Biochimica et Biophysica Acta, 670 (1981) 129–133
Elsevier/North-Holland Biomedical Press

BBA 38715

CONFORMATIONAL CHANGES OF THE SUBUNITS C1q, C1r AND C1s OF HUMAN COMPLEMENT COMPONENT C1 DEMONSTRATED BY ^{125}I LABELING

JOHANN BAUER and GUENTER VALET

Max-Planck-Institut für Biochemie, D-8033 Martinsried (F.R.G.)

(Received February 16th, 1981)

Key words: Complement subunit; C1 activation; Protein conformation; (Human)

C1s and C1r proenzymes and enzymes ($C1\bar{s}$, $C1\bar{r}$) and C1q were labeled with ^{125}I. The distribution of the ^{125}I label between H- and L-chain of C1s was only slightly dependent on the state of activation of C1s, and approx. 90% of the label was found in the H-chain. In the C1r proenzyme molecules 50% of the label was incorporated into the H-chain. The C1r H-chain label was reduced to 10% on activation of C1r to $C1\bar{r}$, while the L-chain label increased to 90% of the total label. The presence of either C1s, C1q or C1qs during labeling reduced the C1r H-chain label, although C1r remained in the proenzyme form. The presence of C1s or C1rs enhanced the ^{125}I uptake of C1q in Ca^{2+} or EDTA medium. This was unexpected because one would have anticipated a diminution of the C1q label due to the apposition of C1r and C1s, similarly as it occurs during C1rs complex and C1s dimer formation for the H-chain label of C1s. The results show that C1r and C1q alter their conformation during activation and C1 complex formation.

Introduction

An important problem of the C1 activation mechanism is how the C1r proenzyme molecule is activated upon binding of C1q to antigen-antibody complexes [1–4]. It has been difficult so far to recognize discrete steps in this intramolecular process.

There are two kinds of experiment from which conclusions can be drawn. The first type of experiment concerns the tendency of isolated C1r to spontaneously activate in buffers containing EDTA. Some authors conclude from these experiments that C1r is capable of autoactivating itself [5–6]. This autoactivation is supposed to be an intramolecular action of C1r proenzyme on itself, since it has been shown that $C1\bar{r}$ is not capable of activating C1r [8]. It was also found that the spontaneous activation of C1r in buffers containing EDTA was due to contaminant proteases which cleave C1r during incubation at 37°C [7–8]. Although these experiments suggest that the spontaneous C1r activation is mainly due to trace contamination of isolated C1r with proteases, it cannot be excluded that isolated C1r in a buffer containing EDTA may alter its conformation and come into a transitional state where it is able to be enzymatically active. This enzymatic activity could be present on both C1r subunits and either the subunits could mutually activate each other or alternatively one C1r proenzyme molecule could activate other surrounding C1r proenzyme molecules.

A second type of experiment investigates the C1r activation within the C1 macromolecule. The interpretation of such experiments was that an altered C1r called C1r* is formed during C1 activation at 37°C. This C1r* is still in the proenzyme form, but is capable of cleaving C1r or binding reversibly the protease inhibitor *p*-nitrophenol-*p'*-guanidino-benzoate [8]. The suggestion that C1r proenzyme can change its

Abbreviations: The nomenclature of complement components and subcomponents is that recommended by the World Health Organisation (1968); activated components are indicated by a bar, e.g. $C1\bar{r}$; SDS, sodium dodecyl sulfate.

conformation is further supported by Ca^{2+} binding experiments [9], where the Ca^{2+} binding capacity of C1r was changed after C1r activation and C1rs complex formation. It was also postulated that C1q changes its conformation during a temperature-dependent process before it becomes able to activate C1r. The action of the altered C1q called $C1\bar{q}$ can be inhibited by poly-pentosan-sulfo-ester [10]. It was furthermore suggested that the conformational changes of the C1 subunits which occur during C1 activation are accelerated by the interaction of fluid phase C1 with cell-bound C1 [11,12].

