Purification and structural studies on the complement-system control protein $\beta_1\text{H}$ (Factor H)

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An efficient procedure for the isolation of the complement-system control protein $\beta_1\text{H}$ (Factor H) from human plasma was developed. The chemical composition and physical characteristics of the protein were studied, and a sequence of 17 amino acid residues at the *N*-terminus was determined. Factor H is a single-polypeptide-chain glycoprotein of mol.wt. 155 000 containing 9.3% carbohydrate. Factor H is cleaved by plasma proteinases to a two-chain form. This cleavage can be mimicked by trypsin, and the two-chain form retains fully the C3b-inactivator cofactor activity of Factor H. The proteolytic fragments of Factor H are compared with those of other proteins (C4b-binding protein and erythrocyte C3b-receptor) that act as cofactors for C3b-inactivator.

The plasma protein $\beta_1\text{H}$ was first described by Nilsson & Muller-Eberhard (1965) as a contaminant that co-purified with the third and fifth components of complement (C3 and C5). The name $\beta_1\text{H}$ defined the mobility of the protein in a standard agar immunoelectrophoresis system. The function of $\beta_1\text{H}$ as a control protein of the complement system was first recognized by Whaley and colleagues (Whaley & Ruddy, 1976*a,b*; Whaley *et al.*, 1978), who demonstrated that $\beta_1\text{H}$ has an important role in regulating the activity of the complement-system activation product, C3b.

C3b is formed, during activation of the complement system, by proteolytic cleavage of the major complement protein, C3. C3b has a number of biological functions. These include interaction with specific C3b-receptors on cell surfaces, and continuation of the cytolytic activity of complement by its participation in formation of C5 convertase enzymes or in formation of the alternative-pathway C3 convertase through interaction with Factor B, Factor D and Properdin (for reviews see Whaley *et al.*, 1978; Reid & Porter, 1981). $\beta_1\text{H}$ binds to C3b and decreases the interaction of Factor B with C3b

(Pangburn & Muller-Eberhard, 1978; Conrad *et al.*, 1978; Nagaki *et al.*, 1978; DiScipio, 1981*a*). This influence of $\beta_1\text{H}$ on the binding of Factor B, or its major proteolytic fragment, Bb, is an important factor in regulating the formation and decay of the C3 convertase enzyme (C3bBb), or C5 convertase enzyme (C3b₂Bb) of the alternative pathway (Whaley & Ruddy, 1976*b*; Weiler *et al.*, 1976; DiScipio, 1981*a,b*).

The complex formed between C3b and $\beta_1\text{H}$ also serves as a substrate for the serine proteinase C3b-inactivator (Factor I), which cleaves C3b at two positions (Harrison & Lachmann, 1980; E. Sim *et al.*, 1981). These proteolytic cleavage reactions destroy all of the activities of C3b, and thus $\beta_1\text{H}$ also serves as an essential cofactor for the proteolytic inactivation of C3b.

Two other apparently dissimilar proteins, namely C4b-binding protein (mol.wt. 450 000–550 000) (Nagasawa & Stroud, 1977; Fujita & Nussenzweig, 1979) and the C3b-receptor of human erythrocytes (mol.wt. 205 000) (Fearon, 1979; Iida & Nussenzweig, 1981) have been shown to possess regulatory properties similar to those of $\beta_1\text{H}$ (mol.wt. 155 000). Both of these other proteins serve as cofactors for C3b-inactivator-mediated proteolysis of soluble C3b, and both regulate the decay of the C3bBb enzyme. Comparison, at a more detailed level, of the structures of these three proteins may provide considerable information on a common mode of interaction with C3b. To initiate such a study, an improved purification procedure for $\beta_1\text{H}$ has been

Abbreviations used: $\alpha_2\text{M}$, α_2 -macroglobulin; the nomenclature of complement-system proteins is, where appropriate, in accordance with the recommendations of the World Health Organisation (1981).

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developed, and chemical and physical characterization of the protein has been performed.

