Isolation of two molecular populations of human complement factor H by hydrophobic affinity chromatography

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(Received 12 December 1983/Accepted 21 March 1984)

Human complement factor H was prepared in highly purified form from fresh serum by euglobulin precipitation, DEAE-Sephacel chromatography and Sephacryl S-300 gel filtration. This preparation allowed the recovery of 37% of the initial factor H. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis revealed that factor H was homogenous both in reduced and non-reduced media and exhibited a molecular mass of 150 kDa. Charge-shift experiments clearly showed the presence of hydrophobic sites in the factor H molecule. Charge shifts were observed with two detergent systems (Triton/sodium deoxycholate and Triton/cetyltrimethylammonium bromide). Factor H was able to bind to phenyl-Sepharose. This property allowed us to study two populations of factor H. These two populations exhibited the same physicochemical parameters, but revealed differences in their ability to aggregate in low- and iso-ionic-strength media. The molecular basis and biological significance of this heterogeneity are discussed.

Human complement component factor H was first described by Nilsson & Muller-Eberhard (1965) and called ' β_1 H-globulin'. The function of this protein in the alternative complement pathway was demonstrated later by Whaley & Ruddy (1976a,b) and Weiler *et al.* (1976). Factor H is necessary to the action of complement component factor I in inactivating C3b (Pangburn *et al.*, 1977) and controls the amplification phase of the alternative pathway by increasing the decay of the C3 convertase C3bBb (Weiler *et al.*, 1976). Factor H blocks the access of factor B and of C5 to C3b (Pangburn & Muller-Eberhard, 1978; Nagaki *et al.*, 1978; Isenman *et al.*, 1980; DiScipio, 1981).

The concentration of factor H is an important parameter for the regulation of the alternative pathway. Low concentrations induce rapid consumption of C3 and factor B, whereas high concentrations totally inhibit the activation of the

alternative pathway (Pangburn *et al.*, 1977; Fearon & Austen, 1977). These observations, made *in vitro* with purified components, were confirmed by the description of a clinical case of factor H deficiency; the patient presented a complete consumption of C3 in his serum (Thompson & Winterborn, 1981).

Factor H may also play an important role in the recognition of activator and non-activator surfaces. It was postulated that the C3b molecule possesses a discriminatory site that is capable of distinguishing between activators and non-activators. Two hypotheses were proposed to explain how bound C3b performed its discriminatory function: the ' β_1 H antagonist' hypothesis (Pangburn & Muller-Eberhard, 1978) and the ' β_1 H protagonist' hypothesis (Kazatchkine *et al.*, 1979), but no discriminatory role of factor H could be ruled out.

Another important biological function was more recently discovered by Lambris *et al.* (1980). Factor H was able to trigger B-lymphocytes through a specific receptor and to induce the release of lymphocyte endogenous factor I. In order to analyse the interactions between factor H and C3b, we looked at the role of hydrophobic forces between the two proteins, since we demonstrated previously the hydrophobic character of C3b (Fontaine *et al.*, 1982). In the present paper we

Abbreviations used: ϵ -Ahx, 6-aminoheptanoic acid; Ig, immunoglobulin; SDS, sodium dodecyl sulphate; VBS⁺⁺, iso-osmotic veronal-buffered saline, containing 0.15 mM-Ca²⁺ and 0.5 mM-Mg²⁺; DGVBS⁺⁺, VBS⁺⁺ containing 5% (w/v) dextrose and 0.1% gelatin. The nomenclature of complement components is that recommended by the W.H.O. Committee (1981).

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demonstrate the hydrophobic properties of factor H. Furthermore, a fast and improved method of purification of factor H is described that has, in comparison with previous purification procedures (Whaley & Ruddy, 1976b; Weiler *et al.*, 1976; Nagaki *et al.*, 1978; Harrison & Lachmann, 1979; Sim & DiScipio, 1982), the advantages of a very high yield (37%). The hydrophobic properties of factor H were assessed by charge-shift experiments and phenyl-Sepharose chromatography. Moreover, we found that factor H could be separated into two populations by using phenyl-Sepharose chromatography. These two populations were quite different in their ability to aggregate, particularly in low-ionic-strength media.

