ULTRACENTRIFUGATION STUDIES ON THE NATIVE FORM OF THE FIRST COMPONENT OF HUMAN COMPLEMENT (C1)*

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1. Introduction

The first component of complement (C1) exists in serum as a macromolecule with a molecular weight of about 10^6 daltons; C1 can be dissociated by EDTA into three major subcomponents, C1q, C1r and C1s [1–3]. The C1q subcomponent has the binding site for complement-fixing immunoglobulins [4,5]; the C1r and C1s subcomponents exist in native C1 in a proesterase state [6–8]. It has been shown that the proesterase C1s is converted into $C1\overline{s}$, the C1 esterase, by the purified and activated form of C1r, $C1\overline{r}$ [6,8]. Purified precursor C1r can be activated by trypsin [6,8]. In contrast to the activated form, $C\overline{1}$, C1 in serum has no enzymatic activity against C4 and C2, the natural substrates of $C\overline{1}$; C1 as well as $C\overline{1}$ is taken up by antigenantibody complexes in a time and temperature independent reaction [9]. The conversion of precursor C1 to $C\overline{1}$ is a relatively slow, temperature dependent process [9,10].

Recently we presented evidence for an activation step in the C1 molecule that occurs after the binding of C1 and before the appearance of $C\overline{1}$ activity; some data suggested that this internal activation step involves the subcomponents C1q [10]. Furthermore we could demonstrate that in diluted serum there exist two different forms of C1; both are taken up by antigen-antibody complexes, but only one C1 population was activable by itself and by trypsin; the other C1 population was activable by trypsin but not by itself indicating that in this C1 population (C1 - X) a factor ('X') is missing which is necessary for internal activation [11]. Since we could demonstrate that factor 'X' was bound independently from C1 and (C1 - X) we concluded that (C1 - X) is a molecule from which a subunit of C1q is missing.

In this report we present evidence for dissociation of native C1 in serum upon dilution as shown by ultracentrifugation. The macromolecule C1 dissociates into the subcomponents C1q, C1r and C1s. While the hemolytic activity C1r and C1s was detectable in the 7 S and 4 S regions, respectively, no hemolytic C1q activity was found, not even in the 11 S part. The detection of C1q protein in the 7 S and about 2 S fractions suggests that fragmentation of C1q upon dilution may take place.

2. Experimental

The methods for the preparation of sheep erythrocytes (E) are given in [12]. Partially purified IgG (7S) antibodies against sheep red cells were purchased from Cordis Corporation, Miami, Florida. Fresh normal human serum was kindly supplied by DRK Blutspendedienst Bad Kreuznach, FRG. Human C1q was purified from the euglobulin fraction by chromatography on DEAE- and CM-cellose and on hydroxylapatite similar to [13,14]; the preparation and the criteria of the antiserum against human C1q are given in [13]. Human C1r was purified by chromatography on DEAE-, CM- and TEAE-cellulose and by polyacrylamide gel electrophoresis similar to [15]; human $C1\overline{s}$ was purified by chromatography on DEAE- and TEAE-cellulose and by polyacrylamide gel electrophoresis similar to [7]. Partially purified guinea pig C4

*The nomenclature follows the recommendation of the World Health Organization Committee on Complement Nomenclature; Immunochemistry, (1970) 7, 137–142.

and C2 was purchased from Cordis Corporation, Miami, Florida. C-EDTA as the source of late-acting components was prepared as described [12].

The hemolytic activity of C1 and the subcomponents of C1 were assayed using erythrocytes sensitized with IgG antibodies against E (ElgG); ElgG were incubated with C1 or dilutions of the subcomponents to be titrated mixed with an excess of the other two subcomponents for 10 min at 30°C; afterwards the cells were washed twice and the number of C1 sites was determined by successive addition of C4 (10 min 30°C) C2 (10 min 30°C) and C-EDTA (60 min 37°C); the results were expressed as Z-values [12]. All titrations were performed using Veronal-buffered sucrose saline (VBS-S: $\mu = 0.065$, pH 7.3) containing 0.001 M Mg^{2+} and 0.00015 M Ca^{2+} and 0.1% gelatine [12].

