDNA sequence or measured on cell-free-translation products of Hep G_2 mRNA. The discrepancy between the results of Bock *et al.* (1986) and ours is probably due to differences in the methods used for the estimation of these M_r values. In any case it appears that deglycosylation by the joint action of *N*- and *O*-glycanases leads to a largely deglycosylated protein, as the values observed are still lower than another value (78000) reported by Harrison (1983) for the M_r of chemically deglycosylated C1Inh. According to the work of Bock *et al.* (1986), most of the carbohydrates are located at the *N*-terminal end of the C1Inh protein, and the reactive site maps in the *C*-terminal end of the molecule, which does not contain known carbohydrate-attachment sites in the 147 *C*-terminal residues. In keeping with these results, ours show that extensive deglycosylation of C1Inh does not significantly alter its functionality, confirming our previous observations on C1Inh biosynthesis (Prandini *et al.*, 1986).

Effects of C1Inh modification were tested by its ability to form complexes with C1s and also on its capacity to block C1 autoactivation. In both cases close parallelism between these two types of reactivities seems to indicate that the dual role of C1Inh involves the same region of the molecule. With regard to the tests used, the unusually high glycosylation of C1Inh does not seem essential. However, a role of carbohydrates cannot be excluded when C1Inh exerts its control on membrane-bound C1. It has been hypothesized that, when C1 is bound to activator surfaces, it escapes control by C1Inh (Tenner & Frank, 1986); this control might involve the interaction of glycosidic residues.

The functional tests of C1Inh described in the present paper, which take into account the influence of C1Inh on both C1 activation and C1 activity, seem a valid test for monitoring C1Inh production in future programs dealing with gene transfection. Demonstration of the functional activity of deglycosylated C1Inh points to the possibility of C1Inh transfection in non-glycosylating prokaryotic cells. In the search for a minimal effective molecule for therapy, production of active C1Inh polypeptides of reduced length by prokaryotic cells could also be considered.

REFERENCES

Arlaud, G. J., Sim, R. B., Duplaa, A. M. & Colomb, M. G. (1979) Mol. Immunol. **16**, 445–450

Arlaud, G. J., Villiers, C. L., Chesne, S. & Colomb, M. G. (1980) Biochim. Biophys. Acta **616**, 116–129

Bensa, J. C., Reboul, A. & Colomb, M. G. (1983) Biochem. J. **216**, 385–392

Bock, S. C., Skriver, K., Nielsen, E., Thogersen, H. C., Wiman, B., Donaldson, V. H., Eddy, R. L., Marrinan, J., Radziejewska, E., Huber, R., Shows, T. B. & Magnusson, S. (1986) Biochemistry **25**, 4292–4301

Brower, M. S. & Harpel, P. C. (1982) J. Biol. Chem. **257**, 9849–9854

Catanese, J. & Kress, L. F. (1984) Biochim. Biophys. Acta **789**, 37–43

Davis, A. E., Whitehead, A. S., Harrisson, R. A., Dauphinais, A., Bruns, G. A. P., Cicardi, M. & Rosen, F. S. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 3161–3165

Donaldson, V. H. & Evans, R. R. (1963) Am. J. Med. **35**, 37–44

Doolittle, R. F. (1983) Science **222**, 417–419

Forbes, C., Pensky, J. & Ratnoff, O. (1970) J. Lab. Clin. Med. **76**, 809–815

Fraker, P. J. & Speck, J. C. (1978) Biochem. Biophys. Res. Commun. **80**, 849–857

Gigli, I., Mason, J., Colinan, R. & Austen, F. (1970) J. Immunol. **104**, 574–581

Harpel, P. C. & Cooper, N. R. (1975) J. Clin. Invest. **55**, 593–604

Harrison, R. A. (1983) Biochemistry **22**, 5001–5007

Haupt, H., Heimburger, N., Kranz, T. & Schwick, H. (1970) Eur. J. Biochem. **17**, 254–261

Houmard, J. & Drapeau, G. R. (1972) Proc. Natl. Acad. Sci. U.S.A. **69**, 3506–3509

Laemmli, U. K. (1970) Nature (London) **227**, 680–685

Odermatt, E., Berger, H. & Sano, Y. (1981) FEBS Lett. **131**, 283–285

Pensky, J. & Schwick, H. (1969) Science **163**, 698–699

Perkins, S. J. (1985) Biochem. J. **228**, 13–26

Plummer, T. H., Elder, J. H., Alexander, S., Phelan, A. W. & Tarentino, A. L. (1984) J. Biol. Chem. **259**, 10700–10704
Prandini, M. H., Reboul, A. & Colomb, M. G. (1986) Biochem. J. **237**, 93–98
Reboul, A., Arlaud, G. J., Sim, R. B. & Colomb, M. G. (1977) FEBS Lett. **79**, 45–50
Reboul, A., Prandini, M. H. & Colomb, M. G. (1985) FEBS Lett. **190**, 65–68
Reid, K. B. M., Lowe, D. M. & Porter, R. R. (1972) Biochem. J. **130**, 749–763
Shotton, D. M. (1970) Methods Enzymol. **19**, 113–140
Sim, R. B., Porter, R., Reid, K. B. M. & Gigli, I. (1977) Biochem. J. **163**, 219–227
Sim, R. B., Reboul, A., Arlaud, G. J., Villiers, C. L. & Colomb, M. G. (1979) FEBS Lett. **97**,111–115
Tenner, A. J. & Frank, M. M. (1986) J. Immunol. **137**, 625–630
Tosi, M., Duponchel, C., Bourgarel, P., Colomb, M. G. & Meo, T. (1986) Gene **42**, 265–272
Umemoto, J., Bhavanandan, V. P. & Davidson, E. A. (1977) J. Biol. Chem. **252**, 8609–8614
Villiers, C. L., Arlaud, G. J. & Colomb, M. G. (1984) Eur. J. Biochem. **140**, 431–426
World Health Organisation (1968) Bull. W.H.O. **39**, 935–936
Yoshimoto, T., Walter, R. & Tsuru, D. (1980) J. Biol. Chem. **255**, 4786–4792
Ziccardi, R. J. (1982) J. Immunol. **128**, 2505–2508

Received 15 September 1986/9 December 1986; accepted 11 February 1987