CNBr cleavage of the light chain of human complement Factor I and alignment of the fragments

Jing-ming YUAN,* Li-min HSIUNG† and Jean GAGNON‡

M.R.C. Immunochemistry Unit, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.

The light chain and heavy chain of reduced and alkylated human complement Factor I were purified by high-pressure gel-permeation chromatography. CNBr cleavage of Factor I light chain yielded four major fragments, which were purified by gel filtration. *N*-Terminal sequence analysis of the CNBr-cleavage fragments allowed identification of 200 of the approx. 240 amino acid residues of the light chain. An alignment is proposed, based on sequence analysis of peptides obtained after cleavage at arginine residues of the light chain and on homology of the sequence determined with that of other serine proteinases. The sequence around the active-site serine residue was established and three potential attachment sites for carbohydrate moieties were identified.

INTRODUCTION

The essential step of complement activation by either the classical pathway or the alternative pathway is the formation of a C3 convertase enzyme (Reid & Porter, 1981). Modulation of the C3 convertase of the alternative pathway (C3bBb) and of the corresponding enzyme of the classical pathway (C4b2a) is achieved by the action of three regulatory proteins: Factor I, Factor H and C4-binding protein. Factor I is a proteinase responsible for cleavage of the α' -chains of C3b (Whaley & Thompson, 1978) and C4b (Fujita *et al.*, 1978) in the presence of two cofactors, Factor H and C4-binding protein respectively.

The α' -chain of C3b is cleaved in two places by Factor I, resulting in the production of C3bi (Harrison & Lachmann, 1980; Sim *et al.*, 1981). In the case of C4b, Factor I cleaves two peptide bonds to produce fragments C4c and C4d (Nagasawa *et al.*, 1980). It has been shown that the specificity of Factor I on C4b α' -chain is for arginine residues (Press & Gagnon, 1981). The effect of proteinase inhibitors has been studied in an unsuccessful attempt to classify this enzyme (Harrison & Lachmann, 1980; Crossley & Porter, 1980). Despite the lack of inhibition observed when studying serine proteinase inhibitors such as di-isopropyl phosphorofluoridate (10 mM), 7-amino-1-chloro-3-L-tosylamidoheptan-2-one ('TLCK') (5 mM), phenylmethanesulphonyl fluoride (0.1 mM) or benzamidin (5 mM), Factor I has been classified as a serine proteinase on the basis of an *N*-terminal sequence determination by Davis (1981).

Human Factor I is glycoprotein of apparent M_r 88000. It is composed of two disulphide-linked polypeptide chains of M_r 50000 and 38000 as determined by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Pangburn *et al.*, 1977; Crossley & Porter, 1980). Both

chains have been reported to contain carbohydrate moieties (Pangburn *et al.*, 1977). The light chain has been reported to be the catalytic chain on the basis of the homology of the sequence of its first 25 *N*-terminal residues with that of other known serine proteinases (Davis, 1981).

In order to corroborate this finding and investigate in more detail the structure of Factor I, the present work reports the purification and amino acid sequence analysis of the fragments generated by CNBr cleavage of the light chain. On the basis of homology with other serine proteinases, an alignment of the peptides is also proposed.

MATERIALS AND METHODS

Materials

Outdated human plasma was obtained from the Churchill Hospital, Oxford, U.K. Sephadex G-100, Sepharose CL-4B and DEAE-Sephacel were obtained from Pharmacia, Hounslow, Middx., U.K. The TSK-G3000 SW gel-permeation column (7.5 mm $\times$ 600 mm) was purchased from LKB Instruments, South Croydon, Surrey, U.K. The Radial-PAK C₁₈ cartridge and μ Bondapak C₁₈ column (3.9 mm $\times$ 300 mm) were obtained from Waters Associates, Northwich, Cheshire, U.K. ODS-Hypersil (5 μ m-particle-size) packing material was purchased from Shandon Southern Products, Runcorn, Cheshire, U.K. All other materials used have been described previously (Christie & Gagnon, 1982).