The ultracentrifugation experiments were performed in a Spinco L265B ultracentrifuge with a Ti14 or a SW 40 rotor. Linear sucrose density gradients (5 to 30%) were prepared with Veronal-buffered saline (VBS) containing 0.001 M Mg^{2+} and 0.00015 M Ca^{2+}. After the samples had been centrifuged at 4°C for 16 h at 39 000 rev/min fractions were collected. 19 S (IgM) hemolysin and 7 S (IgG) hemolysin as markers were purchased from Cordis Corporation, Miami, Florida, and tested as described in [12]. The immundiffusion was carried out in 1% Agarose in Veronal buffer pH 8.6 containing 0.005 M EDTA.

3. Results

3.1. *Effect of dilution on native C1*

Previous experiments concerning the activation of native C1 led to the conclusion that dilution of serum leads to a dissociation of C1 resulting in C1 molecules which are not able of internal activation [11]. Therefore the effect of dilution on C1 in serum was examined in ultracentrifugation studies whereby a separation of the dissociation products may be possible The experiments were performed on a 11 ml sucrose density gradient from 5% to 30% sucrose dissolved in VBS ($\mu = 0.06$) containing 1 mM Mg^{2+} and 0.15 mM Ca^{2+}.

One gradient was overlayered with 0.2 ml of undiluted human serum and an other gradient with 0.4 ml of a diluted (1:100 in the 5% sucrose buffer) human serum and centrifuged with a SW 40 rotor at 39 000 rev/min for 16 h at 4°C. In a separate gradient 19 S and 7 S hemolysins were used as markers. After this time the gradients were fractionated (5 drops per fraction) and tested for hemolytic C1 activity. The results shown in fig.1A indicate that C1 in undiluted

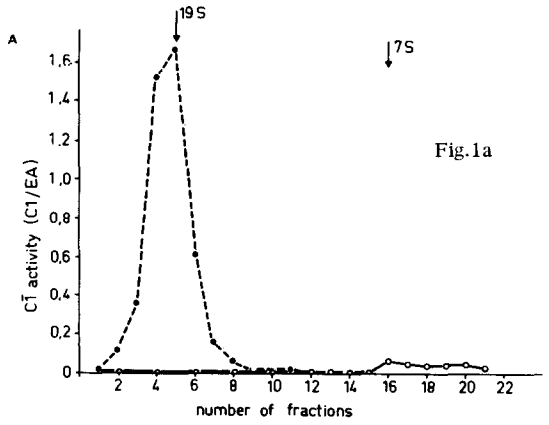

Fig.1a

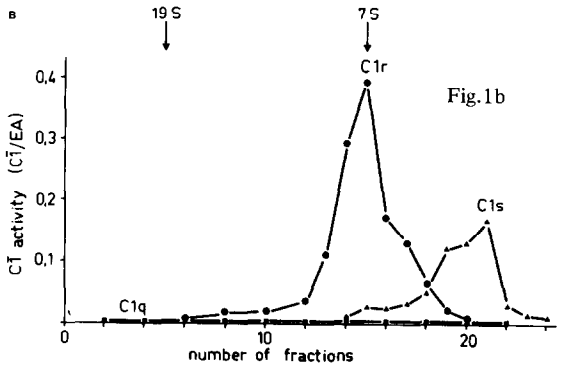

Fig.1b

Fig.1 (A) C1 activity after ultracentrifugation (SW 40 rotor) of undiluted and diluted (1:100) normal human serum on a linear 5% to 30% sucrose gradient; 1 mM Mg^{2+}; 0.15 mM Ca^{2+}; $\mu = 0.06$, 16 h, 39 000 rev/min at 4°C; 19 S (IgM) and 7 S (IgG) hemolysins were used as markers in a separate run. (●- - - -●) Undiluted serum; (○———○) diluted (1:100) serum. (B) The hemolytic activity of C1q, C1r and C1s after ultracentrifugation of diluted (1:100) normal human serum on 5% to 30% sucrose gradient. (■———■) C1q; (●———●) C1r; (▲———▲) C1s.