In a recent revision of complement protein nomenclature (World Health Organisation, 1981), it was recommended that $\beta_1\text{H}$ be re-named 'Factor H', and this name is used in subsequent discussion.

Materials and methods

Materials

Outdated human plasma was obtained from the Regional Blood Transfusion Service, John Radcliffe Hospital, Oxford, U.K. Plasma from at least ten donors was pooled and stored at -20°C until required.

C3 was isolated from plasma as described previously (R. B. Sim *et al.*, 1981). C3b was prepared from C3 by treatment with a fluid-phase C3bBb convertase (R. B. Sim & E. Sim, 1981) or by trypsin treatment (E. Sim & R. B. Sim, 1981). C3b was radio-iodinated with the use of chloramine-T (R. B. Sim *et al.*, 1981) or iodogen (E. Sim & R. B. Sim, 1981) as oxidizing agent. C3b-inactivator (Factor I), isolated as described by Hsiung *et al.* (1982), was generously provided by Dr. Li-Min Hsiung.

Proteins used as standards for determination of molecular weights were purchased from Sigma (London) Chemical Co., Poole, Dorset, U.K. Immunoglobulin was isolated in this laboratory. Trypsin, treated with 1-chloro-4-phenyl-3-tosylamidobutan-2-one, was from the Worthington Division of Millipore, Harrow, Middx., U.K.

Materials for sodium dodecyl sulphate/polyacrylamide-gel electrophoresis were from BDH Chemicals, Poole, Dorset, U.K., and ion-exchange and gel-filtration media were from Pharmacia, Hounslow, Middx., U.K. Hydroxyapatite Ultrogel (HA-Ultrogel) was purchased from IBF Pharm-industrie, Villeneuve-la-Garenne, France. L-Lysine-Sepharose 4B was prepared as described by Deutsch & Mertz (1970). Di-isopropyl phosphorofluoridate was from CP Laboratories, Bishops Stortford, Herts., U.K., and poly(ethylene glycol) 4000 (PEG 4000) was from Sigma Chemical Co.

Monospecific sheep antisera to human Factor H and to C5 were kindly provided by Dr. A. R. Bradwell, Immunodiagnostic Research Laboratory, University of Birmingham, Birmingham, U.K. Monospecific rabbit antisera to human C3, C4b-binding protein, immunoglobulin M and Factor H were prepared in this laboratory, and monospecific rabbit antiserum to human $\alpha_2\text{M}$ was purchased from Dako, Copenhagen, Denmark.

Purification of Factor H

Factor H was isolated from pooled outdated human plasma by poly(ethylene glycol) precipi-

tation followed by five chromatography procedures. All steps were done at 4°C , and all contact with glass vessels was avoided with the end of the lysine-Sepharose chromatography step.

Poly(ethylene glycol) precipitation. A 1-litre volume of citrated plasma was adjusted to pH 7.4, and made 1 mM with respect to di-isopropyl phosphorofluoridate by addition of 0.4 ml of a 2.5 M solution of di-isopropyl phosphorofluoridate in anhydrous propan-2-ol.

A solution (250 ml) of 25% (w/v) poly(ethylene glycol) (PEG 4000) in 50 mM-sodium phosphate/150 mM-NaCl/15 mM-EDTA buffer, pH 7.4, was added over a period of 5 min to the plasma, with constant stirring. The mixture was stirred for 30 min, and the precipitate was removed by centrifugation at 8000 g for 30 min and discarded.

A suspension (190 ml) of 50% (w/v) poly(ethylene glycol) (PEG 4000) in the same buffer was then added slowly to the supernatant, and the mixture was again stirred for 30 min. The precipitate was harvested by centrifugation, as above, and the supernatant was discarded.