Purification of Factor I

Factor I was prepared from human plasma by using the monoclonal antibody MRC OX-21, as previously described (Hsiung *et al.*, 1982). Essentially, an affinity

Abbreviations used: the nomenclature used for the complement components is as recommended by the World Health Organisation (1981). Other abbreviations: CN-, CNBr-cleavage fragment; SP-, staphylococcal-V8-proteinase-digest peptide; T-, tryptic-digest peptide after succinylation (3-carboxypropionylation).

* Present address: Institute of Molecular Science, Shanxi University, Wu-cheng Road, Taiyuan, China.

† Present address: Shanghai Institute of Biochemistry, Academia Sinica, 320 Yue-yang Road, Shanghai, China.

‡ To whom correspondence should be addressed.

column was equilibrated with 0.14 M-NaCl/0.025 M-Tris/HCl/0.02 M-EDTA, pH 7.3. Plasma was applied to the column, washed with the same buffer and then an increasing concentration of NaCl in the same buffer up to 0.5 M. The bound Factor I was eluted with 0.14 M-NaCl/0.05 M-diethylamine/HCl, pH 11.5. The fractions were neutralized with solid glycine to pH 8.2.

Separation of heavy chain and light chain

After reduction and carboxymethylation of Factor I with iodo[2-¹⁴C]acetic acid as described by Christie & Gagnon (1982), the separation of heavy chain and light chain was carried out by high-pressure gel-permeation chromatography on a 7.5 mm × 600 mm column of TSK-G3000 SW equilibrated in 6 M-urea/0.2 M-formic acid as previously described (Arlaud *et al.*, 1982).

CNBr cleavage of light chain

Reduced and carboxymethylated Factor I light chain (250 nmol) was dissolved in 70% (v/v) formic acid (5 ml). CNBr was added in 10-fold excess (w/w) and the mixture incubated in the dark at 4 °C for 24 h. The reaction mixture was diluted 1:10 with water and freeze-dried. The fragments, redissolved in 10% (v/v) acetic acid containing 2.5 M-urea, were separated on a column (3 cm × 100 cm) of Sephadex G-100 equilibrated with 10% (v/v) acetic acid and run at a flow rate of 18 ml/h. Peptides were detected by A_{280} and by radioactivity. Eight pools were made and freeze-dried. The purity of the fragments was assessed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis as described by Laemmli (1970).

V8-proteinase digestion

Fragment CN2 (50 nmol) obtained by CNBr cleavage of reduced and carboxymethylated light chain was further digested with staphylococcal V8 proteinase as previously described (Gagnon & Christie, 1983). Immediately after incubation for 6 h at 37 °C, the peptides were separated by h.p.l.c. on a Radial-PAK C₁₈ cartridge in 0.1% NH₄HCO₃ with a gradient of acetonitrile (Gagnon & Christie, 1983).

Carboxypeptidase-Y digestion

Fragment CN3 was submitted to digestion by carboxypeptidase Y in 0.2 M-pyridine/acetate buffer, pH 5.5, by using the protocol described by Christie & Gagnon (1983).

Succinylation and tryptic cleavage

Reduced and S-carboxy[¹⁴C]methylated Factor I light chain (200 nmol) was dissolved in 6 M-guanidine hydrochloride (3 ml) and the pH adjusted to 8.0 with 4 M-NaOH. Succinylation was carried out by the procedure of Koide *et al.* (1978), with a 10-fold excess (w/w) of succinic anhydride with respect to protein. After dialysis against 0.1 M-NH₄HCO₃ the reduced S-carboxy[¹⁴C]methylated and succinylated light chain was incubated with trypsin [1-chloro-4-phenyl-3-L-tosylamidobutan-2-one ('TPCK')-treated] (60 µg) for 2 h at 37 °C. The incubation was repeated after addition of the same amount of enzyme and terminated by freeze-drying. The digest products redissolved in 0.1 M-NH₄HCO₃ (2.5 ml) were separated on a column (2 cm × 90 cm) of Sephadex G-50 (superfine grade) equilibrated in the same buffer. Peptides eluted at a flow rate of 25 ml/h were detected by A_{206} and

