Vol. 127, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 28, 1985 Pages 264-269

LOCATION OF THE INTER-CHAIN DISULFIDE BONDS OF THE THIRD COMPONENT OF HUMAN COMPLEMENT

Tadashi Matsuda*, Tsukasa Seya**, and Shigeharu Nagasawa*+

*Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, 060, Japan

**Department of Medicine, Division of Rheumatology, Washington University School of Medicine, St. Louis, MO 63110

Received January 10, 1985

Location of the disulfide bonds connecting three polypeptide chains (α3, 27kd; 2, 43kd; β, 75kd) of C3c has been investigated by partial reduction with cysteine followed by alkylation with ^{14}C-monoiodoacetic acid. Treatment of C3c with cystein produced a partially reduced fragment, composed of disulfide-linked β and α3 chains. A single thiol residue was detected on the α3 chain but not on the β chain of the fragment, suggesting that the α2 chain in C3c is linked through a single disulfide bond to the α3 chain but not to the β chain.

The third component of complement, C3, is composed of two disulfide-linked polypeptide chains of 120 kd (α chain) and 75 kd (β chain) (1). Although the primary structure of C3 by sequence analysis of cloned cDNA of murine C3, was reported as this work was in progress (2), the location and number of the disulfide bonds linking the two chains of human C3 still remain uncharacterized.

C3 is cleaved by various serum proteases to yield several fragments termed C3a, C3b, iC3b, C3c, and C3dg (3-6). iC3b (180 kd) is composed of three disulfide-linked polypeptide chains; intact β chain and two α chain fragments of 68 kd (α1) and 43 kd (α2), and yields C3c and C3dg upon limited proteolysis on the 68 kd fragment. C3c (140 kd) is composed of

+To whom all correspondence should be addressed.

Abbreviations used: Complement components are identified according to World Health Organization recommendations (1981); kd, kilo daltons; SDS(PAGE), sodium dodecyl sulfate (polyacrylamide gel electrophoresis).

0006-291X/85 $1.50

three disulfide-linked polypeptide chains; intact β chain and two chain fragments of 43 kd (α2) and 27 kd (α3) (4,6).

Utilizing partial reduction of C3c with cysteine followed by radiolabelling with ^{14}C-monoiodoacetic acid, we obtained evidence suggesting that the α2 chain is linked through a single disulfide bond to the α3 chain, with which the β chain is also disulfide-linked. Based on the present data and the reported primary structure of murine C3 (2), a possible location of disulfide bridges connecting the α and β chains of human C3 will be proposed.

EXPERIMENTAL PROCEDURES

Materials Human complement components, C3(4) and I(7), were purified as reported. Purified H and B were obtained as by-products of the purification of C3(4) and C2(8), respectively (unpublished). iC3b was prepared by incubating elastase treated C3 with I and H for 6 hr at 37°C in 20 mM phosphate buffer, pH 7.5, containing 0.15M NaCl (9). The weight ratio of C3:I:H was 100:1:5. C3c was prepared by incubating iC3b with human plasma kallikrein as reported by Meuth et al. (10) and purified by gel filtration through Sephadex G-200. Plasma kallikrein was purified as reported by Nagase and Barret (11).

Partial reduction iC3b and C3c were partially reduced by incubating with 20 mM cysteine in 1% SDS-10 mM Tris buffer, pH 7.5 at 37°C for 30 min. Partial reduction was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), which was performed with 7.5% acrylamide gels according to the method reported by Fairbanks et al. (12). Acrylamide gels were stained with Coomassie brilliant blue R-250.

Determination of thiol residue Cysteine-treated C3c was subjected to centrifugation through Sephadex G-25 as described by Janatova et al. (13) to remove cysteine. Then, the partially reduced C3c (200 μg) was radiolabeled with 7.5 μCi of ^{14}C-monoiodoacetic acid (56 mCi/mmol) in the presence of 1% SDS-8M urea at pH 8.5 and subjected to SDS-PAGE. One gel was stained with coomassie blue and the remaining unstained gels were sectioned by reference to the stained gel. The gel segment corresponding to α3-β chain complex was extracted with 1% SDS-8M urea by repeated freezing thawing and was followed by gentle stirring for 2 hr at room temperature. The extract was reduced with 1% 2-mercaptoethanol and subjected to SDS-PAGE. The gel was stained with coomassie blue, scanned at 550 nm, and sectioned into 2 mm segments. The gel segments were placed in scintillation vials and incubated with the solubilization cocktail (Protosol-Toluene-Water, 9:10:1 (v/v/v) for 2 hr at 60°C then for 2 hr at -80°C and finally for 2 hr at 60°C. After addition of Econofluor, the radioactivities were determined by liquid scintillation counter. Similarly, ^{14}C-monoiodoacetic acid-treated B which contains a single radiolabeld thiol residue was processed, and the ratio of radioactivity to protein concentration of B was used as a standard for estimation of the thiol residue in the α3 and β chains.