serum appeared in the 19 S region after centrifugation. However, dilution of serum lead to a complete loss of hemolytic C1 activity. When the same fractions were tested for C1q, C1r and C1s, as shown in fig.1B, C1r activity was found in the 7 S region and C1s in the 4 S region; C1q, however, could not be detected by functional assays. The results of this experiment indicate that dilution of serum leads to a dissociation of the 19 S C1 molecule into its subcomponents C1r and C1s. Since C1q was not detectable hemolytically it was looked for with immunochemical methods.

3.2. *Effect of dilution on native C1q*

The following experiment were performed similar to those described above but a Til4 zonal rotor was used. 30 ml of a 1:100 dilution of normal human serum were overlayered on a 5% to 30% sucrose gradient and centrifuged at 39 000 rev/min for 16 h at 4°C. After centrifugation the gradient was fractionated (150 drops per fraction) and analysed for C1, C1q, C1r and C1s activity. In a control experiment 30 ml of purified C1q (46 μg protein per ml) was centrifuged under the same conditions and the fractions were tested for hemolytic C1q activity. The results shown in fig.2A are in accordance with the first experiment; in contrast to C1r and C1s there were no hemolytic activities of C1 and C1q detectable. However, after centrifugation of the purified C1q preparation C1q activity was found in the 11S fractions. To be able to detect C1q protein every two fractions of the gradient of diluted serum were pooled, dialysed, concentrated and analysed in a immunodiffusion test for C1q protein with rabbit anti human C1q antiserum. Fig.2B shows that the C1q protein was detectable in several different fractions of the centrifuged serum dilutions. The major part of the C1q protein was found in the 7S region (fractions 7/8, 9/10 and 11/12); two precipitation lines were seen in the 11S fractions (fractions 13/14 and 15/16) which correspond to the described sedimentation constant of purified C1q [4]. A faint precipitation line was found in the 19S region (fractions 31/32); C1q protein was also found in the first two fractions of the gradient (fraction 1/2). These findings indicate that C1q in serum dissociates into small fragments upon