by radioactivity. Eight pools were made (T1 to T8) and further purified as described by Gagnon & Christie (1983), either by ion-exchange chromatography on DEAE-Sephacel as in the case of pool T2 or by h.p.l.c. for pools T4, T5 and T7.

Amino acid analysis

Hydrolysed samples (Christie & Gagnon, 1982) were analysed on a Durrum D-500 or a LKB model 4400 amino acid analyser, with sodium citrate or lithium citrate buffers.

N-Terminal sequence analysis

This was carried out on a Beckman 890C liquid-phase sequencer fitted with the Beckman cold-trap accessory, by using the procedure described by Christie & Gagnon (1982). Identification of phenylthiohydantoin derivatives was done by reverse-phase h.p.l.c. on a µBondapak C₁₈ column (Christie & Gagnon, 1982) or on an ODS-Hypersil (5 µm-particle-size) column (5 mm × 100 mm) equilibrated in 0.01 M-sodium acetate, pH 4.18, containing 10% (v/v) methanol and eluted with a linear gradient of ethanol/acetonitrile (1:1, v/v) from 5 to 45% (v/v), in 27 min at 1 ml/min.

RESULTS

Separation of heavy chain and light chain of Factor I

The separation of the reduced and carboxymethylated heavy chain and light chain of Factor I is shown in Fig. 1. Two main peaks, eluted at 14.5 ml and 18 ml, were

Fig. 1. Separation of Factor I heavy chain and light chain by high-pressure gel-permeation chromatography

Reduced and S-carboxy[¹⁴C]methylated Factor I (10 nmol) was dissolved in 6 M-urea/0.2 M-formic acid (100 µl) and loaded on a TSK G3000 SW column (7.5 mm × 600 mm) equilibrated in the same buffer and eluted at 1.0 ml/min.

Table 1. Amino acid compositions of peptides used in sequence determination of Factor I light chain

Results are expressed in mol of residues/mol, and values less than 0.2 mol/mol are omitted. Compositions were calculated from 24 h hydrolysates and no correction was done. Cysteine was estimated as *S*-(carboxymethyl)cysteine. Methionine from CNBr-digest fragments was quantified as the sum of homoserine and homoserine lactone values. Numbers in parentheses were obtained from the sequence or from the composition. The low value for valine in peptide CN3 is due to the presence of a Val–Val bond partially resistant under standard hydrolysis conditions. Abbreviation: N.D., not determined.

