ISOLATION OF A COMPLEX OF THE SUBCOMPONENTS OF THE ACTIVATED FIRST COMPONENT OF COMPLEMENT, $C\bar{1}r$–$C\bar{1}s$, FROM ACD-HUMAN PLASMA

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1. Introduction

The first component of complement, Cl, is a Ca^{2+}-dependent complex of a molecular weight of about 10^6 daltons [1] and can be dissociated into three subcomponents, Clq, Clr and Cls, by treatment with EDTA [2]. It is well known that Clq has a binding activity to immune complexes [3] and the active form of $C\bar{1}r$ converts enzymatically Cls into active $C\bar{1}s$ [4] which is an estero-protease and represents the biological activities of the activated $C\bar{1}$ [5].

However, little is known how these subcomponents are interacted to unite the Cl macromolecule.

This paper presents evidence indicating that $C\bar{1}r$ and $C\bar{1}s$ were isolated as a Ca^{2+}-dependent complex form by polyethylene glycol fractionation of ACD*-human plasma followed by chromatographies with IgG-Sepharose 6B and DEAE-Sephadex A-50. This result suggested that the inter-subcomponent bonds in the $C\bar{1}$ macromolecule are not equivalent, and $C\bar{1}r$ and $C\bar{1}s$ have a high affinity via Ca^{2+} for each other but not for Clq. In addition, this isolation procedure seemed to be practical for rapid and concomitant purification of $C\bar{1}r$ and $C\bar{1}s$ from ACD-human plasma.

2. Materials and methods

ACD- and heparinized human bloods were obtained from the blood bank of Japanese Red Cross.

2.1. *Precipitation of $C\bar{1}$ from ACD-human plasma with polyethylene glycol*

One hundred ml of ACD-human plasma were mixed with 10 ml of 50% (W/V) of polyethylene glycol (# 4000) under stirring at 4°C. After 30 min, the precipitate was collected by centrifugation at 3 000 rpm for 15 min and extracted with 50 ml of 0.05 M NaCl. The insoluble materials which contained $C\bar{1}$, fibrinogen and other macromolecular weight plasma proteins were then dissolved in 20 ml of 0.02 M Tris–HCl buffer, pH 8.5, containing 0.15 M NaCl and allowed to clot by the addition of 1 ml of 1 M $CaCl_2$ overnight at 4°C. The defibrinated fraction was used as the starting material for further purification of $C\bar{1}$.

2.2. *Determination of $C\bar{1}$*

$C\bar{1}$ activity was determined by measuring either $C\bar{1}s$ esterase activity with ATEE as substrate [5] or hemolytic activity with EAC4 cells [6]. A functional activity of $C\bar{1}r$ to activate Cls was determined by the method of Naff and Ratnoff [4] with slight modifications. In the most case, aliquots of 10 µl of fractions were incubated with Cls (about 2 µg) in 1 ml of 0.01 M Tris–HCl buffer, pH 8.5, containing 0.10 M NaCl and 5 mM EDTA-3Na at 37°C for 10 min, and the esterase activity of $C\bar{1}s$ thus activated was determined with AGLME [4] as substrate. The value was corrected for the hydrolysis of AGLME by the fractions alone. Human Cls was purified from heparinized plasma according to the method of Sakai and Stroud [7].

* Abbreviations: ATEE, *N*-α-acetyl-L-tyrosine ethyl ester; AGLME, *N*-α-acetylglycyl-L-lysine methyl ester; ACD, acid citrated dextrose.

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3. Results

When ACD-human plasma was fractionated with

Fig. 1. Chromatographic procedure. a) The C1 fraction obtained from 200 ml of human p ma was diluted with equal volume of water and subject d to the affinity chromatography with a column (2 × 5 cm) of IgG-Sepharose as described by Bing [9]. C1 activity was eluted with 0.01 M diaminobutane–HCl, pH 8.5, in 0.01 M Tris–HCl buffer, pH 8.5, containing 0.075 M Na and 5 mM $CaCl_2$. Fractions of each 5 ml were collected at room temperature. The amounts of ATEE hydrolyzed by $C\bar{1}$ were determined with the hydroxamate reagents [10], and one unit of esterase activity was defined as the activity capable of hydrolyzing 1 μmole of the ester per min. b) The $C\bar{1}$ fraction eluted with the diamine solution was dialyzed against 0.01 M Tris–HCl buffer, pH 8.5 containing 0.075 M NaCl and 5 mM $CaCl_2$ overnight and applied to a column (2 × 5 cm) of DEAE-Sephadex A-50 equilibrated with the dialysis buffer. The column was eluted by linear gradient increase of NaCl concentration with each 200 ml of 0.075 M and 0.30 M NaCl in the buffer. Fractions of each 4 ml were collected at 4°C. The activity of $C\bar{1}$ was expressed as the ACT.ME esterase activity of $C\bar{1}$ developed by the action of $C\bar{1}$ r.). The peak fractions, 70–80, were pooled, concentrated by ultrafiltration to 5 ml and gel filtrated with a column (3 × 80 cm) of Sephadex G-B equilibrated with 0.01 M Tris–HCl buffer, pH 7.0, containing 0.15 M NaCl and 5 mM $CaCl_2$. The molecular weight was estimated according to the method of Andrews [12] with the standard proteins of known molecular weights (Boehringer's protein calibration kit I). Fractions of each 4 ml were collected at 4°C.