L-Lysine-Sepharose 4B chromatography. The precipitate was redissolved in 300 ml of 50 mM-sodium phosphate/150 mM-NaCl/15 mM-EDTA buffer, pH 7.4, and passed, at a flow rate of 5 ml/min, through a column (15 cm $\times$ 5 cm diam.) of L-lysine-Sepharose 4B equilibrated in the same buffer. The column was washed with 300 ml of the starting buffer, and the total eluate (600 ml) was pooled and dialysed against two 5-litre volumes of 25 mM-potassium phosphate/5 mM-EDTA buffer, pH 7.0. The lysine-Sepharose column was regenerated as described by Deutsch & Mertz (1970).

DEAE-Sephadex A-50 chromatography. The dialysed material from the previous stage was loaded on to a column (50 cm $\times$ 5 cm diam.) of DEAE-Sephadex A-50 equilibrated in 25 mM-potassium phosphate/5 mM-EDTA buffer, pH 7.0. The column was washed with 2 litres of starting buffer, and then Factor H was eluted by running a 4-litre linear gradient of 0–500 mM-NaCl. Fractions containing Factor H were pooled, and solid poly(ethylene glycol) (PEG 4000) was dissolved in this solution to give a final concentration of 14% (w/v). The material was stirred for 30 min, and the precipitate was collected by centrifugation at 8000 g for 30 min. The supernatant was discarded, and the pellet was redissolved, by use of a hand-operated homogenizer, in 20 ml of 100 mM-potassium phosphate/150 mM-NaCl/5 mM-EDTA buffer, pH 7.4.

Sepharose 6B chromatography. The redissolved protein was clarified by centrifugation at 15 000 g for 30 min, and fractionated on a column (90 cm $\times$ 5 cm diam.) of Sepharose 6B equilibrated in 100 mM-potassium phosphate/150 mM-NaCl/5 mM-EDTA buffer, pH 7.4. Fractions containing Factor H were

pooled and dialysed against two 5-litre volumes of 10 mM-potassium phosphate buffer, pH 6.8.

HA-Ultrogel chromatography. The dialysed material was loaded on to a column (20 cm $\times$ 1.5 cm diam.) of HA-Ultrogel equilibrated in the same buffer. The column was washed with 400 ml of the starting buffer, and then proteins were eluted by running a linear 500 ml gradient of phosphate to a final concentration of 200 mM-potassium phosphate buffer, pH 6.8. The average flow rate was 40 ml/h. Fractions containing Factor H were pooled and dialysed against 10 mM-Tris/25 mM-NaCl/0.5 mM-EDTA buffer, pH 8.4.

DEAE-Sephacrose CL-6B chromatography. Factor H was finally purified to homogeneity by applying it to a column (25 cm $\times$ 2 cm diam.) of DEAE-Sephacrose CL-6B in the Tris buffer noted above. The column was washed with 200 ml of starting buffer, and then developed with a 1.4-litre linear gradient of NaCl to a final concentration of 400 mM. Fractions containing Factor H were pooled, concentrated by ultrafiltration to about 1 mg of protein/ml and stored at -20°C .

Assay of Factor H

Factor H was detected in column eluates by single and double radial immunodiffusion against antiserum to Factor H, and by examination of band patterns on sodium dodecyl sulphate/polyacrylamide-gel electrophoretograms in comparison with known standards.

Factor H activity was determined by measuring its capacity to act as obligatory cofactor for proteolysis of C3b by C3b-inactivator (Factor I). The rate of conversion of soluble ^{125}I -labelled C3b into the cleaved product iC3b in the presence of Factor H and a fixed quantity of C3b-inactivator was determined as described previously (E. Sim *et al.*, 1981). The rate of C3b cleavage is proportional to the concentration of Factor H over a defined range.

Amino acid and carbohydrate compositions

The amino acid composition of Factor H was determined on a Durrum D500 analyser. Protein samples were hydrolysed under vacuum for 24 h, 48 h and 72 h at 110°C in constant-boiling HCl. Serine and threonine values were extrapolated to zero hydrolysis time. Cysteine was determined as cysteic acid after performic acid oxidation (Hirs, 1967) and as *S*-carboxymethylcysteine after acid hydrolysis of reduced and alkylated samples in the presence of 0.05% (v/v) 2-mercaptoethanol. Tryptophan was determined spectrophotometrically (Beaven & Holiday, 1952; Leggett-Bailey, 1962). Hexosamines, neutral sugars and sialic acid were determined by the methods of Allen & Neuberger (1975), Dubois *et al.* (1956) and Warren (1959) respectively.