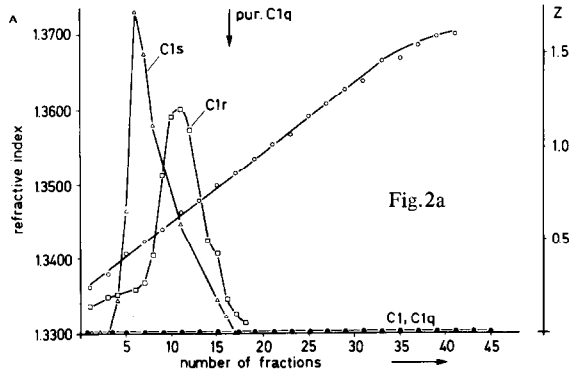

Fig.2a

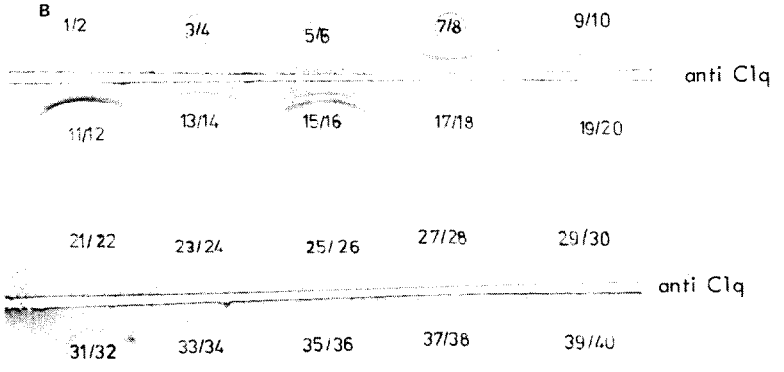

Fig.2b

Fig.2. (A) Zonal ultracentrifugation of diluted (1:100) normal human serum. Ti14 rotor; $\mu = 0.06$; 5% to 30% sucrose gradient containing 1 mM Mg^{2+} and 0.15 mM Ca^{2+}. 39 000 rev/min for 16 h at 4°C. Arrow indicates direction of sedimentation. (●——●) C1 and C1q activity; (△——△) C1 activity; (□——□) C1r activity; (○——○) refractive index. The indicated position of sedimentation of purified human C1q was tested in a separate run. (B) Immunodiffusion analysis of the different, concentrated fractions of the sucrose gradient of fig.2A for C1q protein with rabbit anti-human C1q.

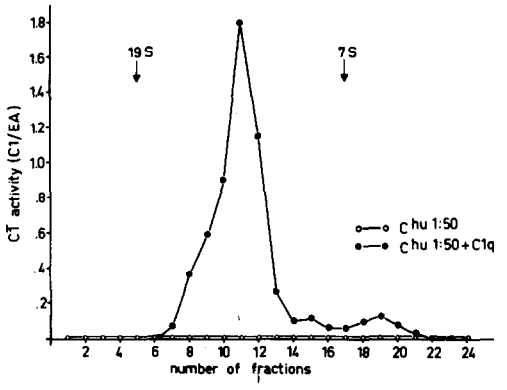

Fig.3. C1 activity after ultracentrifugation (SW 40 rotor) of diluted (1:50) serum to which purified C1q was added. 5% to 30% sucrose gradient containing 1 mM Mg^{2+} and 0.15 mM Ca^{2+}; μ = 0.06; 39 000 rev/min for 16 h at 4°C.

dilution. Although C1q protein was detected immunochemically in the 19 S and 11 S position; C1 and C1q could not be detected hemolytically, this has to be further examined.

3.3. *Recombination of C1 in diluted serum with purified C1q*

In a series of experiments the effect of purified C1q in diluted normal human serum was studied. Equal volumes of purified C1q (46 μg/ml) and of human serum diluted 1:50 in VBS (μ = 0.06) containing 5% sucrose, 1 mM Mg^{2+} and 0.15 mM Ca^{2+} were mixed and incubated for 30 min at 30°C; samples of this mixture and of untreated serum were centrifuged in the descirbed manner using a SW40 rotor. The fractionated gradients were analysed for C1 activity by addition of ElgG cells for 10 min at 30°C. Then the cells were washed three times and tested for C1 activity by successive addition of C4, C2 and C-EDTA. Fig.3 shows that addition of purified C1q to diluted serum leads to an appearance of C1 hemolytic activity in the 12.5 S region (this is a mean value of six different experiments).

4. Discussion

Thompson and Hoffman reported that the dose response curve serum C1 is non-linear in contrast to that of activated C1 ($C\bar{1}$) [16]. These authors could demonstrate that the non-linearity is not due to the effect of the $C\bar{1}$ inactivator which is present in normal serum [17]; they postulated a dissociation of serum C1 into the subcomponents C1q, C1r and C1s through dilution [17]. In previous experimens [11] we treated EAC14 cells (prepared with different concentrations of serum C1) with trypsin and subsequently observed a linear dose response curve. From this and a series of other experiments we concluded that a factor necessary for internal activation of C1 is missing from a portion of the C1 molecules. The activating factor missing from C1 was found to be bound independently from the rest of the C1 molecule and was therefore postulated to be a portion of C1q; this conclusion assumes that C1q is dissociable [11]. The experimental evidence for this interpretation is presented in this report by the ultracentrifugation studies which show that the native C1q subcomponent is dissociated into fragments by dilution. This finding is in good accordance with the studies on the effect of ionic strength on C1 by Colten et al. [18] which allowed the assumption that C1 is composed of more than the three known subcomponents. Furthermore our experiments are also compatible with the observation that purified C1q itself is composed of nine non-covalently bound subunits, each subunit being a disulphide-linked dimer of two polypeptide chains [19–22].