Materials and methods

Sera

Blood collected (with informed consent) from donors (Centre Régional de Transfusion Sanguine, Bois-Guillaume, France) was allowed to clot at room temperature for 4 h and centrifuged at 2500 rev./min (1000g) at 4°C. The serum was mixed with 0.4 M-Na₂EDTA, pH 7.2, and 1 M-ε-Ahx, pH 7.2, to final concentrations of 0.02 M and 0.05 M respectively. Sera were used immediately or stored at -80°C (1 month maximum).

Immunochemical techniques

Immuno-electrophoreses and immunodiffusions were carried out in 1.3% (w/v) 'Agar Noble' (Difco) buffered with 0.05 M-sodium barbital, pH 8.6 (Scheidegger, 1955; Ouchterlony, 1958). Crossed immuno-electrophoresis (Laurell, 1966) were performed in 1% agarose A-37 (I.B.F., Villeneuve-la-Garenne, France) in 0.072 M-Tris/0.024 M-diethylbarbituric acid/0.4 mM-calcium lactate/0.2 mM-Na₂N₃, pH 8.6. Charge-shift electrophoreses were performed by the method of Helenius & Simons (1977). Immuno-electrophoresis was performed in 1% agarose A-37 in 0.05 M-sodium diethylbarbitate/HCl, pH 8.6, containing 0.5% Triton X-100 (Touzart et Matignon, Paris, France), 0.5% Triton X-100/0.25% sodium deoxycholate (Serva, Tebu, Le Perray, France) or 0.5% Triton X-100/0.05% cetyltrimethylammonium bromide (Serva). Protein samples were incubated overnight at 4°C in electrophoretic buffer containing 4-fold the gel concentrations of detergent.

Antisera

Anti-(whole human serum) antiserum was from Organon Teknika (Serifontaine, France), anti-C5 was from Meloy (Eurobio Laboratories, Paris, France), anti-factor I was from Kent (also Eurobio

Laboratories, Paris, France), and anti-μ, anti-α and anti-γ were from the Centre Régional de Transfusion Sanguine (Bois-Guillaume, France). Anti-C4bp was generously given by Dr. C. L. Villiers and Dr. M. G. Colomb (Grenoble, France). Anti-factor H and anti-C3c were prepared in our laboratory by injection of purified antigens into rabbits. Our anti-factor H immune serum showed a complete identity with the anti-factor H immune serum kindly provided by Dr. M. D. Kazatchkine.

Assays of proteins

Purified C3 was obtained as described by Al Salihi *et al.* (1982b). C3 and C5 were quantified by haemolytic assays (Fontaine *et al.*, 1980; Al Salihi *et al.*, 1982a). Human factor H was detected and quantified during chromatographic steps by crossed immuno-electrophoresis using our anti-factor H immune serum. The plates were calibrated with purified factor H whose concentration was determined by colorimetric determination using Coomassie Brilliant Blue (Bio-Rad Laboratories; from Touzart et Matignon). Immunoglobulins (IgG, IgA, IgM), C4bp and factor I, were semi-quantitatively estimated by immunodiffusion of dilutions of each fraction against their respective antisera. The presence of trace amounts of factor I was checked by inhibition of immune adherence of EA C1423 cells with blood-group-O, rhesus-positive human red cells as described by Lachmann *et al.* (1973).

SDS/Polyacrylamide-gel electrophoresis

Samples were treated with 1% SDS/4 M-urea, for 5 mn at 90°C before electrophoresis. In some cases, samples were reduced with 5% (v/v) β-mercaptoethanol. Calibration proteins were purchased from Pharmacia, Bois-d'Arcy, France: hog thyroglobulin (molecular mass 330 kDa), albumin (67 kDa), catalase (60 kDa) and lactate dehydrogenase (36 kDa). Purified C3 was included in each electrophoretic run. Each step of purification was monitored by SDS/polyacrylamide-gel electrophoresis, the system of Laemmli (1970) being used, with 10% (w/v) polyacrylamide resolving gel. *M_r* determinations were done in polyacrylamide-gel slabs (4–30%, w/v, acrylamide) (Pharmacia) according to the manufacturer's instructions.

Chromatographic media

DEAE-Sephacel, Sephacryl S-300 and phenyl-Sepharose were obtained from Pharmacia. The column of Sephacryl S-300 was calibrated with purified IgM and IgG (prepared in our laboratory) and human albumin (Behring Institute, Paris, France).

