EVIDENCE THAT C3d IS AN AMPHIPHILIC PROTEIN. APPLICATION TO ITS PREPARATION BY HYDROPHOBIC AFFINITY CHROMATOGRAPHY

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1. Introduction

The third component of human complement (C3) is composed of two polypeptide chains, α (110 k daltons) and β (75 k daltons) [1–3]. When C3 is activated, a polypeptide (C3a) of 9 k daltons is removed from the N-terminal part of the α chain [4] and a labile binding site on the remaining molecule (C3b) appears [5] and can react with cell membranes [6,9] and with a wide variety of materials such as: zymosan [9], oil droplets coated with *Escherichia coli* lipopolysaccharide [10] and Sepharose [11]. Furthermore, this binding is extremely stable. This interaction might involve hydrophobic interactions [12]; an ester linkage is involved between the labile binding site of C3b and receptive surfaces [13]. The labile binding site was localized in a segment containing the C3d antigenicity.

Here, we investigated the hydrophobic properties of human C3 and we demonstrated that C3d was a true amphiphilic protein, easily prepared from aged sera by hydrophobic affinity chromatography.

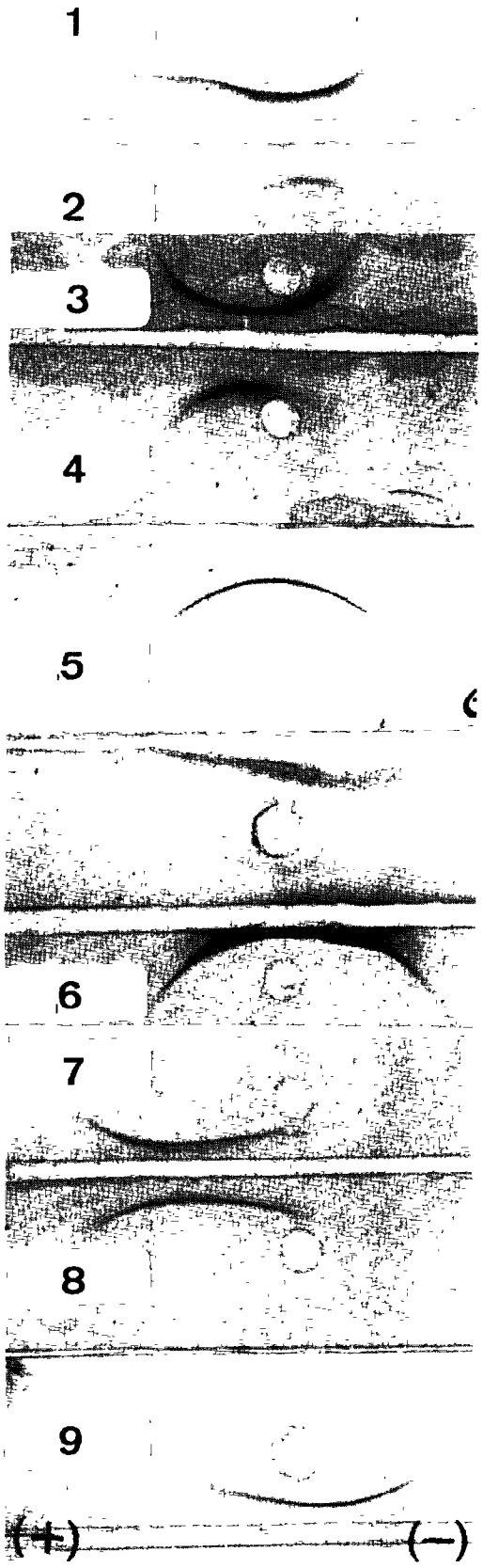
2. Materials and methods

Charge shift electrophoresis experiments were done as in [14]. Immuno-electrophoresis and immunodiffusion were carried out on 1.3% agar in 0.05 M barbital buffer (pH 8.2) by using anti-whole human serum, anti-C3d (Organon Teknika) and anti-C3c immunesera (prepared in our laboratory).

C3 and C3c were purified as in [15]. Protein concentrations were determined by radial immunodiffusion (Partigen plate anti-C3c, Behring Werke AG) and by a dye-binding assay using Coomassie brilliant blue G-250 (BioRad Labs. Richmond, CA). Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was done following [16]. Sera from healthy blood donors were incubated for 8 days at 37°C with 0.1% NaN_3 then, precipitated by 0.5% (w/v) Rivanol®. The precipitate was redissolved in a 0.5 M NaCl solution and intensively dialyzed against 10 mM sodium acetate buffer (pH 5.3) [17]. The pseudoglobulin fraction was dialyzed against 0.8 M ammonium sulphate, 0.15 M NaCl, 0.01 M sodium phosphate buffer (pH 7.2) then, loaded on the top of a phenyl–Sepharose (Pharmacia Fine Chemicals) column chromatography. Elution was developed by decreasing the ionic strength with 50% (v/v) ethylene glycol (EG) solution in distilled water. Gel filtration of C3d rich fractions were performed on AcA 44 (IBF) equilibrated with 10% (v/v) EG, 0.1 M Tris, HCl buffer (pH 8).