In contrast to native C1q purified C1q was found in the 11 S portion after ultracentrifugation as described in the literature [4]. Therefore it seems to be reasonable that the inter-subunit bonds are stronger in the purified form than in the native C1q as it is present in serum. This assumption is supported by the recombination experiment (fig.2) with purified C1q in serum which resulted in a 12.5 S hemolytically active C1 molecule. A sedimentation constant of 12 S was also found for the recombined homologous human, guinea pig and hybrid C1 molecules made with purified subcomponents [3].

The major points of this report can be summarized as follows: First, native C1 is, in contrast to activated C1 ($C\bar{1}$), a very labile molecule which can be dissociated into its subcomponents by dilution. Second, the most sensitive part of the native C1 molecule is C1q which, in contrast to purified C1q, may be partially dissociated into its subunits or fragments leading to a loss of hemolytic C1q activity.

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References

[1] Naff, G. B., Pensky, J. and Lepow, I. H. (1964) J. Exp. Med. 119, 593–612.
[2] Lepow, I. H., Naff, G. B., Todd, E. B., Pensky, J. and Hinz, C. F. (1963) J. Exp. Med. 117, 983–1008.
[3] Sassano, F. G., Colten, H. R., Borsos, T. and Rapp, H. J. (1972) Immunochemistry 9, 405–412.
[4] Müller-Eberhard, H. J. and Kunkel, H. G. (1961) Proc. Soc. Exp. Biol. Med. 106, 291–295.
[5] Allan, R. and Isliker, H. (1974) Immunochemistry 11, 175–180.
[6] Valet, G. and Cooper, N. R. (1974) J. Immunol. 112, 1667–1673.
[7] Sakai, K. and Stroud, R. M. (1973) J. Immunol. 110, 1010–1020.
[8] Naff, G. B. and Ratnoff, O. D. (1968) J. Exp. Med. 128, 571–593.
[9] Borsos, T., Rapp, H. J. and Walz, U. L. (1964) J. Immunol. 92, 108–112.
[10] Loos, M., Borsos, T. and Rapp, H. J. (1972) J. Immunol. 108, 683–688.
[11] Loos, M., Borsos, T. and Rapp, H. J. (1973) J. Immunol. 110, 205–212.
[12] Rapp, H. J. and Borsos, T. (1970) Molecular Basis of Complement Action, Appleton-Century-Crofts, New York.
[13] Reid, K. B. M., Lowe, D. M. and Porter, R. R. (1972) Biochem. J. 130, 749–763.
[14] Calcott, M. A. and Müller-Eberhard, H. J. (1972) Biochemistry 11, 3443–3450.
[15] de Bracco, M. M. and Stroud, R. M. (1971) J. Clin. Invest. 50, 838–848.
[16] Thompson, J. J. and Hoffmann, L. G. (1971) Immunochemistry 8, 169–184.
[17] Hoffmann, L. G. and Stevland, N. P. (1971) Immunochemistry 8, 499–508.
[18] Colten, H. R., Borsos, T. and Rapp, H. J. (1968) J. Immunol. 100, 808–813.
[19] Yonemasu, K. and Stroud, R. M. (1972) Immunochemistry 9, 545–554.
[20] Reid, K. B. R. (1974) Biochem. J. 141, 189–203.
[21] Reid, K. B. R. and Porter, R. R. (1976) Biochem. J. 155, 19–23.
[22] Knobel, H. R., Villiger, W. and Isliker, H. (1975) Eur. J. Immunol. 5, 78–82.