Amino acid sequence analysis

Factor H was desalted by dialysis against aq. 50 mM- NH_3 , freeze-dried, then redissolved to a concentration of 2 mg/ml in 8 M-guanidinium chloride in 0.2 M-Tris/HCl/40 mM-dithiothreitol buffer, pH 8.0, and incubated at 37°C for 1 h. The sample was then made 90 mM with respect to sodium iodoacetate, pH 8.0, incubated at 37°C for 20 min and dialysed against aq. 50 mM- NH_3 . The *N*-terminal residue of Factor H was determined by the dansyl method (Gray, 1972). A 50 nmol sample of Factor H was subjected to automated Edman degradation in a Beckman 890C sequencer. Conditions for sequencing and for identification of residues were as described by Johnson *et al.* (1980).

Determination of molecular weight

The apparent molecular weight of Factor H was calculated from sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. The electrophoresis system of Laemmli (1970) with 7.5% polyacrylamide gels, or that of Kiesel *et al.* (1976) with 5% polyacrylamide gels, was used. Protein samples were prepared for electrophoresis, and the gels were stained with Coomassie Blue and destained, as described previously (Gigli *et al.*, 1976). Reduced and alkylated myosin, phosphorylase A, C3, catalase, ovalbumin and carboxypeptidase A were used as calibration standards.

The diffusion coefficient of Factor H was determined from gel filtration on a calibrated column (95 cm $\times$ 2.6 cm diam.) of Sephacryl S-300 (Ackers, 1975). The column was run in 10 mM-imidazole/HCl/350 mM-NaCl buffer, pH 7.3. Calibration standards were (with $D_{20,w}$ values in parentheses) immunoglobulin M ($1.7 \times 10^{-7} \text{ cm}^2/\text{s}$), apoferritin ($3.6 \times 10^{-7} \text{ cm}^2/\text{s}$), catalase ($4.2 \times 10^{-7} \text{ cm}^2/\text{s}$), bovine serum albumin ($5.9 \times 10^{-7} \text{ cm}^2/\text{s}$), ovalbumin ($7.6 \times 10^{-7} \text{ cm}^2/\text{s}$) and immunoglobulin G ($4.0 \times 10^{-7} \text{ cm}^2/\text{s}$).

The sedimentation coefficient of Factor H was determined by using a Beckman model E analytical ultracentrifuge equipped with a photoelectric scanner and electronic speed control. Factor H (0.4 mg/ml) in 10 mM-4-morpholine-ethanesulphonic acid/150 mM-NaCl buffer, adjusted to pH 7.3 by addition of Tris base, was centrifuged at 52 000 rev./min at 20°C in a 12 mm double-sector cell. Absorption optics were used to monitor the rate of sedimentation. The sedimentation coefficient was calculated and corrected to standard conditions ($s_{20,w}$) as described by Emes (1978).

The molecular weight of Factor H was calculated from $s_{20,w}$ and $D_{20,w}$ values by using the Svedberg equation (see Schachman, 1957). The partial specific volume of Factor H was calculated from the amino acid and carbohydrate compositions by the method of Cohn & Edsall (1943) and Gibbons (1966). The

molecular weight of Factor H was also determined by a meniscus-depletion sedimentation-equilibrium method (Yphantis, 1964). The equilibrium run was performed at 13000 rev./min and 20°C in 12 mm double-sector cells. The protein concentration was 300 µg/ml in the morpholine-ethanesulphonic acid buffer noted above.

The frictional ratio (f/f_0) of Factor H was calculated from the molecular weight, diffusion coefficient and partial specific volume, as described by Ackers (1975).

The absorption coefficient of Factor H was determined with the use of a Beckman model E analytical ultracentrifuge to determine protein concentration from synthetic-boundary experiments (Babul & Stellwagen, 1969). The absorbance at 280 nm was corrected for Rayleigh light-scattering as described by Shapiro & Waugh (1966).