RESULTS

Partial reduction of iC3b and C3c. Fig. 1 shows the SDS-PAGE of paritally reduced iC3b and C3c. Mild reduction of iC3b with cysteine yielded an intermediate fragment of 143 kd, which was assigned as α1-β chain complex from its molecular weight. A fragment corresponding to α2-β chain complex (118 kd) was not detected. In the case of C3c, an intermediate fragment of 102 kd was detected. This was assigned as α3-β chain complex from its molecular weight and subsequent SDS-PAGE after extraction and reduction of the fragment. In this case too, α2-β chain complex was not detected. These results suggested that the β chain in C3 was linked to the α chain only through the cysteinyl residue on the 27kd α3 fragment and that there is no disulfide linkage between the 43 kd α2 fragment and the β chain.

Radiolabelling of partially reduced C3c Alternative possibility was that, although there are disulfide bonds between the 43kd α2 chain and β chain, these disulfide bonds are rapidly reduced with cysteine so that the α2-β chain complex could not be detected upon SDS-PAGE. If this were the case, free cysteine residues would be detected on the β chain of α3-β chain complex.

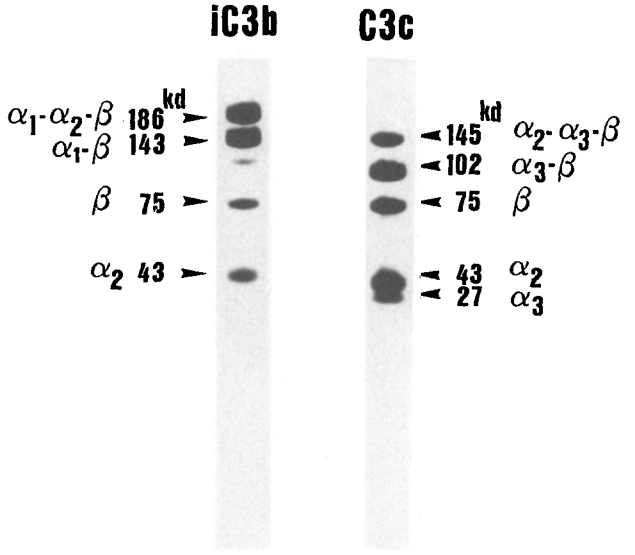

Fig. 1. SDS-PAGE of partially reduced iC3b and C3c. iC3b and C3c were reduced with cysteine and subjected to SDS-PAGE as described in Methods.

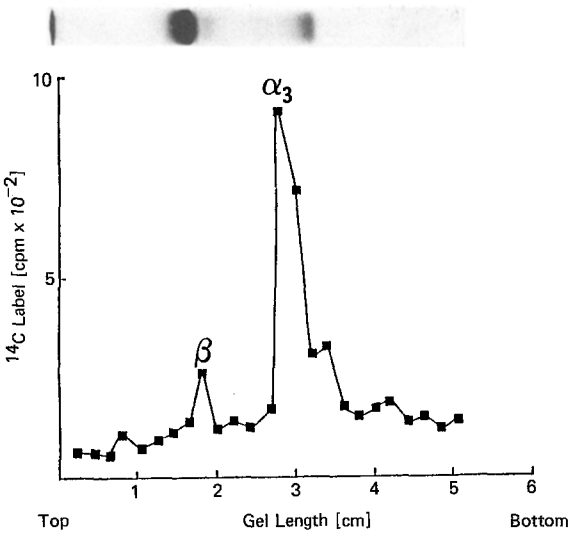

Fig. 2. SDS-PAGE of reduced, radiolabeled α3-β chain complex. ^{14}C-moniodoacetic acid treated α3-β chain complex was reduced with 1% mercaptoethanol and subjected to SDS-PAGE. The gel was stained with coomassie blue (upper panel) and sliced into 2 mm segments. The radioactivity of each segment was determined as described in Methods (lower panel).

To address this possibility, partially reduced C3c was treated with ^{14}C-monoiodoacetic acid and the radiolabeled α3-β chain complex was purified by SDS-PAGE. The purified complex was reduced with mercaptoethanol and subjected to SDS-PAGE. As shown in Fig. 2, two bands of 75kd (β chain) and 27kd (α3 chain) were stained with coomassie blue. The gel was sliced into 2 mm segments and determined for the radioactivity. As shown in Fig. 2, the radioactivity was found to be incorporated mostly into the 27 kd α3 fragment and only slightly into the β chain. Utilizing ^{14}C-monoiodocetic acid-treated B as a standard having a single radiolabelled thiol residue, it was estimated that the radioactivity in the 27kd band represented 0.5 thiol residue per mol, while that in the chain represented 0.1 thiol residue per mol. This results suggests that α3-β chain complex has a single thiol residue on the α3 fragment but not on the β chain and thus makes it unlikely that there is an inter-chain disulfide bond between β and α2 chain.