Cellular intermediates

Sensitized sheep red blood cells coated with complement components C1, C4, C2 and C3 (EA C1423b) were prepared in a two-step procedure using an R3 reagent (human serum depleted of C3 by immunoadsorption) and highly purified C3 as described by Fontaine *et al.* (1983). EA C1423b intermediates were washed three times in cold DGVBS⁺⁺, then stored at +4°C. EA C1423bH intermediates were prepared as follows: EA C1423b cells were suspended in DGVBS⁺⁺ at a concentration of 10^8 cells/ml; 0.5 ml samples of EA C1423b suspension were incubated 20 min at 37°C with different amounts (1, 5, 10, 25, 50, 100 µg) of factor H. EA C1423bH intermediates were washed three times with DGVBS⁺⁺ and then immediately used for agglutination assays.

Agglutination experiments

EA C1423bH cells prepared as described above were resuspended in the starting volume either in DGVBS⁺⁺ (half-iso-osmotic) or in VBS⁺⁺ (iso-osmotic). Then 100 µl of each sample was distributed on to a microtitration plate (C.M.L., Neumours, France). Plates were examined after 12 h at +4°C.

Radioiodination procedures

Factor H was radioiodinated with ^{125}I by using the Bolton & Hunter (1973) reagent. Specific radioactivities were 100 000 c.p.m./µg for the two populations of ^{125}I -labelled factor H.

Radioactivity binding assays

For assays of factor H binding to cell-bound C3b, samples of EA C1423b cells were incubated 20 min at 37°C with various amounts of ^{125}I -labelled factor H (0, 5, 10, 25, 50 and 100 µg). The cells were washed three times with DGVBS⁺⁺, then the pellet was counted for radioactivity in a γ -radiation counter (NE 1600; Nuclear Enterprises).

Amino acid analysis and sequence determination

Before sequence determination, factor H (10 mg/ml) was reduced with 0.05 M-dithiothreitol in 0.35 M-Tris/HCl/8 M-urea buffer, pH 8.3, and alkylated with iodoacetic acid (final concn. 0.11 M). The reduced alkylated protein was dialysed against 1 M-formic acid and freeze-dried. The *N*-terminal amino acid sequence of factor H (15 mg) was determined by automatic Edman degradation in a Beckman 890C protein sequencer with 1 M-Quadrol buffer program. Phenylthiohydantoin derivatives were identified by high-pressure liquid chromatography on C18 µBondapak (Waters Associates) (Hermann *et al.*, 1978) and by g.l.c. (Pisano & Bronzert, 1969).

Results

Purification of factor H

Euglobulin precipitation. Serum, treated as described in the Materials and methods section, was dialysed against 20 vol. of 5 mM-EDTA/2 mM- ϵ Ahx, pH 5.3, overnight at 4°C. The final pH of the dialysed serum was 6.1. After centrifugation at 11 000 *g* (8000 rev./min) for 30 min at 4°C, the precipitate was washed twice with the cold dialysis buffer and redissolved in one-tenth of the starting volume of 0.15 M-NaCl/0.05 M- ϵ Ahx/0.02 M-EDTA, pH 7.2. A second euglobulin precipitation was obtained from the supernatant by a second dialysis (final pH 5.4). The precipitate was treated as described above.

DEAE-Sephacel ion-exchange chromatography. The euglobulin fraction was diluted with distilled water to lower the conductivity to 2 mS/cm² (20°C). The diluted fraction was loaded on to a DEAE-Sephacel column (90 cm × 2.6 cm) equilibrated with 5 mM-Tris/5 mM-EDTA/0.01 M- ϵ Ahx, pH 7.2. Elution of protein was monitored by measuring A_{280} , and 10 ml fractions were collected. The washing of the column with the equilibrating buffer was maintained until A_{280} was zero. Then a NaCl gradient (0–0.25 M) presenting two different linear steps (Fig. 1) (Gilson gradient former) was applied at a flow rate of 80 ml/h during 24 h. C3 and C5 were detected by haemolytic assays and factor H by rocket immunoelectrophoresis (Fig. 1). Factor H was eluted at a conductivity of 5 mS/cm² (20°C). The fractions rich in factor H were pooled and concentrated by ultrafiltration on a PM 30 membrane (Amicon).

