THE NH_2-TERMINAL SEQUENCES OF A SUBUNIT OF THE FIRST COMPONENT OF HUMAN COMPLEMENT, Cls, AND ITS ACTIVATED FORM, $\overline{Cls}$

Kazuhiko TAKAHASHI, Shigeharu NAGASAWA and Jiro KOYAMA
Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan

Received 3 October 1974
Revised version 2 December 1974

1. Introduction

The first component of complement, C1*, is a Ca^{++}-dependent complex of three subunits, Clq, Clr and Cls, and triggers a series of immunological reaction, upon activation by binding to immune complexes [1]. At least two intramolecular sequential reactions are known to be related with the activation of C1; activation of Clr [2] and activation of Cls by activated $\overline{Clr}$ [3].

Recently, Sakai and Stroud [4] and Valet and Cooper [5] reported that the activation of Cls by $\overline{Clr}$ is a proteolytic process; the zymogen Cls, which is a single polypeptide chain, is converted to active $\overline{Cls}$, which is composed of two polypeptide chains connected by disulfide bond. $\overline{Cls}$ is a DFP-sensitive serine protease and represents the biological activities of activated $\overline{C1}$ [6].

There is general agreement about the activation reaction of various zymogens in blood plasma; activation of zymogen is associated with the formation of a new NH_2-terminal sequence which is homologous to those of pancreatic serine proteases [7,8].

So, it is of interest to see whether a homologous NH_2-terminal sequence is also liberated as the new NH_2-terminal after the activation of Cls to $\overline{Cls}$.

To this end, we here describe a simple method for purification of $\overline{Cls}$ from ACD-human plasma and determine the NH_2-terminal sequences of $\overline{Cls}$ and the zymogen Cls.

The data indicate that human $\overline{Cls}$ has two polypeptide chains; the heavy chain having the NH_2-terminal sequence of the zymogen Cls and the light chain having a new NH_2-terminal sequence which is homologous to those of other plasma and pancreatic proteases.

2. Materials and methods

Out-dated human ACD-blood was obtained from the Blood Center of the Japanese Red Cross, Hokkaido.

IgG-Sepharose 6B which was used in the affinity chromatography of $\overline{C1}$ was prepared by the method of Bing [9]. The zymogen Cls was purified from human plasma according to the method of Sakai and Stroud [4] with a slight modifications; the precipitation of euglobulins from human plasma and extraction of Cls from the euglobulins were done in the presence of 5 mM benzamidine, in order to avoid the spontaneous activation of Cls during the isolation procedure.

Two pure polypeptide chains of $\overline{Cls}$ were isolated by analytical scale polyacrylamide gel electrophoresis [10] of $\overline{Cls}$ which had been treated with 0.1 M 2-mercaptoethanol in 0.1 M phosphate buffer, pH 7.5, containing 1% SDS at 37°C for 1 hr.

* The symbols for complement components used in this paper conform to the recommendations of the World Health Organization Committee on complement nomenclature; (1970) Immunochemistry 7, 137–142. Activated components are indicated by placing a bar over the numeral which refers to the active component or subunit. Other abbreviations used are ACD, acid citrated dextrose; ATEE, N-α-acetyl-*L*-tyrosine ethylester; SDS, sodium dodecylsulfate; DFP, diisopropylfluorophosphate; EDTA, ethylenediamine tetraacetate.

North-Holland Publishing Company – Amsterdam

The NH_2-terminal sequences of Cls and $\overline{Cls}$ were determined by the SDS-dansyl-Edman microtechnique of Weiner et al. [10]. Dansyl-amino acids were identified by thin-layer microchromatography on Polyamide layer (Chen Chin Co.), using solvent systems reported [10,11]. Dansylchloride and standard dansyl-amino acids were obtained from Seikagaku Kogyo Co., Japan. Molecular weights of the polypeptide chains were estimated by the method of Weber and Osborn [12] with standard proteins of known molecular weight (protein calibration kit II, Boehringer Co.).