Most of the evidence for conformational changes of the C1 subunits is indirect. In this study we tried to get more direct evidence for conformational changes of the C1 subunits during C1 complex formation and C1 activation by labeling the C1 subunits, either alone or in various combinations in the presence of Ca^{2+} or EDTA with ^{125}I.

Material and Methods

Isolation. C1r and C1s were isolated as proenzymes or enzymes as described [2] and C1q as described by Zubler et al. [13] from human serum. C1r and C1s enzymes were obtained by incubation of the pH 6.9 euglobulin fraction for 20 min at 37°C. The apparent molecular weight of the subunits was determined by a Sephacryl S-300 molecular sieve. C1q had a molecular weight of 400 000, C1r of 180 000, and C1s of 85 000.

Labeling. Carrier free ^{125}I (2 125 mCi/mM) from New England Nuclear (Boston, MA) was used for labeling C1r and C1s proenzymes and enzymes, and C1q according to the Chloramin T method [14]. Specific activities around 0.8 mCi/mg protein were obtained and approx. 40% of the offered ^{125}I was incorporated into the protein. The degree of iodination was such that about 4% of the protein molecules were labeled. The unbound ^{125}I was removed by dialysis twice against a 2 000-fold excess of 0.2 M NaCl/10 mM Tris-HCl buffer, pH 7.5. All labeling and dialysis steps were strictly performed between 0–4°C. Aliquots of the dialysed protein containing between 40 000 and 80 000 cpm were either applied to a polyacrylamide gel or bound to sensitized sheep erythrocytes (EA cells) after C1 reconstitution.

SDS-polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis of radioactive protein samples was performed in 8% polyacrylamide slab gels with 0.1% SDS. The applied protein was pretreated for 7 min at 100°C with 1.5% SDS, at pH 7.2, with or without 2.5% (v/v) mercaptoethanol [15]. Each trace of the slab was cut after electrophoresis into 20 equal slices. The ^{125}I radioactivity of each slice was determined in a Gamma 7000 spectrometer (Beckman, Irvine, CA).

C1r and C1s activation. The labeled proenzyme subunits C1r or C1s were reconstituted with cold C1q, C1s or C1q, C1r subunits and 2 mM Ca^{2+} to C1. This was important for the subsequent experiments where the labeled proenzyme within the reconstituted C1 was activated on EA cells in order to obtain cleavage of C1r and C1s into the respective H- and L-chains. The C1 macromolecule was bound for this purpose to optimally sensitized sheep erythrocytes suspended in a low ionic strength veronal buffered saline with sucrose with 0.03% gelatin [16] and incubated for 15 min at 37°C to activate the cell-bound C1. The cells were separated from the suspension buffer after this incubation by centrifuging the samples into a cushion of dibutyl-phthalate in order to separate quantitatively the cell-bound C1 from the unbound C1 in the supernatant. The cell pellet was suspended in a veronal buffered saline/EDTA with gelatin to dissociate the C1 into the single subcomponents C1q, C1r and C1s. The sample was recentrifuged and the supernatant which contained the ^{125}I labeled and activated C1r or C1s subunits was applied to an SDS-polyacrylamide gel.

Results

^{125}I-labeled C1s proenzyme on SDS-polyacrylamide gel under reducing condition exhibited one major peak at 85 000 and two minor peaks at 60 000 (H-chain) and 25 000 (L-chain) indicating that C1s activation during isolation and radioactive labeling was less than 15% (Fig. 1:1). C1s enzyme, however, showed one major peak at 60 000 (H-chain) and one minor peak at 25 000 (L-chain) (Fig.1:2–5). The distribution of the ^{125}I label between H- and L-chain was slightly dependent on the state of activation of C1s. Approx. 95% of the label was incorporated into the H-chain, if C1s was labeled as a proenzyme (Fig. 1:4) and 90% if it was labeled in the activated form