Gel filtration. The fraction obtained after the ion-exchange chromatography appeared little contaminated (Fig. 3 below). The major contami-

Fig. 1. Elution profile of the DEAE-Sephacel chromatography

—, A_{280} ; ----, [NaCl]; ■, factor H activity determined by rocket immunoelectrophoresis; ▨, C5 haemolytic activity; ▩, C3 haemolytic activity.

nants were immunoglobulins and C4bp. These contaminants were easily removed by gel filtration on a column (90cm $\times$ 2.6cm) of Sephacryl S-300 equilibrated with 0.15M-NaCl/0.02M-EDTA/0.05M- ϵ Ahx, pH 7.2; fractions (2ml) were collected. Elution of factor H was monitored by rocket immunoelectrophoresis. C4bp and polymeric immunoglobulins appeared near the void volume of the column. Factor H appeared in the major peak of the chromatogram (Fig. 2), exhibiting a molecular mass of 300 kDa. Monomeric immunoglobulins were eluted later. The factor H-positive fractions were pooled and concentrated by ultrafiltration on a PM 30 membrane. The fraction represented 37% of the initial serum factor H (Table 1).

Quality control of factor H preparations

The fraction obtained from gel filtration was checked to determine the presence of contaminating complement components and other plasma proteins. This preparation exhibited a single precipitin line against anti-(whole human serum) anti-serum identical with that obtained with monospecific anti-(factor H) immune serum. Haemolytic assays and double immunodiffusion failed to detect C3, C5 and inactivated fragments.

Fig. 2. Chromatography of factor H on Sephacryl S-300 —, A_{280} ; —, factor H activity; protein markers [IgM, IgG, albumin(Alb.)] are indicated by arrows.

We never found any C4bp or factor I. This preparation did not inhibit the immune adherence phenomenon. Only traces of IgA could be observed.

Fig. 3 shows SDS/polyacrylamide-gel electrophoretic analysis of the successive steps of purification. We used the Laemmli's (1970) system, and samples were reduced before electrophoresis. Electrophoresis conditions were chosen to reveal high- M_r proteins. Track 1 represents the euglobulin precipitate, track 2 the factor H fraction from DEAE-Sephacel, and track 3 the factor H fraction obtained after Sephacryl S-300 gel filtration. Track 4 was obtained with purified C3 and track 5 with M_r markers. This electrophoresis indicated the considerable enrichment in factor H after DEAE-Sephacel chromatography.

The M_r of factor H was estimated by SDS/4–30%(w/v)-polyacrylamide-gradient gel electrophoresis (Fig. 4).

Fig. 3. SDS/polyacrylamide-gel electrophoresis monitoring of the purification of factor H

Samples were reduced before electrophoresis. Track 1, euglobulin precipitate; 2, DEAE-Sephacel fraction; 3, factor H after Sephacryl S-300 gel filtration; 4, purified C3; 5, phosphorylase (top band) and albumin (bottom band).

Table 1. Purification of human factor H

The concentration of factor H was determined by crossed immunoelectrophoresis using monospecific anti-(factor-H) antibodies

Step	Volume (ml)	[Factor H] (mg/ml)	Total factor H (mg)	Yield (%)
Serum	500	0.45	225	100
Euglobulin precipitate	100	1.38	138	61.3
DEAE-Sephacel eluate	100	0.90	90	40
Sephacryl S-300 gel-filtration eluate	80	1.03	83	36.8

Fig. 4. Determination of factor H molecular mass by SDS/4–30% (w/v)-polyacrylamide-gradient-gel electrophoresis

(a) Non-reduced samples: track 1, purified C3; track 2, purified factor H; (b) reduced samples: track 1, purified factor H; track 2, C3; track 3, thyroglobulin (T), albumin (A) and catalase (C). In the graph, (c), electrophoretic mobility is expressed relative to the migration of the dye front.

In a reducing or in a non-reducing medium, factor H exhibited a single polypeptide chain that migrated like a 150 kDa species. The functional activity of this preparation was kindly checked by Dr. M. Kazatchkine and compared with a standard preparation of factor H. The two preparations exhibited the same specific activity indicating no loss of activity of our preparation during the purification process. Both preparations dissociated factor B from C3b–B complex with the same efficiency.

A monospecific anti-(factor H) immune serum was obtained by injection of purified factor H to rabbits. The immune serum was used to quantify factor H in normal human sera by crossed immunoelectrophoresis, with purified factor H as standard. An average value of $450 \pm 50 \mu\text{g/ml}$ was found for factor H concentration in normal sera.