DISCUSSION

Isenman et al. (14) reported that in the absence of denaturing agent the three intra-chain disulfide bonds on the α chain of C3 were selectively reduced upon treatment of C3 with 2mM dithiothreitol but the inter-chain disulfide bond connecting the α and β chain were only partially reduced, even with 60 mM dithiothreitol. This result suggested that the inter-chain disulfide linkages connecting the α and β chains of C3 are more stable to reduction than the intra-chain disulfide bond.

In the present study, partial reduction of C3c with cysteine produced a complex composed of disulfide-linked α3 and β chains. A free cysteine residue appears on the α3 fragment of α3-β chain complex. This result suggests that the α2 fragment is linked to the α3 fragment via a single disulfide bond and the disulfide bond linking the two α chain fragments is highly susceptible to reduction with cysteine.

Evidence that no free thiol residue was detected on the β chain of α3-β chain complex is strong evidence against the possibility that the α2 chain is also disulfide-linked to the β chain. Recently, sequence analysis of cDNA of murine C3 has revealed that there are two cysteine residues in the 27kd counter part of the α chain and three cystein residues in the vicinity of the C-terminal of β chain (2). If this hold also for human C3, it seems that there are two cysteinyl residues on the α3 fragment; one is forming a disulfide linkage with the β chain and the other is forming a disulfide linkage with the α2 fragment. In addition, it suggests that the two remaining cysteinyl residues on the β chain form an intra-chain disulfide linkage. The inter- and intra-chain disulfide linkages of C3 are diagrammatically presented in Fig. 3.

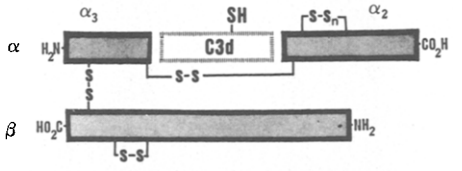

Fig. 3. A possible model of the disulfide-linked C3c. The exact positions of thiol residues forming inter-chain disulfide bonds are unknown.

ACKNOWLEDGEMENTS We are grateful to Prof. J. Koyama for encouragement during this work and to Prof. J.P. Atkinson for his critical reading. We also thank Mrs. P. Hart and L. Whiteley for typing the mansucript. This work was supported by a Grant-in Aid for Scientific Research from Ministry of Education, Science and Culture of Japan, and a grant of Houansha.

REFERENCES

1. Tack, B.F., and Prah, J.W. (1976) Biochemistry 15, 4513-4521.
2. Wetsel, R.A., Lundwall, A.B., Tack, B.F., and Fey, G.H. (1984) Fed. Proc. 43, 1449.
3. Hugli, T.E., Vallota, E.H., and Muller-Eberhard, H.J. (1975) J. Biol. Chem. 250, 1472-1478.
4. Nagasawa, S. and Stroud, R.M. (1977) Immunochemistry 14, 749-756.
5. Pangburn, M.K., Schreiber, R.D., Muller-Eberhard, H.J. (1977) J. Exp. Med. 146, 257-270.
6. Lachmann, P.J., Pangburn, M.K., Oldroyd, R.G. (1982) J. Exp. Med. 156, 205-216.
7. Nagasawa, S., Ichihara, C., and Stroud, R.M. (1980) J. Immunol. 125, 578-582.
8. Nagasawa, S., Kobayashi, C., Yamashita, N., Maki-Szuki, T., and Koyama, J. (1985) J. Biochem. in press.
9. Seya, T. and Nagasawa, S. (1985) J. Biochem. in press.
10. Meuth, J.L., Mergan, E.L., DiScipio, R.G., and Hugli, T.E. (1983) 130, 2605-2611.
11. Nagase, H., and Barrett, A.J. (1981) Biochem. J. 193, 182-192.
12. Fairbanks, G., Steck, T.L., and Wallach, D.F.H. (1971) Biochemistry 10, 2606-2617.
13. Janatova, J., Lorenz, P.E., Schechter, A.N., Prahl, J.W., and Tack, B.F. (1980) Biochemistry 19, 4471-4478.
14. Isenman, D.E., Sundsmo, J.S., and Cooper, N.R. (1980) J. Immunol. 125, 1798-1805.