	CN1	CN2	CN3	CN4	Total	Light chain	CN2-SP4	T2-D4	T4-4
Cys	2.6 (3)	3.7 (4)	1.2 (1)	2.7 (3)	11	10.6	0.9 (1)	2.5 (3)	1.0 (1)
Asx	9.0 (9)	8.7 (9)	6.6 (7)	3.1 (3)	28	24.7	2.1 (1)	6.3 (5)	6.6 (7)
Thr	4.7 (5)	1.4 (1)	1.8 (2)	0.9 (1)	9	11.5	—	2.2 (2)	0.9 (1)
Ser	4.0 (4)	5.6 (6)	2.8 (3)	1.8 (2)	15	14.3	1.7 (2)	2.6 (3)	0.4
Glx	8.2 (8)	8.1 (8)	3.5 (4)	1.5 (1)	21	21.9	1.4 (1)	3.5 (3)	3.9 (4)
Pro	2.7 (3)	5.3 (5)	2.8 (3)	1.6 (1)	12	10.2	0.4	0.9 (1)	1.0 (1)
Gly	8.4 (8)	5.4 (5)	5.2 (5)	4.7 (5)	23	24.3	1.2 (1)	6.5 (6)	2.1 (2)
Ala	5.9 (6)	1.5 (1)	2.2 (2)	2.0 (2)	11	12.9	0.3	3.4 (3)	2.1 (2)
Val	7.3 (7)	4.2 (4)	5.9 (7)	1.3 (1)	19	19.4	1.2 (1)	4.9 (5)	0.6
Met	1.0 (1)	1.4 (1)	—	0.6 (1)	3	3.2	—	1.6 (2)	1.1 (1)
Ile	8.4 (9)	2.8 (3)	2.1 (2)	1.0 (1)	15	16.6	0.5 (1)	1.8 (1)	2.8 (4)
Leu	5.6 (6)	4.1 (4)	—	1.1 (1)	11	12.2	1.0 (1)	1.4 (1)	1.9 (2)
Tyr	4.5 (5)	3.1 (3)	4.6 (5)	1.0 (1)	14	13.8	1.5 (2)	3.1 (3)	1.5 (2)
Phe	2.7 (3)	3.7 (4)	2.7 (3)	—	10	8.7	1.4 (2)	2.2 (1)	1.0 (1)
His	2.6 (3)	—	1.1 (1)	—	4	5.7	—	0.3	0.8 (1)
Lys	5.3 (5)	8.2 (8)	2.2 (2)	1.1 (1)	16	15.2	1.8 (2)	2.4 (2)	3.2 (4)
Arg	4.6 (5)	3.7 (4)	0.7 (1)	—	10	9.9	1.0 (1)	—	1.0 (1)
Trp	(1)	(3)	(3)	—	(7)			(2)	
Total	(91)	(73)	(51)	(24)	239	235.1	(17)	(44)	(34)
GlcNAc	1.4	1.5	1.3	—		N.D.	N.D.	0.6	—
Yield (%)	28	50	64	59			40	20	22

Fig. 2. Fractionation of CNBr-cleavage fragments from reduced and *S*-carboxy[¹⁴C]methylated Factor I light chain

The CNBr digest of 250 nmol of Factor I light chain was loaded on a Sephadex G-100 column (3 cm × 100 cm) equilibrated in 10% acetic acid and eluted at a flow rate of 18 ml/h. Fractions of volume size 3.5 ml were collected. Samples (50 μl) were taken for determination of radioactivity. —, A_{280} ; ----, radioactivity.

detected by absorbance at 280 nm. The highest of the two peaks (18 ml) represented the light chain, which, when judiciously pooled, was pure. Amino acid analysis of the light chain is shown in Table 1. The heavy chain (14.5 ml) is slightly contaminated with aggregated material and some IgG heavy chain. The pools were dialysed against dilute acetic acid and subsequently deionized water, and freeze-dried.

Sequence analysis of CNBr-digest fragments of the light chain

The CNBr-digest fragments were separated by gel filtration on a Sephadex G-100 column (3 cm × 100 cm) equilibrated with 10% (v/v) acetic acid (Fig. 2). Eight pools were made and analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. Pools 2, 4,

Fig. 3. Partial amino acid sequence of Factor I light chain

CN-, CNBr-cleavage peptide; T-, tryptic peptide after succinylation; SP-, peptide from V8-proteinase digestion; CP-Y, carboxypeptidase-Y digestion.

6 and 8 contained a single peptide each, which were called CN1, CN2, CN3 and CN4 respectively. Their amino acid compositions and yields are presented in Table 1; the sum of the residues of these peptides is in close agreement with the composition of the light chain. These four CNBr-cleavage fragments were used directly for *N*-terminal sequence analysis. Pool 1 contained aggregated material and Pool 3 an unequal mixture of peptides CN1 and CN2, the amount of peptide CN1 being greater. The *N*-terminal amino acid sequences of the CNBr-cleavage peptides of the light chain of Factor I are presented in Fig. 3.