Presence of hydrophobic sites in the factor H molecule

Fig. 5 shows experiments of charge-shift electrophoresis. Factor H treated with Triton X-100, Triton X-100/sodium deoxycholate and Triton X-100/cetyltrimethylammonium bromide was submitted to agarose electrophoresis in the corresponding medium. Triton X-100 did not modify the

Fig. 5. Charge-shift experiments performed on purified factor H

(a) Triton X-100 + sodium deoxycholate; (b) Triton X-100; (c) Triton X-100 + cetyltrimethylammonium bromide. Factor H was revealed by a monospecific anti-(factor H) immune serum.

Fig. 6. Chromatography of factor H on phenyl-Sepharose —, A_{280} ; —, factor H activity. The arrow indicates the start of elution with ethylene glycol.

electrophoretic mobility of factor H. Addition of sodium deoxycholate or cetyl-trimethylammonium bromide led to anodic and cathodic shifts respectively. This phenomenon argued for the presence of hydrophobic sites in the factor H molecule. Factor H exhibited more pronounced shifts than C3 (Fontaine *et al.*, 1982).

Microheterogeneity of factor H: evidence for two populations with different hydrophobic reactivities

Hydrophobicity of factor H was confirmed by affinity chromatography on phenyl-Sepharose. In these experiments, we used a fraction of factor H derived from DEAE-Sephacel eluates. This fraction contained some contaminants. Fig. 6 shows

the elution pattern (A_{280}) of proteins from phenyl-Sepharose and the specific elution of factor H monitored by crossed-immunoelectrophoresis. The experiment was performed at 20°C. Phenyl-Sepharose was equilibrated with 0.15M-NaCl/0.02M-EDTA/0.05M- ϵ Ahx, pH 7.2. The resin was washed with this buffer until the A_{280} was less than 0.1. Proteins that bound to the column were eluted with a 50% (v/v) ethylene glycol solution in distilled water. Factor H appeared heterogeneous on phenyl-Sepharose. A fraction with factor H antigenicity ($\phi 1$) appeared after a first peak of proteins without factor H antigenicity. These proteins were eluted in the void volume of the column, thus indicating that factor H was retarded on the column. A second fraction with factor H antigenicity ($\phi 2$) was eluted by ethylene glycol. Fractions $\phi 1$ and $\phi 2$ were dialysed and concentrated by ultrafiltration on a PM 30 membrane at 1–2 mg/ml.

Fraction $\phi 1$ in the experiment depicted in Fig. 6 contained 40% of the initial input of factor H, and fraction $\phi 2$, 60%. Fractions $\phi 1$ and $\phi 2$ had similar molecular parameters. SDS/polyacrylamide-gel electrophoresis failed to demonstrate proteolytic cleavage. The two factor H species appeared homogeneous (even under reducing conditions) and exhibited an M_r of 150000. When rechromatographed under the same conditions on phenyl-Sepharose, the two populations, $\phi 1$ and $\phi 2$, behave differently. $\phi 2$ bound to the column and was eluted by ethylene glycol only. $\phi 1$ could be further separated in two populations: a minor one (25%), which behaved like $\phi 2$, and a major one (75%), which was retarded on the column like $\phi 1$.

Different behaviour of $\phi 1$ and $\phi 2$ populations in low-ionic-strength media

In high salt concentrations or under physiological conditions, fractions $\phi 1$ and $\phi 2$ exhibited identical M_r values when they were submitted to gel filtration on Sephacryl S-300. They were eluted with an apparent molecular mass of 300 kDa. On the other hand, under very-low-ionic-strength conditions [50% (w/v) glycerol/1 mM-EDTA/0.5 mM- ϵ Ahx, pH 7.2], $\phi 2$ was eluted with an

apparent molecular mass of 1600 kDa, whereas $\phi 1$ was eluted with an apparent molecular mass of 700 kDa (Fig. 7). These experiments showed that the more hydrophobic factor H was, the more it could aggregate in low-ionic-strength buffer.