Results and discussion

Isolation of Factor H

Factor H was purified as described in the Materials and methods section. The yield of protein at each step, and the principal contaminants of Factor H, are listed in Table 1. Initial fractionation of the plasma with poly(ethylene glycol) resulted in about 80% of Factor H antigen being concentrated in the second precipitate. Plasmin and plasminogen were then removed from this fraction by passage on lysine-Sepharose. Use of the serine proteinase inhibitor di-isopropyl phosphorofluoridate and avoidance of contact with glass was considered necessary during the early stages of the preparation in order to avoid proteolytic fragmentation of Factor H (as discussed below). These precautions were generally found to be unnecessary after completion of

lysine-Sepharose chromatography, provided that the rest of the purification was performed rapidly.

Fractionation on DEAE-Sephadex A-50 (Fig. 1) gave a high purification factor. Factor H is eluted as a distinct peak between the two major peaks in the gradient, which represent α_2 M and C3. The pool of Factor H made after this purification step contained about 55% of the original Factor H antigen, as some Factor H was deliberately discarded in order to limit contamination by C3 and α_2 M. Concentration of this pool by precipitation with 14% (w/v) poly(ethylene glycol) resulted in elimination of albumin from the sample, and decreased the contamination by α_2 M. Subsequent fractionation on Sepharose 6B (Fig. 2) completely removed the high-molecular-weight contaminants immunoglobulin M and C4b-binding protein, with partial removal of α_2 M, C3 species and C5 (Table 1). Some Factor H was again discarded, to minimize contamination with C3 and C5, and the recovery of Factor H antigen at this stage was 40–45% of the plasma content.

Chromatography on HA-Ultrogel (Fig. 3) was used to remove the remaining α_2 M, which is eluted in very pure form as a distinct peak preceding Factor H. Factor H, representing 30–35% of the plasma antigen, was separated from the remaining traces of C3 degradation products and C5 on DEAE-Sepharose CL-6B (Fig. 4). The final yield of Factor H was about 80 mg. This material was homogeneous when examined by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, with or without reduction of disulphide bridges (Fig. 5), and no evidence of impurities was found in the analytical ultracentrifugation procedures described in the Materials and methods section.

The average plasma concentration of Factor H has previously been estimated to be 120 µg/ml

Table 1. *Purification of Factor H*

For experimental details see the Materials and methods section. The total quantity of protein, the percentage yield of Factor H as assessed by single radial immunodiffusion, and the major contaminants present in the Factor H fraction at each stage are shown. Results shown are averages for four preparations.

Material	Total protein (mg)	Protein (% of total)	Yield of Factor H (%)	Major contaminants present
Plasma (1 litre)	70 000	100	100	—
Poly(ethylene glycol) precipitate	16 195	23.1	80	Many
Post lysine-Sepharose	15 070	21.5	80	Many
DEAE-Sephadex pool	890	1.3	55	α_2 M, immunoglobulin M, albumin, C4b-binding protein, C5, C3, C3 degradation products
Concentrate after DEAE-Sephadex	444	0.6	55	α_2 M, immunoglobulin M, C4b-binding protein, C5, C3, C3 degradation products
Sepharose-6B pool	144	0.2	40–45	α_2 M, C5, C3 degradation products
HA-Ultrogel pool	97	0.14	30–35	C5, degraded C3
DEAE-Sepharose CL-6B pool	80	0.11	25	None

Fig. 1. *Chromatography of Factor H on DEAE-Sephadex A-50*

Conditions were as described in the Materials and methods section. Fractions of volume 30 ml were collected. —, A_{280} ; ----, NaCl concentration in the gradient. The start of gradient elution is marked by a vertical arrow. The fractions taken for further purification of Factor H are indicated by a horizontal bar. Elution positions of other proteins of interest are marked by arrowed horizontal lines. Abbreviations: IgM, immunoglobulin M; C4bp, C4b-binding protein; Alb, albumin.