3. Results

3.1. *Purification of human $\overline{Cls}$*

The various steps in the purification of human $\overline{Cls}$ are shown in table 1. As reported in a previous paper [13], Cl in human plasma was precipitated as the spontaneously activated form with polyethylene glycol and adsorbed on a column of IgG-Sepharose. $\overline{Cls}$ was then released from $\overline{Cl}$ on IgG-Sepharose with EDTA as described by Sledge and Bing [14] (fig.1a) and finally purified by chromatography on DEAE-cellulose (fig.1b). $\overline{Clr}$, which was simultaneously released from $\overline{Cl}$ on IgG-Sepharose, was recovered in the unadsorbed fraction of DEAE-cellulose chromatography.

The purification was about 200-fold with an overall yield of 23%, relative to the first precipitate with polyethylene glycol. The entire procedure is reproducible and can be done within 72 hr.

$\overline{Cls}$ prepared in this manner yielded a single band after polyacrylamide gel electrophoresis at pH 9.5 (fig.2a) or at pH 7.4 in the presence of SDS (fig.2b). The two-chain structure of $\overline{Cls}$ was evident on SDS-polyacrylamide gel electrophoresis of the 2-mercapto-ethanol-treated $\overline{Cls}$ (fig.2c). The mol wts of $\overline{Cls}$ and the two chains, termed heavy and light chains, were estimated to be 10.5×10^4, 6.8×10^4 and 3.4×10^4, respectively.

3.2. *The NH_2-terminal sequences of the heavy and light chains of $\overline{Cls}$ and the zymogen Cls*

The first four NH_2-terminal sequences of the isolated two polypeptide chains of $\overline{Cls}$ (figs.2d and e) were determined by the SDS-dansyl-Edman microtechnique [10] to be as follows; Glx–Ile–Thr–Met for the heavy chain and Ile–Ile–Gly–Gly for the light chain.

In addition, the first two NH_2-terminal sequence of human Cls was determined by the SDS-dansyl-Edman technique to be Glx–Ile.

These results indicate that the heavy chain is

Table 1
Purification of $\overline{Cls}$

Purification Step	Total volume (ml)	Total protein (A280 nm)	Total activity[a] (units)	Specific activity (units/A280 nm)
Plasma	230	15,890	N.D.[b]	–
Polyethylene glycol precipitate[c]	41	710	16.0	0.02
Defibrination[d]	41	189	15.3	0.08
IgG-Sepharose	34	15.1	7.1	0.47
DEAE-cellulose	43	1.3	5.0	3.84

[a] $\overline{Cls}$ was determined by measuring the esterase activity with ATEE as the substrate [6]. One unit of $\overline{Cls}$ is defined as the activity capable of hydrolyzing 1 μmole of the ester per min at 37°C.

[b] The exact content of $\overline{Cls}$ or Cls can not be determined.

[c] Proteins precipitated from ACD-plasma at 4.5% (w/v) polyethylene glycol concentration were collected by centrifugation, washed with 200 ml of 0.05 M NaCl and then dissolved in 0.02 M Tris–HCl buffer, pH 8.5, containing 0.15 M NaCl [13].

[d] Fibrinogen, the major constituent of polyethylene glycol precipitate, was removed as fibrin clot by the addition of $CaCl_2$ [13].