When performing agglutination experiments with cellular intermediates EA C1423bH ($\phi 1$), EA C143bH ($\phi 2$) and anti-(factor H) serum, we observed that higher concentrations of antibodies were necessary to agglutinate EA C1423bH ($\phi 1$) intermediates than to agglutinate EA C1423bH ($\phi 2$). Anti-(factor H) serum reacted identically with the two factor H populations, as revealed by a complete identity between anti-(factor H) and fractions $\phi 1$ and $\phi 2$ on Ouchterlony (1958) analysis. The difference of agglutinability observed lay in the fact that EA C1423bH ($\phi 2$) intermediates had a noticeably higher tendency to agglutinate than EA C1423bH ($\phi 1$), particularly under low-salt conditions. To illustrate this phenomenon, agglutination experiments were performed as described in the Materials and methods section by using either EA C1423bH ($\phi 1$) or EA C1423bH ($\phi 2$) intermediates. Results showed

Fig. 7. Elution profiles on fractions $\phi 1$ (○) and $\phi 2$ (●) in low-ionic-strength medium [50% (w/v) glycerol/1 mM-EDTA/5 mM- ϵ Ahx, pH 7.2]

Protein markers [aldolase, 158 kDa (A), catalase (tetrameric) 240 kDa (C) and ferritin 450 kDa (F)] are indicated.

Table 2. Agglutinability of EA C1423bH ($\phi 1$) and EA C1423bH ($\phi 2$) cells in VBS⁺⁺ and in DGVBS⁺⁺. EA C1423b cells were coated with increasing amounts of either factor H ($\phi 1$) or factor H ($\phi 2$). Agglutination was estimated semi-quantitatively. 0, No agglutination; + + + +, maximal agglutination. Agglutination of EA C1423bH ($\phi 1$) was zero either in VBS⁺⁺ or in DGVBS⁺⁺ whatever the H($\phi 1$) input.

	Level of agglutination						
	0	1	5	10	25	50	100
Factor H ($\phi 2$) input (μ g) ...	0	1	5	10	25	50	100
Agglutination of EA C1423b H ($\phi 2$)							
In VBS ⁺⁺	0	0	0	0	0	++	++
In DGVBS ⁺⁺	0	++	+++	++++	++++	++++	++++

Fig. 8. Analysis of the interaction of increasing amounts of ^{125}I -factor H ($\phi 1$) and ^{125}I -factor H ($\phi 2$) with EA C1423b cells

The amount of ^{125}I -factor H ($\phi 1$) and ^{125}I -factor H ($\phi 2$) bound is shown as a function of the amount of ^{125}I -factor H ($\phi 1$) (●) and ^{125}I -factor H ($\phi 2$) (○) added.

(Table 2) that, in a low-ionic-strength medium (DGVBS⁺⁺), EA C1423bH ($\phi 2$) underwent spontaneous agglutination for the lowest amount of $\phi 2$ used ($1\mu\text{g}/5 \times 10^7$ EA C1423b cells). For $10\mu\text{g}$ of $\phi 2/5 \times 10^7$ cells, agglutination was maximal. In physiological ionic-strength medium agglutination of EA C1423bH ($\phi 2$) intermediates was also observed, but only for a large amount of $\phi 2$ ($50\mu\text{g}$). No agglutination was observed with EA C1423bH ($\phi 1$) cells either in low- or in physiological-ionic-strength medium.

Ability of ^{125}I -labelled $\phi 1$ and $\phi 2$ populations to bind to cell-bound C3b

When ^{125}I -factor H ($\phi 1$) and ^{125}I -factor H ($\phi 2$) were used, no difference was found between the two factor H populations in their ability to bind to cell-bound C3b. When a constant number of EA C1423b cells (0.5×10^8) was incubated with increasing amounts of ^{125}I -factor H ($\phi 1$) and ^{125}I -factor H ($\phi 2$), curves through the data points were concave to the abscissa. For the two populations, at weak concentrations of factor H, the uptake of ^{125}I -labelled factor H grew proportionally with the input of factor H. This uptake then reached a plateau for input amounts of factor H ranging from 5 to $10\mu\text{g}$ (Fig. 8).

N-Terminal amino acid sequence

This determination was performed on a factor H preparation not fractionated on phenyl-Sepharose. A single N-terminal amino acid (glutamic acid) was found. We obtained a unique seven-residue sequence by automated Edman degradation. This sequence was identical with that obtained by Sim

& DiScipio (1982), who obtained a unique 17-residue N-terminal sequence. The following sequence was obtained:

Discussion

The present paper describes the presence of hydrophobic sites in complement factor H and the possibility of isolating two populations of factor H that showed (i) different reactivities against hydrophobic surfaces such as phenyl-Sepharose, (ii) different aggregation properties, particularly in low-ionic-strength medium.