3. Results and discussion

As illustrated in fig.1, incubation with Triton X-100 did not change the electrophoretic mobilities of C3 and C3c. C3 presented a cathodic line (fig.1(1,2)) and C3c, which did not migrate on agar immuno-electrophoresis, exhibited its characteristic precipitin line under the well (fig.1(5)). On the contrary, adding sodium deoxycholate to the Triton–protein mixtures [14] induced large modifications of electrophoretic mobilities for C3 and C3d (fig.1(3,4,7,8)), but the mobility of C3c was unchanged (fig.1(6)). These results showed that C3 had hydrophobic sites that bound the non-ionic detergent Triton X-100. The analysis of C3 fragments (C3c, C3d) clearly indicated that those hydrophobic sites were located on the C3d part of the molecule. These findings are consistent



with [18] where the binding of C3b to Sepharose was enhanced by the presence of non-ionic detergents, suggesting the presence of hydrophobic sites near the labile binding sites of C3b.

The hydrophobic properties of C3d were used in the present study to devise an improved method for its purification from aged sera by hydrophobic affinity chromatography.

Serum was incubated for 8 days at 37°C with 0.1% (w/v) NaN_3. Under these conditions, C3 was completely converted into C3c and C3d fragments.

The pseudoglobulins (see section 2) were fractionated on a phenyl–Sepharose column chromatography. The ionic strength was increased with ammonium sulphate (0.8 M) to ensure a good fixation of proteins bearing hydrophobic sites. Elution was carried out both by decreasing the ionic strength and increasing the concentration of EG (fig.2). Immunodiffusion analysis showed that C3d eluted in fraction IV and V. A protein of 25 k daltons was detected by SDS–PAGE in fraction V only. Gel filtration of fraction V permitted us to purify this protein (fig.3) and to identify it as C3d by immunochemical criteria. In SDS–PAGE, its mobility was not affected by the presence of mercaptoethanol (fig.3). These findings confirmed that C3d consisted of a single polypeptide chain of 25 k daltons, as preliminary in [19]. C3d was isolated [2] by gel filtration of a trypsin digest of C3 and C3d was found made of two polypeptide chains. The chosen experimental procedure to purify C3d could account for these discrepancies. In [15], we showed that trypsin produced a high number of small polypeptides coming from C3c and from the polypeptide that joined C3d and C3c in native C3 and therefore, could contaminate C3d preparations.

The preparation described herein is reproducible and ensures a good yield (~25%). For example, we purified 3 mg C3d from 130 ml serum that contained 0.68 mg native C3/ml.

C3d is an important catabolic fragment of C3 and

Fig.1. Immuno-electrophoresis of C3 and of its fragments (C3c and C3d) in the presence of Triton X-100 and sodium deoxycholate (DOC): (1–6) anti-C3c in the trough; (7–9) anti-C3d in the trough; (1,2) purified C3, Triton X-100 alone; (3,4) purified C3, Triton X-100 + DOC; (5) purified C3c, Triton X-100 alone; (6) purified C3c, Triton X-100 + DOC; (7,8) pseudoglobulins, Triton X-100 + DOC; (9) pseudoglobulins, Triton X-100 alone.

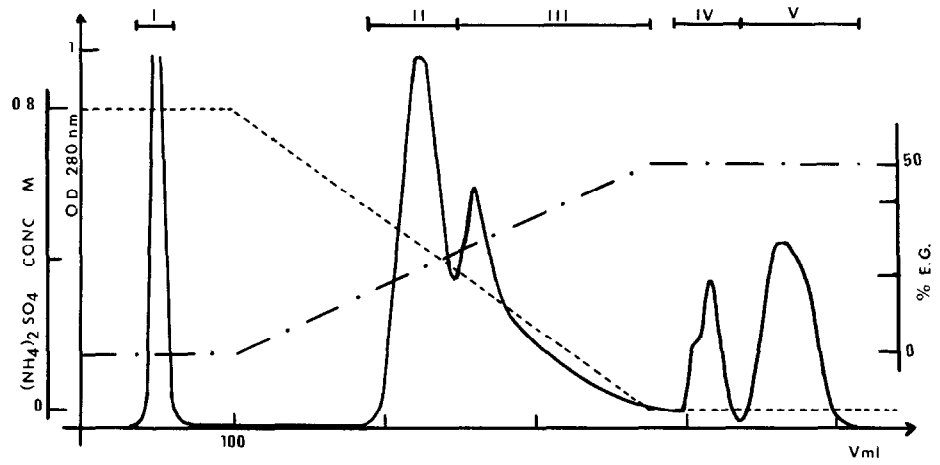


Fig.2. Phenyl–Sepharose affinity chromatography of the pseudoglobulin fraction. Elution was carried out both by decreasing the ionic strength (– – –) and increasing EG concentration (—— · ——). Fractions were pooled as indicated by the solid bars.