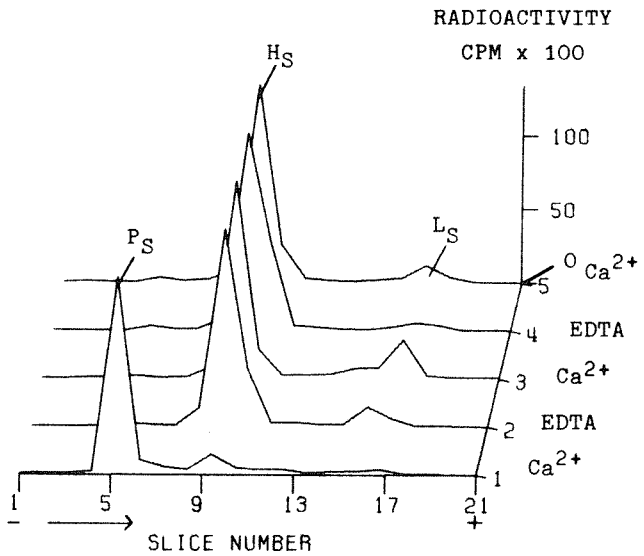

Fig. 1. SDS-polyacrylamide gel electrophoresis of ^{125}I-labeled C1s proenzyme (1) and enzyme (2–5). C1s of curves 2, 3 was labeled in its activated form, C1s of curves 4, 5 was labeled as proenzyme.

(Fig. 1:2), both times in the presence of 5 mM EDTA. The H-chain label decreased to 90% in the proenzyme (Fig. 1:5) and to 80% in the enzyme (Fig. 1:3), if labeling was performed in the presence of Ca^{2+} at a concentration of 5 mM.

^{125}I-labeled C1r proenzyme exhibited one major peak at 90 000 and two minor peaks at 60 000

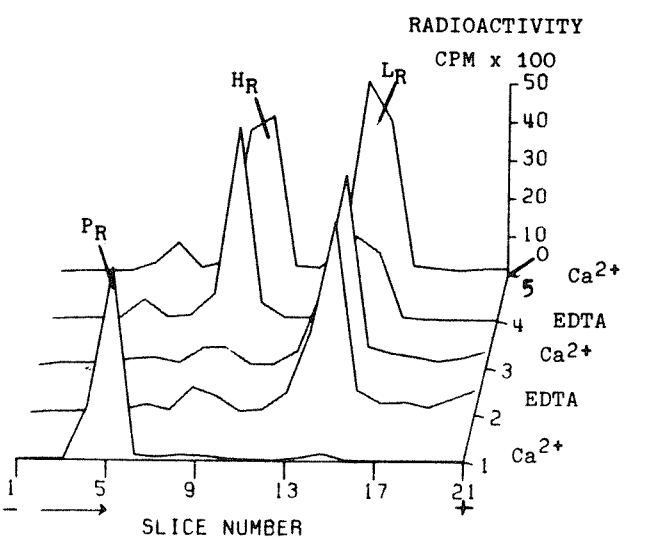

Fig. 2. SDS-polyacrylamide gel electrophoresis of ^{125}I-labeled C1r proenzyme (1) and enzyme (2–5). C1r of curves 2, 3 was labeled in its activated form, C1r of curves 4, 5 was labeled as proenzyme.

(H-chain) and 35 000 (L-chain) indicating that the activation of C1r to $\overline{C1r}$ enzyme was less than 8%. Approx. 50% of the label was found in the H-chain, if C1r was labeled in the proenzyme form in the presence of Ca^{2+} (Fig. 2:5). Labeling of the C1r proenzyme in the presence of EDTA enhanced the H-chain label to 60% (Fig. 2:4). A decrease to 10% of the label was, however, observed in the H-chain, if C1r was labeled in the activated form irrespective of the presence of Ca^{2+} or EDTA (Fig. 2:3, 2). The results show that ^{125}I labeling of the H-chain is diminished to one-fifth on activation of C1r to $\overline{C1r}$.