Fig. 2. *Sepharose-6B chromatography of Factor H*
Conditions were as described in the Materials and methods section. Fractions of volume 12 ml were collected. The fractions pooled for further purification of Factor H are indicated by a horizontal bar.

Fig. 3. *HA-Ultrogel chromatography of Factor H*
Conditions were as described in the Materials and methods section. Fractions of volume 8 ml were collected. —, A_{280} ; ----, phosphate concentration in the gradient. The start of gradient elution is marked by a vertical arrow. Fractions pooled for further purification of Factor H are indicated by a horizontal bar.

Fig. 4. *Chromatography of Factor H on DEAE-Sephacel CL-6B*

Conditions were as described in the Materials and methods section. Fractions of volume 15 ml were collected. —, A_{280} ; ----, NaCl concentration in the gradient. The start of gradient elution is indicated by a vertical arrow. The fractions retained as pure Factor H are shown by a horizontal bar.

(Adinolfi *et al.*, 1981), 300 $\mu\text{g}/\text{ml}$ (Whaley *et al.*, 1978), 450–600 $\mu\text{g}/\text{ml}$ (Weiler *et al.*, 1976) and 620–790 $\mu\text{g}/\text{ml}$ (Charlesworth *et al.*, 1979). Our results are in agreement with those of Whaley and colleagues, and the yield from this preparative method is therefore about 25%. Preparation methods published by Whaley & Ruddy (1976a,b) and Nagaki *et al.* (1978) also yielded very pure Factor H, but with low recovery (2–3% and 5% respectively). Weiler *et al.* (1976) and Harrison & Lachmann (1979) have developed higher-yield methods for Factor H purification (10% and 37%

Fig. 5. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of α_2 M and Factor H. Isolated Factor H (tracks 1 and 2), α_2 M taken from the first peak on HA-Ultrogel (see Fig. 3) (tracks 3 and 4) and Factor H isolated from plasma without use of proteinase inhibitors or plasminogen depletion (tracks 5 and 6) were electrophoresed on a 7.5% Laemmli-system slab gel, as described in the Materials and methods section. Tracks 1, 3 and 5 show unreduced protein; tracks 2, 4 and 6 show reduced and alkylated material. Track 7 is a reduced protein standard containing myosin (mol.wt. 205 000) and C3b (mol.wt.: α' -chain 108 000; β -chain 70 000).

Table 2. Physical characteristics of Factor H

The characteristics of Factor H were investigated as described in the Materials and methods section.

Molecular weight	
On sodium dodecyl sulphate/polyacrylamide-gel electrophoresis	160 000–170 000
Based on $s_{20,w}$ and $D_{20,w}$	154 500
By sedimentation equilibrium	156 500
Sedimentation coefficient, $s_{20,w}$	5.5 S
Diffusion coefficient, $D_{20,w}$	$3.0 \times 10^{-7} \text{ cm}^2/\text{s}$
Partial specific volume, $\bar{v}$	0.711 ml/g
Absorption coefficient at 280 nm ($A_{1\text{ cm}}^{1\%}$)	14.2
Frictional ratio, f/f_0	2.11

respectively), but the final products are not homogeneous.

α_2 M was a persistent contaminant of Factor H preparations, and the HA-Ultrogel chromatography step was developed specifically to remove this contaminant. The extent of contamination with α_2 M was not initially evident, since, after reduction of disulphide bridges and alkylation, α_2 M co-runs exactly with Factor H on both systems of sodium dodecyl sulphate/polyacrylamide-gel electrophoresis used in the present study (Fig. 5).

Chemical and physical characterization of Factor H

The physical characteristics of Factor H are shown in Table 2. Molecular weights found by sedimentation equilibrium and by calculation from $s_{20,w}$ and $D_{20,w}$ are in close agreement, and are comparable with the value of 150 000 found by Whaley & Ruddy (1976b). Factor H has a very high frictional ratio (Table 2), and is therefore likely to have an elongated shape.