Fig. 2. Polyacrylamide gel electrophoresis. a) The peak fraction (75 in fig. 1b); b) The peak fraction after addition of EDTA-3Na to 5 mM; c) The EDTA-treated fraction as in b) after recalcification to final 10 mM. Disc gel electrophoresis was performed with 5% polyacrylamide gels [11] and proteins were stained with Coomassie blue. d) Electrophoresis of the peak fraction (45 in fig. 1c) in the presence of 0.2% sodium dodecyl sulfate with a 5% polyacrylamide gel [17].

poly ethylene glycol, a mild protein precipitant [8], $C\bar{1}$ as measured by the hemolytic activity and $C\bar{1}\bar{s}$ esterase activity was found to be precipitated at the polyethylene glycol concentration of 4.5%. The $C\bar{1}$ in the precipitate was partially purified by the affinity chromatography with IgG-Sepharose 6B as described by Bing [9] (fig. 1a). The $C\bar{1}$ fraction eluted with the diamine solution was then chromatographed with DEAE-Sephadex A-50. As shown in fig. 1b, the functional activities of $C\bar{1}$ and $C\bar{1}\bar{s}$ were eluted as a superimposed peak, and the peak fraction exhibited a single band on disc electrophoresis (fig. 2a).

However, the $C\bar{1}$ thus isolated was found to be lacking in the subcomponent C1q from the results of gel filtration on Sepharose 6B and polyacrylamide gel electrophoresis in the presence of EDTA or sodium dodecyl sulfate.

As shown in fig. 1c, the $C\bar{1}$ as measured by $C\bar{1}\bar{r}$ and $C\bar{1}\bar{s}$ activities was eluted from the calibrated column of Sepharose 6B as a single peak of the molecular weight of 3.2×10^5 daltons. This value was smaller than those of the C1 macromolecule, 10^6 daltons [1], or C1q, 4×10^5 daltons [13], and was comparable to the sum of the molecular weights of $C\bar{1}\bar{r}$, 1.8×10^5 daltons [14], and $C\bar{1}\bar{s}$, 1.1×10^5

daltons [15]. Gel electrophoresis of the peak fraction in the presence of sodium dodecyl sulfate exhibited two protein components of the molecular weights of 1.3×10^5 daltons and 1.1×10^5 daltons, respectively. The protein components corresponding to Clq itself or its non-covalent subunit of the molecular weight of 7×10^4 daltons [16] were not detected in the electrophoretogram (fig. 2d). In addition, EDTA-treatment of the peak fraction resulted in the dissociation into two protein components (fig. 2b), and $\overline{Cl}$ and $\overline{Cls}$ activities eluted from the unstained sectioned gels were found to be associated with the protein components in the β- and α-globulin regions, respectively. The dissociated two components were found to be complexed again with each other by the addition of Ca^{2+} (fig. 2c).

From these results, it was proposed that the $\overline{Cl}$ thus isolated was a Ca^{2+}-dependent complex of $\overline{Clr}$ and $\overline{Cls}$.

4. Discussion

As presented above, the $\overline{Cl}$ thus isolated from ACD-human plasma was found to be lacking in Clq subcomponent and was proposed to be a Ca^{2+}-dependent complex of $\overline{Clr}$ and $\overline{Cls}$.

As an additional information to support this conclusion, experiments in progress indicate that the purified $\overline{Clr}$-$\overline{Cls}$ can not bind to the IgG-Sepharose 6B and needs the addition of Clq for its binding to the IgG-Sepharose 6B. In the present experiment, Clq was found to be recovered in the unadsorbed fraction of the DEAE-Sephadex A-50 chromatography. The fact that Clq and the $\overline{Clr}$–$\overline{Cls}$ complex were separated from each other by DEAE-Sephadex A-50 chromatography even in the presence of Ca^{2+} may suggest the affinity of Clq for the $\overline{Clr}$–$\overline{Cls}$ complex to be very weak.

The question on the origin of the $\overline{Clr}$–$\overline{Cls}$ complex remains to be solved. However, the precursor complex, Clr–Cls, can be obtained from ACD-human plasma by DEAE-Sephadex A-50 chromatography and gel filtration on Sephadex G-200 (unpublished data). So, it seems likely that the Cl macromolecule is partly dissociated by the chelating action of anticoagulant citrate into Clq and the Clr–Cls complex and then activated during polyethylene glycol fractionation. Activation of Cl by polyethylene glycol was not unexpected matter, since polyethylene glycol was known as a non-immunological effector of complement system in human serum [18].

In relation to the present observation, Valet and Cooper have recently observed the affinity of Clr for Cls to be so high as to form a stable Ca^{2+}-dependent complex [19].

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