In further experiments we tested whether various incubation conditions would alter the labeling behaviour of C1r proenzyme. Varying the concentration of Ca^{2+} between 3 and 20 mM did not affect C1r labeling. Similar observations were obtained for labeling in buffers containing EDTA indicating that the difference of incorporation between Ca^{2+} and EDTA buffers could not be further increased by a 7-fold increase of the respective Ca^{2+} or EDTA concentrations. However, the simultaneous presence of C1q or C1s in the reaction mixture significantly influenced the labeling of C1r proenzyme. The presence of C1s during labeling decreased the H-chain label of C1r to 42%, the presence of C1q to 36% and the presence of C1qs to 25%. The influence of the subunits on C1r was independent of the presence of Ca^{2+} or EDTA. This indicates that an interaction of the C1 subunits in EDTA occurred without firm complex formation, which changed the ^{125}I labeling of C1r proenzyme in such a way as to simulate activated C1r although C1r remained in the proenzyme form as was shown by subsequent electrophoresis of the labeled C1r in SDS-polyacrylamide gels.

The specific radioactivity of all samples was similar whether they contained only one subunit (C1q, C1r, C1s) or various combinations of C1 subunits (C1qr, C1qs, C1rs, C1qrs). Although the specific radioactivity of the total protein of the samples was similar, the specific activity of the individual C1 subunits changed. If equal quantities of C1q were labeled in the presence of 3 mM Ca^{2+} together with C1r (molar ratio 1 : 2), C1s (molar ratio 1 : 4), or with C1rs complex (molar ratio 1 : 1), the result was that 50% (Fig. 3:4), 70% (Fig. 3:3) or 75% (Fig. 3:2) of the label was incorporated into the C1q molecule. This corresponds to an increase of the C1q specific activity to 1.4- and

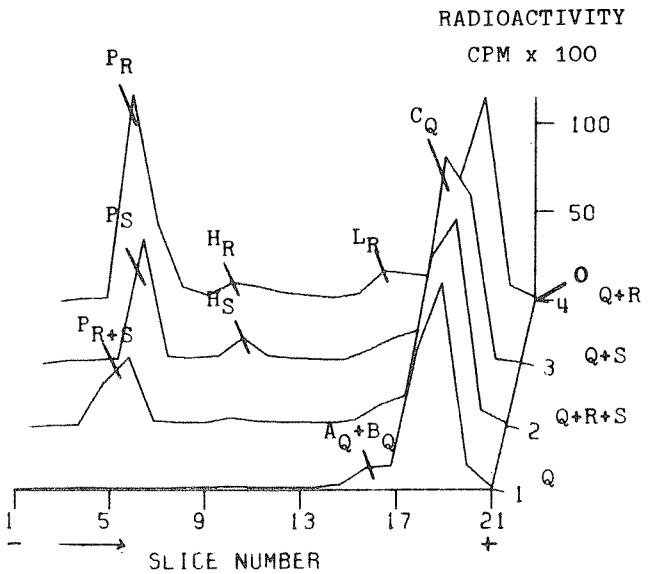

Fig. 3. SDS-polyacrylamide gel electrophoresis of ^{125}I-labeled C1q. C1q was labeled alone (1) and together with equivalent amounts of C1r (4), C1s (3) and C1rs (2).

1.5-fold of its normal value, while the specific activities of C1s and C1rs decreased to 0.6 and 0.5 of the initial specific activity. The result indicates that the specific activity of C1q increases as the interaction of the C1 subunits increases. In further experiments C1q, C1r and C1s were labeled in the presence of Ca^{2+} in a molar ratio of 1 : 2 : 4 in order to maximize the interaction of C1r and C1s with C1q. 58% of the label was in C1q although C1q represented only 33% of the total protein. This corresponds to 1.7-fold increase of the C1q specific activity.