The apparent molecular weight of reduced Factor

H as determined by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was 160 000 (system of Kisiel *et al.*, 1976) to 170 000 (system of Laemmli, 1970). Since Factor H is a glycoprotein (Table 3), this high apparent molecular weight on

Table 3. Amino acid and carbohydrate compositions of Factor H

The chemical composition of Factor H was determined as described in the Materials and methods section. The amino acid composition is compared with the mean of two analyses published by Gardner *et al.* (1980). The carbohydrate composition is shown as a percentage of the total glycoprotein weight. For this calculation, hexosamine was calculated as *N*-acetylhexosamine and sialic acid as *N*-acetylneuraminic acid. N.D., Not determined. The number of amino acid residues/molecule was calculated from the amino acid composition established in the present study on the basis of a total molecular weight of 155 500 for Factor H, of which 141 000 is protein and 14 500 is carbohydrate.

Amino acid	Residues/100 residues		Residues/ molecule
	Present study	Gardner <i>et al.</i> (1980)	
Asp	8.54	9.37	109
Thr	6.66	6.25	85
Ser	9.63	6.48	123
Glu	11.09	11.47	141
Pro	8.79	9.10	112
Gly	8.64	8.59	110
Ala	4.71	3.25	60
Cys	3.93	5.13	50
Val	5.85	4.86	75
Met	1.33	1.27	17
Ile	4.31	5.08	55
Leu	5.66	4.92	72
Tyr	4.55	5.52	58
Phe	2.43	2.33	31
His	2.12	2.25	27
Lys	5.64	6.60	72
Arg	3.30	4.71	42
Trp	2.82	N.D.	36
	100.00	97.18	1275
Carbohydrate	% of total glycoprotein weight		Residues/ molecule
Hexose	3.8		36
Hexosamine	2.5		19
Sialic acid	3.2		17

sodium dodecyl sulphate/polyacrylamide-gel electrophoresis is not unexpected. As shown in Fig. 5, Factor H migrates on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis as a single polypeptide chain, both with and without reduction of disulphide bridges.

The absorption coefficient of Factor H at 280 nm ($A_{1\%}^{1\text{cm}}$) was found to be 14.2, which is consistent both with a value of 13.1 provided by Whaley *et al.* (1978) and with the relatively high tyrosine and tryptophan content of the protein (Table 3). These observations suggest strongly that the value of 9.04 for the absorption coefficient reported by Nagaki *et al.* (1978) is an underestimate.

The carbohydrate and amino acid compositions of Factor H are shown in Table 3. Factor H contains 9.3% carbohydrate. The amino acid analysis is in general agreement with the results obtained by Gardner *et al.* (1980), although there are differences in the estimates of serine, alanine, cysteine and the basic amino acids. The content of cysteine found in the present study, although lower than the value determined by Gardner *et al.* (1980), was reproducible and consistent, when measured either as *S*-carboxymethylcysteine or as cysteic acid. On the basis of the chemical composition and the molecular weight found by analytical ultracentrifugation (Table 2), the Factor H polypeptide chain was estimated to contain about 1275 amino acids (Table 3).

N-Terminal amino acid sequence

A single *N*-terminal amino acid, glutamic acid/ glutamine, was found for Factor H by the dansyl technique. An unique 17-residue *N*-terminal sequence (Fig. 6) was obtained by automated Edman degradation. No relationship between this sequence and other known protein sequences has so far been noted. The sequence does not resemble the *N*-terminal sequence of the complement-system control protein C4b-binding protein (Dahlbäck & Stenflo, 1981; Reid & Gagnon, 1982).

Fragmentation of Factor H

If Factor H is purified without addition of proteinase inhibitors at any stage, or if crude preparations are stored for long periods at 4°C, a proportion of the Factor H undergoes proteolytic

Fig. 6. *N*-Terminal amino acid sequence of Factor H

The *N*-terminal amino acid sequence of Factor H was determined as described in the Materials and methods section. The yield at the first step was 40%. The repetitive yield was 87%, on the basis of recoveries of residues at steps 1, 5 and 13.

cleavage. This cleaved form of Factor H is indistinguishable from intact Factor H when subjected to sodium dodecyl sulphate/polyacrylamide-gel electrophoresis without prior reduction (Fig. 5). However, on reduction, it is seen to consist of two polypeptide chains with apparent mol.wts. 141 000 and 34 000 (Fig. 5).