Peptide CN1 was estimated to be some 90 amino acid residues long (Table 1), and *N*-terminal sequence analysis gave the position of the first 32 residues. This sequence was found to be identical with that of the whole light chain, indicating that CN1 is the *N*-terminal peptide.

Peptide CN2 was estimated to be composed of some 70 amino acid residues (Table 1). The first 67 residues were identified with two exceptions in the sequences -Pro-Xaa-Asp-Thr-Cys- and Ser-Xaa-Cys-Ser-, which could be *N*-linked carbohydrate-attachment sites, as the presence of *N*-acetylglucosamine was detected in fragment CN2. To extend this sequence a V8-proteinase digest of CN2 was performed and the peptides were separated directly by reverse-phase h.p.l.c. Six peaks were collected and further analysed. Five of these, called CN2-SP2 to CN2-SP6, contained peptide material. Peptides CN2-SP3, CN2-SP5 and CN2-SP6 were identified by amino acid analysis and sequence determination from within the sequence of the first 66 residues of fragment CN2. Peptide CN2-SP6 contained some glucosamine and represented the first 40 residues of fragment CN2. The sequence of peptide CN2-SP4, also containing glucosamine, extended the sequence of peptide CN2 by three residues: -Phe-Tyr-Glu (Fig. 3). Peptide CN2-SP2 contained, by amino acid analysis, only lysine, glutamic acid and homoserine.

Peptide CN3 was the only peptide containing no homoserine or homoserine lactone. It was found to consist of 50 amino acid residues and was completely

Fig. 4. Carboxypeptidase-Y digestion of CNBr-digest fragment CN3 from Factor I light chain

Fragment CN3 (40 nmol) in 50 mM-pyridine/acetate buffer, pH 5.5 (0.3 ml), was digested with carboxypeptidase Y (25 μ g) at 37 °C. Samples (25 μ l) were taken at various time intervals, freeze-dried and subjected to amino acid analysis. Norleucine was used as an internal standard. Symbols: $\circ$, asparagine; $\triangle$, tyrosine; $\bullet$, glutamine; $\blacktriangle$, serine; $\square$, isoleucine.

sequenced. In order to corroborate this result the whole peptide CN3 was submitted to carboxypeptidase-Y digestion. The analysis, shown in Fig. 4, confirmed the C-terminal sequence to be Ile-Ser-Gln-Tyr-Asn. The glucosamine content of peptide CN3 indicated also a *N*-linked carbohydrate-attachment site in the sequence -Asn-Xaa-Val-Thr-Tyr-.

Finally, peptide CN4, containing only 24 residues, was sequenced in its entirety. This short peptide was found to

Fig. 5. Initial fractionation of a tryptic digest of reduced and *S*-carboxy[^{14}C]methylated and succinylated Factor I light chain

The tryptic digest (200 nmol) of Factor I light chain was loaded on a Sephadex G-50 (superfine grade) column (2 cm $\times$ 90 cm) equilibrated in 0.1 M-NH $_4$ HCO $_3$ and eluted at a flow rate of 28 ml/h. Fractions of volume size 5 ml were collected. Samples (50 μ l) were taken for determination of radioactivity. ———, A_{206} ; ----, radioactivity.

contain the sequence -Gly-Asp-Ser-Gly-Gly-, which is invariable at the active site of serine proteinases. With these data and homology to known serine proteinases, the CNBr-digest fragments of Factor I light chain can be aligned tentatively in the sequence CN1-CN2-CN4-CN3.

Sequence analysis of tryptic-digest peptides of the light chain

To ascertain the alignment of the CNBr-digest fragments, overlap peptides were isolated in the following way. Reduced and *S*-carboxy[^{14}C]methylated light chain was cleaved at arginine residues by digestion with trypsin after succinylation of the chain. The digest was separated by gel filtration on Sephadex G-50 (superfine grade) equilibrated with 0.1 M-NH $_4$ HCO $_3$ (Fig. 5). Eight pools, designated T1 to T8, were made and analysed for their methionine content. Only pools T2 and T4 were found to contain methionine residues.