The same result (58% label for 33% protein) was obtained when the experiment was performed in the presence of EDTA, showing that the enhanced specific C1q radioactivity was not due to a diminution of the C1rs label because of firm apposition of C1rs to C1q during C1 complex formation in the presence of Ca^{2+}. Alteration of the labeling behaviour caused by Ca^{2+}-dependent complex formation was, however, shown for the C1r and C1s proenzyme molecules. C1s labeled together with equal amounts of C1r incorporated 50% of the label in presence of EDTA, but only between 35 and 40% of the label in presence of Ca^{2+}. The Ca^{2+}-dependent diminution of the C1s label concerned the C1s H-chain suggesting that C1rs complex formation occurs via the C1s H-chains. The decrease of the H-chain label from 95 to 90%, observed when labeling C1s in presence of Ca^{2+} or EDTA (Fig. 1:5, 4), suggests that the Ca^{2+}-dependent C1s dimer formation also occurs via the H-chains.

Discussion

The main result of this study is that C1r and C1q change their ^{125}I labeling behaviour quite obviously upon variation of the ionic and protein environment during labeling, while the labeling behaviour of C1s is less dependent on these conditions. The C1r H-chain label decreases to one-fifth on activation of C1r to $C1\bar{r}$. It is of interest that the C1r molecule can be forced by the presence of the subunits C1q, C1s or C1qs and Ca^{2+} or EDTA environment into a conformation, which in its labeling behaviour resembles closely activated C1r, although the molecule remains in the proenzyme form. These results may indicate that a C1r* molecule is formed within the C1 macromolecule [8]. The same change of the C1r conformation cannot be induced, if C1r proenzyme alone is labeled in buffers containing EDTA. On the contrary an increase of the ^{125}I label in the H-chain of C1r proenzyme from 50 to 60% in presence of EDTA is observed. The H-chain becomes more accessible to the attachement of ^{125}I, which may explain the higher speed of spontaneous C1r activation in buffers containing EDTA [7]. The H-chain is cleaved into smaller pieces on prolonged incubation, a fact which is also suggestive of a higher accessibility of the C1r H-chain for the attack by contaminant proteases. Similar observations are obtained with trypsin, where the C1r H-chain is cleaved into small peptides in presence of EDTA but not in buffers containing Ca^{2+} [17].

The diminution of the C1s H-chain label by C1rs complex formation was earlier described by Villiers et al. [18], who labeled C1r and C1s enzymes in presence of Ca^{2+} and EDTA. Our results obtained from the labeling of the proenzymes agree with them. The conclusion from Colomb's and our results would be that the C1s H-chain comes by apposition into the interior of the C1rs complex or the C1s dimer and is therefore less accessible to ^{125}I.

The alteration of the C1q labeling behaviour upon interaction of C1q with the other C1 subunits, however, cannot be explained in the same way, because it does not depend on the presence of Ca^{2+} and because a 2-fold excess of C1rs molecules increases the speci-

fic radioactivity of C1q from 1.5- to 1.7-fold. These results suggest that the presence of C1s or C1rs enchances the accessibility of C1q for ^{125}I. The largest part of the ^{125}I is incorporated into the C-chain of C1q [19,20] and probably within the C-chain the tyrosine residue at position 5 is mainly labeled by ^{125}I [19,21]. It seems likely that the interaction of the C1rs complex with the collagen part of C1q [22, 23] changes the conformation of the NH_2 terminal region of the C1q molecule near the S-S linkages.