Purification of factor H was performed with an improved method that had the following advantages; (i) fast procedure (three steps only), (ii) high yield (37%), (iii) high purity without loss of activity. Chromatography on DEAE-Sephacel appeared to be a crucial step and confirmed that this resin is a powerful tool for the purification of complement components (Hammer *et al.*, 1981). The presence of proteinase inhibitors at all stages of the purification was necessary, since factor H was susceptible to proteolysis. Proteolysed forms of factor H are often found in various preparations of this protein (Harrison & Lachmann, 1979; Hong *et al.*, 1982; Sim & DiScipio, 1982). A minor degradation product could be seen after Sephacryl S-300 gel filtration in our factor H preparation, but on SDS/polyacrylamide-gel electrophoresis under non-reducing conditions, no fragmentation was observed. Charge-shift experiments clearly demonstrated the amphiphilic character of factor H. The hydrophobicity of factor H was confirmed by affinity chromatography on phenyl-Sepharose. Factor H could bind to phenyl-Sepharose. More than 60% of the different preparations used were bound and could be eluted by ethylene glycol, the remainder being only retarded by the column. This allowed us to separate and to study these two populations: H ($\phi 2$), which bound, and H ($\phi 1$), which was only retarded. As we mentioned in the Results section, $\phi 2$ retained its capacity to bind to the phenyl-Sepharose, whereas $\phi 1$ could be further separated on this resin.

We did not find any structural differences between the two populations. Both populations were homogeneous on SDS/polyacrylamide-gel electrophoresis, each of them exhibiting a molecular mass of 150kDa. This ruled out the possibility of proteolysis. N-Terminal sequence analysis did not reveal any heterogeneity in this part of the protein.

The two factor H populations showed no difference in their ability to bind to cell-bound C3b, as was demonstrated by the similar uptake of

^{125}I -labelled $\phi 1$ and ^{125}I -labelled $\phi 2$ by EA C1423b cells.

Major differences between the two populations were found in their behaviour in low-ionic-strength medium. $\phi 2$ had a greater ability to aggregate in low-ionic-strength medium, as was demonstrated by gel-chromatographic experiments. Furthermore, the tendency of $\phi 2$ to aggregate was so strong that it was responsible for agglutination of EA C1423b intermediates. In physiological-ionic-strength medium (VBS $^{++}$), agglutination of EA C1423bH ($\phi 2$) intermediates was also observed with large amounts of $\phi 2$. No agglutination was observed for EA C1423b intermediates with $\phi 1$ in low- and physiological-ionic-strength media.

Self-aggregation of factor H was observed by different authors (Schmitt *et al.*, 1981; Lambris *et al.*, 1980), and an important function, such as release of endogenous factor I from B-lymphocytes via the binding of factor H to its receptor, was observed with preparations containing polymeric forms of factor H (Lambris *et al.*, 1980). To explain the paradox that C3b and H receptor seemed to have the same binding site on factor H and C3b-H complex could trigger factor H receptor, it was postulated that factor H could aggregate after interaction with bound C3b (Lambris & Ross, 1982). In this context it is noteworthy that $\phi 2$, when bound to C3b, could induce agglutination of EA C1423b intermediates even in physiological-ionic-strength medium, whereas it does not itself seem to undergo self-aggregation at such an ionic strength. Possibly $\phi 2$ could correspond to an activated form of factor H. Hydrophobic interactions might play an important role in the relationship between factor H and its physiological substrate C3b, since dramatic enhancement in surface C3 hydrophobicity has been observed on conversion of C3 into C3b (Isenman, 1983). In any case, such a capacity to agglutinate cells to which factor H is bound may have an important role in the bridging phenomenon between immuno-competent cells, since some of these cells possess factor H receptors (B-lymphocytes, phagocytic cells) (Lambris *et al.*, 1980; Schmitt *et al.*, 1981).

We thank Dr. C. L. Villiers and Dr. M. G. Colomb for generously providing the anti-C4bp, Dr. M. D. Kazatchkine, who determined the biological activity of our preparation, and Mrs. A. Chaube and Mrs. M. M. Fontaine for the preparation of the manuscript. This work was supported by the University of Rouen and INSERM (C.R.L. n° 82.10.40). A. A. S. received financial support from the French Foreign Office.

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