

PARTIAL PURIFICATION AND AUTOCATALYTIC ACTIVATION OF THE SUBUNIT OF THE FIRST COMPONENT OF HUMAN COMPLEMENT, C1r

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1. Introduction

The first component of complement, C1, is a Ca^{2+} -dependent complex of three subunits, C1q, C1r and C1s [1]. When C1 binds to immune complex via C1q, the proenzyme C1r acquires a protease activity to activate C1s to C1s̄ [2,3,4]. However, the mechanism of activation of C1r to its enzyme form C1r̄ still has remained conjectural. Recently, we proposed on the basis of structural similarities of C1r̄ to C1s̄ that the activation of C1r also may be mediated by some proteolytic reaction, as is the case of the activation of C1s by C1s̄ [5].

On the other hand, the activation of C1 macro-molecule has been shown to be an autocatalytic reaction [6]. Then, we have attempted to investigate the possibility of autocatalytic activation of C1r to C1r̄. In this communication, we report a simple method for isolation of C1r in its zymogen form and spontaneous activation of C1r.

2. Experimental

CPD-human plasma was obtained from the Blood Bank of Japanese Red Cross, Sapporo.

The proenzyme form of C1r was detected by the two-step activation system, as described by Valet and Cooper [7]. Aliquots (0.2–0.5 ml) to be tested were incubated with 2 μg of trypsin for 5 min at 37°C, and then 4 μg of soy bean trypsin inhibitor was added to neutralize the trypsin activity. After further incubation for 5 min, the reaction mixture was reacted with 10 μg of C1s for 15 min at 37°C, and the esterase activity of C1s̄ thus developed was determined with 5 mM of AGLME as a substrate. The activator activity of C1r̄ was expressed as the esterase activity of C1s̄ thus evolved under the standard condition [5]. The C1s used was purified from CPD-plasma by a newly developed method which will be reported elsewhere.

Autocatalytic activation of C1r was measured with the pH-stat method at pH 7.5, using 40 mM AAME in 4 mM phosphate buffer containing 0.15 M NaCl in a final volume of 2.5 ml since AAME is a well-known substrate for C1r̄ [8]. The reaction mixture was maintained at 37°C and pH 7.5 using 20 mM NaOH.

3. Results

3.1. Partial purification of C1r

CPD-plasma was incubated with 0.5 mM DFP for 2 h at room temperature. After additions of benzamidine, EDTA-3Na and 0.2 M phosphate buffer, pH 8.0, at final concentrations of 5, 5 and 20 mM, respectively, the plasma was applied to a column of

Abbreviations: The symbols for complement components used in this paper conform to the recommendations of the World Health Organization Committee on Complement Nomenclature; *Immunochemistry*, 7, 137–142 (1970). Activated components were indicated by placing a bar over the numeral which refers to the active component. Other abbreviations used are DFP, diisopropylfluorophosphate; CPD, citrate-phosphate dextrose; AGLME, acetylglycyl-L-lysine methylester; AAME, N- α -acetyl-L-arginine methylester; EDTA, ethylenediamine tetraacetate; SDS, sodium dodecyl-sulfate.

QAE-Sephadex A-50 (Pharmacia Fine Chemicals) equilibrated with 20 mM phosphate buffer, pH 8.0, containing 0.15 M NaCl and 5 mM EDTA 3Na. After extensive washing of the column, the bound proteins were eluted by a linear gradient increase of NaCl concentration.

The pooled C1r fraction again was incubated with 0.5 mM DFP for 2 h at room temperature and applied to a column of CM-Sephadex C-50 (Pharmacia Fine Chemicals) after dialysis against 20 mM phosphate buffer, pH 6.0. After washing of the column, the bound proteins were eluted by a linear gradient increase of NaCl concentration. The fractions containing C1r were pooled and used as partially purified C1r in the following experiments. By these purification procedures, C1r was completely separated from C1s and C1 $\bar{r}$ inhibitor.

Although this preparation still showed several bands on polyacrylamide disc electrophoresis [9] (fig.1), C1r activity was found to be associated with the main component which migrated as a β -globulin, as reported with C1r [7] and C1 $\bar{r}$ [5,10,11]. In addition, this preparation showed a precipitin arc against rabbit anti-human C1 $\bar{r}$ serum which was kindly donated by Professor R. M. Stroud, University of Alabama. The mol. wt. of C1r thus purified was estimated to be 240 000 by the gel filtration on a

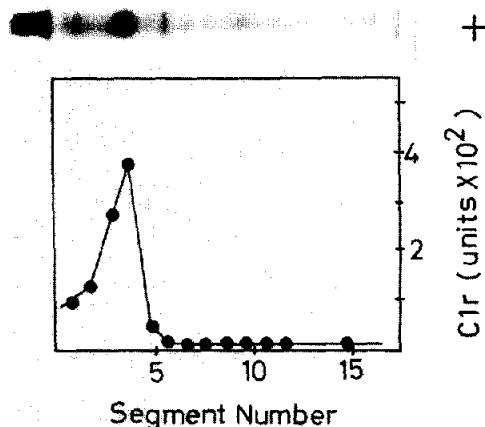


Fig.1. Electrophoretograms. Partially purified C1r was electrophoresed in 6% polyacrylamide gel using Tris-glycine buffer, pH 8.6 [9]. The gel was stained with Coomassie blue. The C1r activity was measured using the extract from each 2 mm segment of a parallel unstained gel.