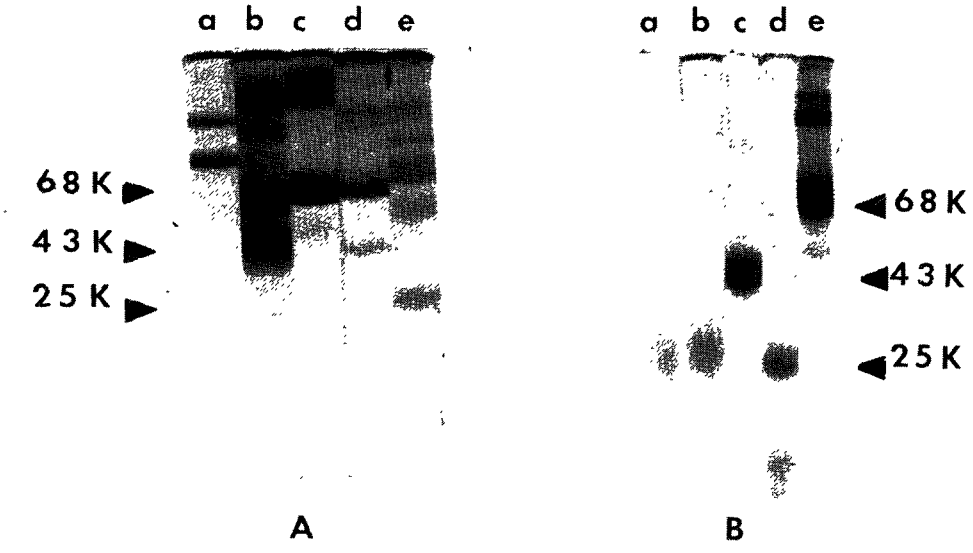


Fig.3. SDS–polyacrylamide gel electrophoresis. (A) Fractions from the phenyl–Sepharose affinity chromatography were analyzed in 5% polyacrylamide gels containing 0.1% SDS. (a) fraction I; (b) fraction II; (c) fraction III; (d) fraction IV; (e) fraction V. (B) SDS–PAGE of purified C3d in 7.5% polyacrylamide gels. (a) C3d recovered from the gel filtration on AcA 44 without mercaptoethanol; (b) with mercaptoethanol; (c–e) protein markers (ovalbumin 43 k daltons, trypsin 25 k daltons and albumin 68 k daltons).

the knowledge of its serum level in patients affected with renal diseases is very important. The use of phenyl–Sepharose facilitates the purification of C3d and obtaining specific immuneserum. Furthermore, it will be possible to obtain more complete informations about the structure of this C3 fragment that probably plays an important part in the biological properties of C3.

Acknowledgements

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References

[1] Nilsson, V. and Mapes, J. (1973) J. Immunol. 111, 293–294.
[2] Molenaar, J. L., Helder, A. W., Muller, M. A. C., Gorismulder, M., Sonker, L. S., Brouwer, M. and Pondman, K. W. (1975) Immunochemistry 12, 359–364.
[3] Tack, B. F. and Prahl, J. W. (1976) Biochemistry 15, 4513–4521.
[4] Bokisch, V. A., Cochrane, C. G. and Muller-Eberhard, H. J. (1975) J. Exp. Med. 129, 1109.
[5] Muller-Eberhard, H. J., Dalmasso, R. P. and Calcott, M. A. (1966) J. Exp. Med. 123, 33.
[6] Lachmann, P. J. (1973) MTP Int. Rev. Sci. Biochemistry, ser. 1, vol. 10, pp. 361–396, Butterworth, London.
[7] Fearon, D. T. and Austen, K. F. (1976) Essays Med. Biochem. 2, 1–35.
[8] Muller-Eberhard, H. J. (1975) Annu. Rev. Biochem. 44, 697–724.
[9] Nicholson, A., Brade, V., Lee, D. G., Shin, H. S. and Mayer, M. M. (1974) J. Immunol. 112, 1115–1123.
[10] Stossel, T. P., Field, R. J., Gitlin, J. D., Alper, C. A. and Rosen, F. S. (1975) J. Exp. Med. 141, 1329–1347.
[11] Goldstein, I. M., Kaplan, H. B., Radin, A. and Frosch, M. (1976) J. Immunol. 117, 1282–1287.
[12] Law, S. K. and Levine, R. P. (1977) Proc. Natl. Acad. Sci. USA 74, 2701–2705.
[13] Law, S. K., Lichtenberg, N. A. and Levine, R. P. (1979) J. Immunol. 123, 1388–1394.
[14] Helenius, A. and Simons, K. (1977) Proc. Natl. Acad. Sci. USA 74, 529–532.
[15] Fontaine, M. and Rivat, C. (1979) Ann. Immunol. Inst. Pasteur 130C, 349–366.
[16] Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412.
[17] Steinbuch, M. (1964) Vox Sang. 9, 96–98.
[18] Capel, P. J. A., Groeneboer, O., Grosveld, G. and Pondman, K. W. (1978) J. Immunol. 121, 2566–2572.
[19] Bokisch, V. A., Dierich, M. P. and Muller-Eberhard, H. J. (1975) Proc. Natl. Acad. Sci. USA 72, 1989–1993.