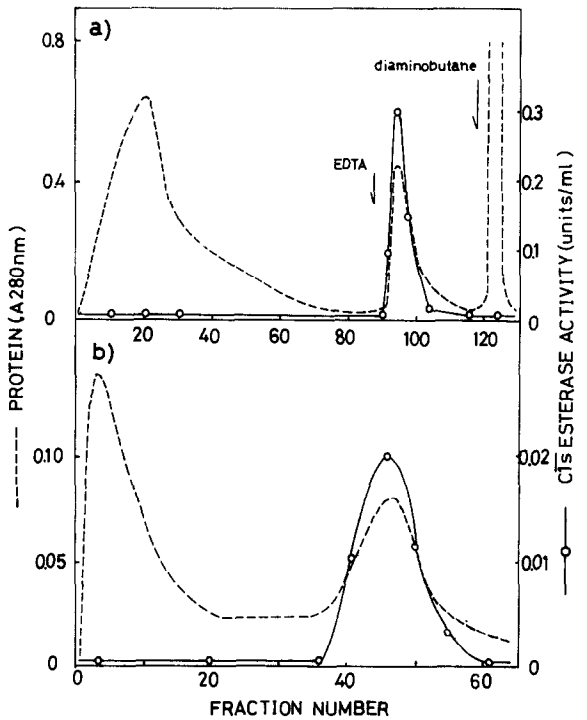


Fig.1. Purification of $C\bar{1}s$. a) The $C\bar{1}$ fraction prepared from 230 ml of ACD-human plasma with polyethylene glycol [13] was applied to a column (1.5 × 4 cm) of IgG-Sepharose 6B [9] equilibrated with 0.02 M Tris–HCl buffer, pH 8.5, containing 0.075 M NaCl and 5 mM $CaCl_2$. After washing the column with the equilibration buffer, the column was eluted with 100 ml of 10 mM EDTA in 0.02 M Tris–HCl buffer, pH 8.5, containing 0.075 M NaCl and with 50 ml of 10 mM diaminobutane in the buffer. Fractions of each 5 ml were collected at the cold room. b) The $C\bar{1}s$ fraction eluted with EDTA was applied to a column (1.5 × 5 cm) of DEAE-cellulose (DE-52) equilibrated with 0.02 M Tris–HCl buffer, pH 8.5, containing 0.075 M NaCl and 5 mM EDTA. After washing the column with 50 ml of the equilibration 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 5 ml were collected at the cold room. Fractions, 42–50, were pooled and used as the purified $C\bar{1}s$.

derived from the NH_2-terminal portion of the zymogen Cls molecule and that the light chain, which is derived from the remaining COOH-terminal portion of Cls, has a new NH_2-terminal sequence homologous to those of other mammalian serine proteases.

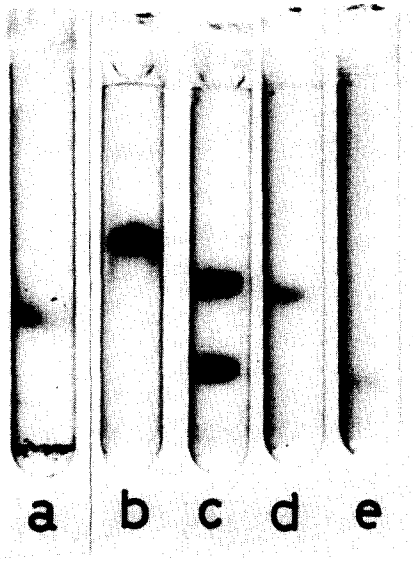


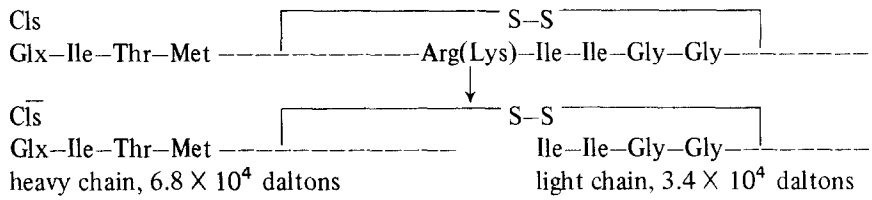
Fig.2. Electrophoretograms. Purified Cls was electrophoresed with (a) 5% polyacrylamide gel [15], (b) 0.2% SDS-5% polyacrylamide gel [12] and (c) 0.2% SDS-5% polyacrylamide gel after treatment with 0.1 M 2-mercaptoethanol. The isolated heavy (d) and light (e) chains of $C\bar{1}s$ were electrophoresed as in (b). Proteins were stained with Coomassie Blue.

4. Discussion

The present experiments provide informations on the molecular events associated with the activation of human Cls.

The first four NH_2-terminal sequence of the light chain of $C\bar{1}s$ shows homology with those of other serine proteases; Ile–Val–Gly–Gly for bovine trypsin [16], plasmin [17] and Factor Xa [18], and Ile–Val–(Asn or Glu)–Gly for bovine chymotrypsin [19] or thrombin [20]. The newly appeared α-amino group on isoleucine is essential for the proteolytic activity of bovine trypsin [21,22], chymotrypsin [23] and thrombin [24], so the cleavage of a specific peptide bond, X–Ile, in the zymogen Cls molecule is probably an essential step for the formation of functionally active $C\bar{1}s$. Considering that $C\bar{1}r$ has a specificity similar to that of trypsin (3), the amino acid 'X' in the cleaved peptide bond is probably Arg or Lys.