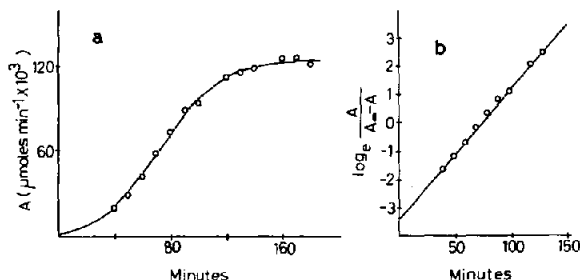


Fig.2. Autocatalytic activation of C1r. (a) About 0.5 unit of C1r was incubated at 37°C in 4 mM phosphate buffer, pH 7.5, containing 0.15 M NaCl. The C1 $\bar{r}$ activity evolved was monitored by the pH-stat titration using 40 mM AAME as a substrate. The curve was calculated from the equation for an autocatalytic reaction, where $k = 3.60 \times 10^{-4}$, $A = 126$ and $A_0 = 4.2$; open circles show the observed activity. (b) The data in fig.2a is compared with the integrated form of the autocatalytic equation.

calibrated column of Sephadex G-200 [12]. This value corresponds to the double of that (115 000) of activated C1 $\bar{r}$ estimated by the SDS gel electrophoresis [5].

3.2. Autocatalytic activation of C1r

Incubation of the purified C1r alone at 37°C and pH 7.5 led to spontaneous activation of C1r (fig.2a). A classical S-shaped curve was obtained for appearance of the esterase activity, suggesting that the activation proceeds autocatalytically following the equation [13];

$$\log_e A/(A_\infty - A) = k \cdot A_\infty \cdot t + \log_e A_0/(A_\infty - A_0)$$

where k , A_∞ and A_0 are a second-order autocatalytic rate constant, final and initial activities of C1 $\bar{r}$, respectively. Furthermore, fig.2b shows that the esterase activity of C1r was evolved following a second-order autocatalytic rate law. Namely, a slow initial formation of C1 $\bar{r}$ by autocatalytic activation of C1r may be followed by rapid acceleration due to catalysis by the product, C1 $\bar{r}$. When highly purified C1 $\bar{r}$ [5] was added to the C1r, the lag phase period of the sigmoidal activation curve was apparently shortened, indicating that C1 $\bar{r}$, itself, has an ability to activate C1r to C1 $\bar{r}$. In addition, the increase of C1r concentration also shortened the lag phase of activation curve, suggesting that the autocatalytic activation may be of an intermolecular reaction.

4. Discussion

Because of its propensity of activation, the presence of a zymogen form of C1r had been lacking in the confirmation. Recently, Valet and Cooper [7] reported for the first time the purification of a zymogen form of C1r and mentioned that C1r could be precipitated in the euglobulin fraction, not accompanied by the activation to C1 $\bar{r}$, provided the temperature was strictly kept at 0°C. On the other hand, the critical point on our purification procedure is the treatment with DFP, prior to every step of the purification. In particular, QAE-Sephadex A-50 chromatography of DFP-treated plasma was found to be highly reproducible and effective for the recovery of a zymogen form of C1r. The protective effect of DFP can now be explained as the prevention of autocatalytic activation of C1r by the inhibition of C1 $\bar{r}$ which was contaminating the C1r preparation.

The kinetic analysis indicated that the spontaneous activation of C1r is an autocatalytic reaction. In a previous paper [5], we suggested that activation of C1r should involve a proteolytic process, since C1 $\bar{r}$ also is a DFP-sensitive serine protease consisting of two polypeptide chains connected by disulfide bonds, as is the case of C1s. Recently, Ziccardi and Cooper [14] and Gigli and Porter [15] have presented independently evidence supporting our proposition; C1r is a single polypeptide chain and converted into C1 $\bar{r}$ of two polypeptide chains. Therefore, the activation of C1r seems to be similar to those of various zymogens such as trypsinogen [16], pepsinogen [17], prorennin [18] and C1s [13].

The autocatalytic activation of these zymogens have been defined as a process triggered by the inherent proteolytic activity of each zymogen itself and then accelerated by the activated enzyme [19]. As to the second step, it was confirmed with purified C1 $\bar{r}$ that C1 $\bar{r}$ was capable of catalyzing the activation of its zymogen form. At present, we can not conclude whether the autocatalytic activation of C1r is triggered by its zymogen form itself or by a trace amount of C1 $\bar{r}$ which might present as a contaminant in the C1r preparation. However, even in the zymogen form, C1r may have an inherent capacity to activate itself.

To explain the autocatalytic activation of trypsinogen, the formation of 'active trypsinogen' by a bimolecular interaction has been proposed [19];

two molecules of trypsinogen may come together and cause the formation, in an occasional molecule, of a trypsin-like active site which can active one or the other of trypsinogen molecule or a third trypsinogen molecule. This bimolecular interaction seems to be the case also for autoactivation of C1r, since Ziccardi and Cooper [14] reported recently that C1r takes a non-covalently linked dimer, and Gigli and Porter [15] reported that two moles of C1r are binding to one mole of C1q in C1 macromolecule. If so, one possible mechanism of activation of C1 by immune complex may be as follows; binding of immune complex to C1q causes some conformational change in C1q to allow the close association of two C1r molecules so that the activate site is formed in the associated dimer of C1r and activates one or the other of C1r or C1r in other C1 macromolecule.

The effect of C1q and immune complex on the autocatalytic activation of C1r are now being in progress.

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