Preparations of Factor H containing various proportions of the fragmented form all have the same specific activity as cofactors for degradation of ^{125}I -labelled C3b by C3b-inactivator, and so cleavage of Factor H does not affect this activity. Similar proteolysed forms of Factor H have been observed by others (Harrison & Lachmann, 1979; Charlesworth *et al.*, 1979; Gardner *et al.*, 1980). Charlesworth *et al.* (1979) also observed that a cleaved form of Factor H was active in several assay systems.

We have also investigated the sensitivity of undenatured Factor H to trypsin digestion, as a means of producing defined fragments suitable for further structural studies. 'Native' Factor H was found to be resistant to trypsin digestion, but at high trypsin concentrations cleavage into two disulphide-linked fragments of apparent mol.wts. 34 000 and 141 000 was observed. This product was indistinguishable, on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, from the cleaved form isolated from plasma, and it had the same specific cofactor activity as intact Factor H.

On prolonged trypsin digestion, the fragment of apparent mol.wt. 141 000 from cleaved Factor H was slowly degraded to a fragment of apparent mol.wt. 88 000 with simultaneous formation of two fragments of apparent mol.wt. 24 000–26 000. All four fragments remain disulphide-linked together, and, even when degradation of Factor H to the stable four-chain form is complete, there is no alteration in the C3b-activator cofactor activity in the assay system used in the present work. The breakdown pattern of Factor H is shown in Fig. 7.

Bitter-Suermann *et al.* (1981) have shown that guinea-pig Factor H also undergoes cleavage, by serum enzymes, to a two-chain form. This cleavage pattern could be mimicked by trypsin, and, as is the case for the human protein, proteolysis did not cause loss of the C3b-inactivator cofactor activity of Factor H.

Proteolytic cleavage of Factor H without loss of cofactor activity is a feature in which it resembles both of the other proteins known to act as cofactors for C3b-inactivator (Factor I). The 75 000-mol.wt. monomer polypeptide chain of C4b-binding protein has been shown (Reid & Gagnon, 1982) to be cleaved by trypsin into two major disulphide-linked fragments of apparent mol.wts. 37 500 and 36 000. This occurs without loss of activity as cofactor for the breakdown of soluble C4b by C3b-inactivator. The 205 000-mol.wt. single polypeptide chain of

Fig. 7. Pattern of breakdown of Factor H by trypsin. Undenatured Factor H, at a concentration of $200\mu\text{g}/\text{ml}$ in 10mM -sodium phosphate/ 140mM -NaCl buffer, pH 7.4, was digested at 37°C with trypsin at an enzyme/substrate molar ratio of (a) 1:10 or (b) 1:1. Factor H was initially cleaved into a two-chain form, and then into a stable four-chain form, as discussed in the text. The times for 50% completion of cleavage reactions (t_1) for conditions (a) and (b) are shown. Disulphide bridges are represented by thin lines. The order of fragments and position of disulphide bridges is arbitrary. Estimates of apparent molecular weight, as determined by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, are shown.

human erythrocyte C3b-receptor can also be cleaved to an active form with two disulphide-linked chains of apparent mol.wts. about 35 000 and 170 000 (R. B. Sim, unpublished work).

The features of proteolysis without loss of activity, and without dissociation of non-covalently linked fragments, together with the formation in each case of a major fragment of apparent mol.wt. 35 000, may indicate regions of structural similarity among the three proteins.

The preparative method described in the present paper permits, for the first time, isolation of large quantities of Factor H suitable for structural studies. Further comparison of this unusual elongated protein with C4b-binding protein and C3b-receptor can now be undertaken.

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