So, the molecular event associated with the activation of human Cls can be written as follows:



Although the activation reaction of Cls is very similar to those of other zymogens, it should be emphasized that the cleavage of an NH_2-terminal peptide does not occur during the activation of Cls to $\overline{Cls}$. This is in contrast to the activation reaction of other zymogens; the NH_2-terminals of bovine trypsinogen [25], Factor X [18] and Factor XIII [26] are released as the 'activation peptide' in accompanied with the formation of active enzymes, and that of human plasminogen [27] is released as the 'pre-activation peptide' prior to the concomitant cleavage of the sensitive peptide bond necessary for the formation of active plasmin.

As additional evidence on homology of $\overline{Cls}$ with other serine proteases, Barkas et al. [28] recently reported that the DFP-sensitive active site of $\overline{Cls}$ resides in the light chain and the amino acid sequence surrounding the active site seems to be similar to those of chymotrypsin and thrombin.

Acknowledgements

The authors thank Professor T. Suzuki, Institute for Protein Research, Osaka University, for his interest in our work. We also thank Dr S. Kimura for his advice regarding the dansyl-Edman technique. This work was supported by a grant from Houansha, Japan.

References

[1] Lepow, I. H., Naff, G. B., Todd, E. W., Pensky, J. and Hinz, C. F. (1963) J. Exp. Med. 117, 983–1008.
[2] Valet, G. and Cooper, N. R. (1974) J. Immunol. 112, 1667–1673.
[3] Naff, G. B. and Ratnoff, O. D. (1968) J. Exp. Med. 128, 571–593.
[4] Sakai, K. and Stroud, R. M. (1973) J. Immunol. 110, 1010–1020.
[5] Valet, G. and Cooper, N. R. (1974) J. Immunol. 112, 339–350.
[6] Heines, A. L. and Lepow, I. H. (1964) J. Immunol. 94, 456–467.
[7] Robbins, K. C., Bernabe, P., Arzadon, L. and Summaria, L. (1972) J. Biol. Chem. 247, 6757–6762.
[8] Titani, K., Hermodson, M. A., Fujikawa, K., Ericsson, L. H., Walsh, K. A., Neurath, H. and Davie, E. W. (1972) Biochemistry 11, 4899–4903.
[9] Bing, D. H. (1971) J. Immunol. 107, 1247–1249.
[10] Weiner, A. M., Platt, T. and Weber, K. (1972) J. Biol. Chem. 247, 3242–3251.
[11] Kimura, S. (1974) Japan Analyst 23, 563–575.
[12] Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412.
[13] Nagasawa, S., Takahashi, K. and Koyama, J. (1974) FEBS Lett. 41, 280–282.
[14] Sledge, C. R. and Bing, D. H. (1973) J. Immunol. 111, 661–666.
[15] Davis, B. J. (1964) Ann. N.Y. Acad. Sci. 121, 404–427.
[16] Walsh, K. A. and Neurath, H. (1964) Proc. Natl. Acad. Sci. 52, 884–889.
[17] Nagasawa, S. and Suzuki, T. (1970) Biochem. Biophys. Res. Commun. 41, 562–567.
[18] Fujikawa, K., Legaz, M. E. and Davie, E. W. (1972) Biochemistry 11, 4892–4898.
[19] Hartley, B. S. and Kauffman, D. L. (1966) Biochem. J. 101, 229–231.
[20] Magnusson, S. (1970) Thromb. Diath. Heamor. suppl. 38, 97–104.
[21] Scrimger, S. T. and Hoffman, T. (1967) J. Biol. Chem. 242, 2528–2533.
[22] Robinson, N. C., Neurath, H. and Walsh, K. A. (1973) Biochemistry 12, 420–426.
[23] Dixon, J. W. and Hoffman, T. (1970) Can. J. Biochem. 48, 671–681.
[24] Magnusson, S. and Hoffman, T. (1970) Can. J. Biochem. 48, 432–437.
[25] Davie, E. W. and Neurath, H. (1955) J. Biol. Chem. 212, 507–514.
[26] Nakamura, S., Iwanaga, S., Suzuki, T., Mikuni, Y. and Konishi, K. (1974) Biochem. Biophys. Res. Commun. 58, 250–256.
[27] Rickili, E. and Otavsky, W. I. (1973) Biochim. Biophys. Acta 295, 381–384.
[28] Barkas, T., Scott, K. and Fothergill, J. E. (1973) Biochem. Soc. Trans. 1, 1219–1220.