Although the results show that C1r and C1q change their labeling behaviour very significantly during complex formation and activation, one can only speculate about the molecular aspect of the inherent conformational changes. It is known that the major amount of the hypoiodite generated from I^- during the labeling reaction at pH 7.5 binds to dissociated [24]. The alteration of the labeling behaviour of the protein can then be explained in two ways: (a) Tyrosine residues become more or less accessible by alteration of their position with respect to the protein surface. (b) Tyrosine residues alter their degree of dissociation due to changes of interaction with neighbour amino acids. It is not possible to conclude from our data which of the two mechanisms accounts for the changes of ^{125}I labeling behaviour of the C1 subunits upon complex formation and activation. It is interesting that the increase of the C1r and C1q label occurs both times in parts of the molecule which seem important for the intramolecular activation process. Furthermore the changes of the molecular conformation occur independently of the presence of Ca^{2+} or EDTA. The firm complex formation is therefore apparently not required, the presence of other C1 subunits is, however, necessary. One may speculate that alterations of position or dissociation of tyrosine residues may play a direct role during C1r activation within the C1 macromolecule. As known from other protease reaction mechanisms, tyrosine residues can function as H-donators during cleavage of peptide bonds [25].

References

1 Valet, G. and Cooper, N.R. (1974) J. Immunol. 112, 1667–1673
2 Valet, G. and Cooper, N.R. (1974) J. Immunol. 112, 339–350
3 Ziccardi, R.J. and Cooper, N.R. (1976) J. Immunol. 116, 496–504
4 Gigli, I., Porter, R.R. and Sim, R.B. (1976) Biochem. J. 157, 541–548
5 Ziccardi, R.J. and Cooper, N.R. (1976) J. Immunol. 116, 504–509
6 Arlaud, G.J., Villiers, C.L., Chesne, S., Painter, R.H. and Colomb, M.G. (1980) 4th. Int. Congr. Immunol., Paris 1980, Abst. 15.1.01
7 Bauer, J., Weiner, E.M. and Valet, G. (1980) J. Immunol. 124, 1514–1514
8 Dodds, A.W., Sim, R.B., Porter, R.R. and Kerr, M.A. (1978) Biochem. J. 175, 383–390
9 Villier, C.L., Arlaud, G.J., Painter, R.H. and Colomb, M.G. (1980) FEBS Lett. 117, 289–294
10 Loos, M., Borsos, T. and Kopp, H.J. (1972) J. Immunol. 108, 683–688
11 Medicus, R.G. and Chapuis, R.M. (1980) J. Immunol. 125, 390–395
12 Medicus, R.G. and Chapuis, R.M. (1980) 5th Eur. Compl. Workshop, Lund, 3
13 Zubler, R.H., Lange, G., Lambert, A.H. and Miescher, P.H. (1976) J. Immunol. 116, 232–235
14 McConahey, P. and Dixon, F.J. (1966) Int. Arch. Allergy Appl. Immunol. 29, 185–193
15 Davies, B. (1964) Ann. N.Y. Acad. Sci. 121, 404–427
16 Borsos, T. and Rapp, H.J. (1963) J. Immunol. 91, 826–832
17 Valet, G., Bauer, J. and Kahle, H. (1981) H-S.Z. Physiol. 362, 26–26
18 Villiers, C.L., Arlaud, G.J., Reboul, A., Thielens, N. and Colomb, M.G. (1980) J. Immunol. 124, 1543–1543
19 Lin, T. and Fletcher, D.S. (1978) Immunochemistry 15, 107–117
20 Heusser, C., Boesman, M., Knobel, H.R., Jacot-Guillarmod, H. and Isliker, H. (1975) Immunochemistry 12, 213–219
21 Reid, K.B.M. (1977) Biochem. J. 161, 247–251
22 Reid, K.B.M., Sim, R.B. and Faiers, A.P. (1977) Biochem. J. 161, 239–245
23 Valet, G., Kahle, H. and Timpl, R. (1978) Z. Immun. Imm. 155, 71–71
24 Seon, B.K., Roholt, O.A. and Pressman, D. (1970) Bio-Chim. Biophys. Acta 221, 114–118
25 Blackburn, S. (1976) Enzyme structure and function pp. 169–219, Marcel Dekker Inc., New York