Pool T2 was further purified by ion-exchange chromatography on DEAE-Sephacel with a linear gradient of NH $_4$ HCO $_3$ (0.25–1.0 M). A peptide, T2-D4, of approx. 42 residues was obtained. It contained two methionine residues, some glucosamine but no arginine (Table 1). *N*-Terminal sequence analysis of the first 33 residues (Fig. 3) indicated it to be derived from a chymotryptic-like cleavage, probably after a tryptophan residue. This peptide gave the overlap between CNBr-digest fragments CN2, CN4 and CN3.

Purification of pool T4 by h.p.l.c. yielded peptide T4-4, which consisted of 34 amino acids, including one methionine residue (Table 1). This peptide was sequenced to its *C*-terminal arginine residue (Fig. 3). It presumably provided the overlap between CNBr-digest fragments CN1 and CN2.

During these studies other peptides were isolated and located within the sequence of the light chain by amino

Table 2. Yields and positions of other peptides in the sequence of Factor I light chain

Peptide	Yield (%)	Position in sequence
CN3-SP2	50	164–166
CN3-SP3	30	133–145
CN3-SP5	24	137–145
CN3-SP6	36	93–132
T4-5	22	138–159
T5-13	18	7–44
T1-1	14	132–137
T7-4	16	1–6

acid composition and partial sequence with the exception of peptide T7-4. These are shown in Table 2.

DISCUSSION

The light chain of Factor I was conveniently isolated from reduced and *S*-carboxy[^{14}C]methylated Factor I by h.p.l.c. with a gel-filtration column. From its methionine content (Table 1), four peptides were expected from CNBr cleavage of the light chain. With the exception of CN1, the fragments were obtained in good yield and sequenced nearly to completion (Table 1 and Fig. 3).

Fragment CN1 was located at the *N*-terminus and fragment CN3 at the *C*-terminus of the light chain. On the basis of homology with other serine proteinases (Fig. 6) the other two fragments could be positioned in the sequence CN1-CN2-CN4-CN3.

The presence of *N*-acetylglucosamine was detected in three of the fragments. Amino acid sequence analysis indicated two potential *N*-linked carbohydrate-attachment sites (Neuberger *et al.*, 1972) in fragment CN2

Fig. 6. Homology of Factor I light chain with sequences of other serine proteinases
Sequence data for bovine trypsin and chymotrypsin are from Young *et al.* (1978). - denotes that a gap was left to give maximum homology on alignment of the amino acid residues. The residue numbering indicated above is arbitrary and takes into account the assumed length of fragment CN1 as 92 residues (Table 1 and Fig. 3). The residue numbering below is that of chymotrypsinogen. Residues identical with those of Factor I light chain are enclosed in 'boxes'.

(positions 121 and 151) and one in fragment CN3 (position 193). Presumably there is also at least one carbohydrate moiety in fragment CN1 (Table 1). This fragment was obtained in lower yield, and we were repeatedly unable to obtain meaningful sequence information after residue 32. The sequence of fragment CN1 could not be extended by sequence determination of tryptic peptide T5-13 (Table 2). Attempts to subdigest fragment CN1 with *Staphylococcus aureus* V8 proteinase or chymotrypsin were unsuccessful.

The tentative alignment of the CNBr-digest fragments was in part confirmed by the sequence of two tryptic-digest peptides, namely T4-4 and T2-D4. Although we have no formal proof that the first 21 amino acid residues of peptide T2-D4 were derived from fragment CN1, the amino acid composition can be accommodated by that of fragment CN1. However, if these 21 residues were not part of fragment CN1 then they would come from a CNBr-digest fragment of at least 22 amino acids that remained undetected by us.

Comparison of the partial sequence of human Factor I light chain with that of bovine trypsin and chymotrypsin (Fig. 6) revealed strong blocks of homology around the functionally important residues such as isoleucine-1, aspartic acid-86 and serine-182 (Ile-16, Asp-102 and Ser-195, chymotrypsinogen numbering) as well as close to the cysteine residues. Overall the light chain showed a minimum of 29% homology with trypsin and 25% homology with chymotrypsin. This compared favourably with the homology between trypsin and chymotrypsin.

The presence of an aspartic acid residue at position 176 (189, chymotrypsinogen numbering) was consistent with the known 'trypsin-like' activity of Factor I for C4b (Press & Gagnon, 1981), the complete sequence of which has been reported by Belt *et al.* (1984).

The *N*-terminal sequence of the light chain determined in this study differed somewhat from that reported by Davis (1981), although the *N*-terminal sequence of the heavy chain (results not shown) was similar. We cannot offer an explanation at the present time. Despite this the data presented here indicate that Factor I is a serine proteinase of 'trypsin-like' specificity. The sequence,

although partial, will be useful for further work at the nucleic acid level.

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REFERENCES

- Arlaud, G. J., Gagnon, J. & Porter, R. R. (1982) *Biochem. J.* **201**, 49–59
- Belt, K. T., Carroll, M. C. & Porter, R. R. (1984) *Cell* (Cambridge, Mass.) **36**, 907–914
- Christie, D. L. & Gagnon, J. (1982) *Biochem. J.* **201**, 555–567
- Christie, D. L. & Gagnon, J. (1983) *Biochem. J.* **209**, 61–70
- Crossley, L. G. & Porter, R. R. (1980) *Biochem. J.* **191**, 173–182
- Davis, A. E. (1981) *FEBS Lett.* **134**, 147–150
- Fujita, T., Gigli, I. & Nussenzweig, V. (1978) *J. Exp. Med.* **148**, 1044–1051
- Gagnon, J. & Christie, D. L. (1983) *Biochem. J.* **209**, 51–60
- Harrison, R. A. & Lachmann, P. J. (1980) *Mol. Immunol.* **17**, 9–20
- Hsiung, L.-M., Barclay, A. N., Brandon, M. R., Sim, E. & Porter, R. R. (1982) *Biochem. J.* **203**, 293–298
- Koide, A., Titani, K., Ericsson, L. H., Kumar, S., Neurath, H. & Walsh, K. A. (1978) *Biochemistry* **17**, 5657–5672
- Laemmli, U. K. (1970) *Nature* (London) **227**, 680–685
- Nagasawa, S., Ichihara, C. & Stroud, R. M. (1980) *J. Immunol.* **125**, 578–582
- Neuberger, A., Gottschalk, A., Marshall, R. D. & Spiro, R. G. (1972) in *Glycoproteins* (Gottschalk, A., ed.), 2nd edn., vol. 5, part A, pp. 450–490, Elsevier, Amsterdam
- Pangburn, M. K., Schreiber, R. D. & Müller-Eberhard, H. J. (1977) *J. Exp. Med.* **146**, 257–270
- Press, E. M. & Gagnon, J. (1981) *Biochem. J.* **199**, 351–357
- Reid, K. B. M. & Porter, R. R. (1981) *Annu. Rev. Biochem.* **50**, 433–464
- Sim, E., Wood, A. B., Hsiung, L.-M. & Sim, R. B. (1981) *FEBS Lett.* **132**, 55–60
- Whaley, K. & Thompson, R. A. (1978) *Immunology* **35**, 1045–1049
- World Health Organisation (1981) *Bull. W.H.O.* **51**, 489–490
- Young, C. L., Barker, W. C., Tomaselli, C. M. & Dayhoff, M. O. (1978) in *Atlas of Protein Sequence and Structures* (Dayhoff, M. O., ed.), vol. 5, suppl. 3, pp. 73–93, National Biomedical Research Foundation, Washington