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Structural features of the first component of human complement, C1, as revealed by surface iodination

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Lactoperoxidase-catalysed surface iodination and sucrose-gradient ultracentrifugation were used to investigate the structure of human complement component C1. 1. Proenzymic subcomponents C1r and C1s associated to form a trimeric $C1r_2$–C1s complex (7.6 S) in the presence of EDTA, and a tetrameric $C1r_2$–$C1s_2$ complex (9.1 S) in the presence of Ca^{2+}. Iodination of the 9.1 S complex led to a predominant labelling of C1r (70%) over C1s (30%), essentially located in the b-chain moiety of C1r and in the a-chain moiety of C1s. 2. Reconstruction of proenzymic soluble C1 (15.2 S) from C1q, C1r and C1s was partially inhibited when C1s labelled in its monomeric form was used and almost abolished when iodinated C1r was used. Reconstruction of fully activated $\overline{C1}$ was not possible, whereas hybrid C1q–$C1r_2$–$\overline{C1s}_2$ complex was obtained. 3. Iodination of proenzymic or activated C1 bound to IgG–ovalbumin aggregates led to an equal distribution of the radioactivity between C1q and $C1r_2$–$C1s_2$. With regard to C1q, the label distribution between the three chains was similar whether C1 was in its proenzymic or activated form. Label distribution in the $C1r_2$–$C1s_2$ moiety of C1 was the same as that obtained for isolated $C1r_2$–$C1s_2$, and this was also true for the corresponding activated components. However, two different labelling patterns were found, corresponding to the proenzyme and the activated states.

The first component of human complement, C1, is a Ca^{2+}-dependent complex of glycoproteins C1q, C1r and C1s (Müller-Eberhard, 1979; Reid & Porter, 1981). It is largely agreed that C1 consists of one molecule of C1q (mol.wt. 410000), two molecules of C1r (mol.wt. 83000–95000) and two molecules of C1s (mol.wt. 85000–88000) (reviewed by Sim, 1981). These molecules are thought to be organized in two weakly interacting moieties, C1q and a tetrameric $C1r_2$–$C1s_2$ association (Nagasawa *et al.*, 1974; Gigli *et al.*, 1976; Arlaud *et al.*, 1979). Activation of C1 occurs upon binding, through C1q, to antigen–antibody aggregates, triggering sequential modification of proenzymic C1r and C1s into active serine proteinases. The activation proceeds through cleavage of at least one peptide bond in each monochain subcomponent, resulting in two-chain active proteinases. The *N*-terminal a-chain is thought to mediate protein–protein interactions (Arlaud *et al.*, 1980*a*), whereas the di-isopropyl phosphorofluoridate-sensitive catalytic site is located in the *C*-terminal b-chain (Barkas *et al.*, 1973; Takahashi *et al.*, 1975; Sim & Porter, 1976).

Lactoperoxidase-catalysed iodination of proteins is thought to reveal tyrosine residues exposed to the solvent and can be used to evaluate accessible areas in protein associations. This approach was used to study C1 structure, in search of possible conformational changes resulting from C1 activation.

Abbreviations used: The nomenclature of complement components is that recommended by the World Health Organisation (1968); activated components are indicated by an overbar; SDS, sodium dodecyl sulphate; IgG, immunoglobulin G.

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Experimental

Materials

C1q, activated $\overline{C1r}$ and activated $\overline{C1s}$ were purified as described by Arlaud *et al.* (1979).

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Proenzyme C1s and proenzyme C1r were prepared as described by Arlaud *et al.* (1980*b*) and Villiers *et al.* (1982) respectively.

Purified C1r (C1r), C1s (C1s) and C1q were determined from their A_{280} by using respectively $A^{1\%}_{1cm} = 11.5$ (Sim *et al.*, 1977), 9.5 (Sim *et al.*, 1977) and 6.8 (Reid *et al.*, 1972). Molecular weights were taken as 85000 for $\bar{C}1r$ (C1r), 85000 for $\bar{C}1s$ (C1s) and 410000 for C1q.

Insoluble immune aggregates were prepared as described by Arlaud *et al.* (1979). Yeast alcohol dehydrogenase, horse spleen apoferritin, ox liver catalase and lactoperoxidase were obtained from Calbiochem. $Na^{125}I$ was purchased from Amersham International.

Methods

Sucrose-density-gradient ultracentrifugation. Samples were sedimented at 4°C for 15h (110000*g*; $r_{av.}$ 9cm) as described by Martin & Ames (1961) by using a TST 54 rotor in a Kontron TGA 50 ultracentrifuge. Proteins were determined by the method of Bradford (1976). Yeast alcohol dehydrogenase (7.6S), ox liver catalase (11.4S) and horse spleen apoferritin (17.6S) were used as standards for the estimate of $s_{20,w}$.

SDS/polyacrylamide-gel electrophoresis. Unreduced samples were prepared as described previously (Arlaud *et al.*, 1980*a*). Reduced samples were incubated for 1h at 37°C in 4M-urea/1% (w/v) SDS/50mM-dithiothreitol/0.1M-Tris/HCl, pH8.0, and then alkylated by 140mM-iodoacetamide for 20min at 37°C. Gels containing 6 (C1r or C1s) or 12% (C1q) acrylamide were prepared as described by Fairbanks *et al.* (1971). Proteins were stained by Coomassie Blue and scanned at 550nm.

Iodination of individual C1 subcomponents and $C1r_2$–$C1s_2$ complexes. $C1r_2$–$C1s_2$ and $\bar{C}1r_2$–$\bar{C}1s_2$ complexes were prepared from C1r ($\bar{C}1r$) and C1s ($\bar{C}1s$) incubated in equal amounts for 30min at 0°C in 5mM-triethanolamine/145mM-NaCl/5mM-$CaCl_2$, pH7.4.

$\underline{C}1q$, $\underline{C}1r$, $\bar{C}1r$, C1s, $\bar{C}1s$, $C1r_2$–$C1s_2$ and $\bar{C}1r_2$–$\bar{C}1s_2$ were labelled with ^{125}I by a method originally described by Heusser *et al.* (1973) and modified by Arlaud *et al.* (1980*a*). The average labelling was estimated to be 0.1–0.2 atom of iodine per protein molecule. SDS/polyacrylamide-gel electrophoresis was used to evaluate the distribution of radioactivity in the different proteins and their chains. Before electrophoresis, proenzymic C1r and $C1r_2$–$C1s_2$ were activated for 60min at 37°C in 5mM-triethanolamine/145mM-NaCl/10mM-EDTA, pH7.4; proenzyme C1s was activated under the same conditions, but with 10% (w/w) activated $\bar{C}1r$. Gels were cut into 1mm slices and counted for radioactivity.

Iodination of activated C1 bound to IgG–ovalbumin aggregates. (IgG–ovalbumin)–$\bar{C}1$ complexes were prepared from 50ml of human serum and 50mg of immune complexes as described previously (Arlaud *et al.*, 1979) and suspended in 1ml of 120mM-NaCl/5mM-$CaCl_2$/20mM-Tris/HCl, pH7.0, at 0°C. Iodination was performed as described by Arlaud *et al.* (1980*a*) and led to an average labelling of 0.02 atom of iodine per protein molecule. Labelled (IgG–ovalbumin)–$\bar{C}1$ complexes were centrifuged for 5min at 10000*g* ($r_{av.}$ 8.7cm) and washed three times with 5ml portions of 120mM-NaCl/5mM-$CaCl_2$/20mM-Tris/HCl, pH7.0. C1r plus $\bar{C}1s$, then C1q, were extracted as described previously (Arlaud *et al.*, 1979). For each extract the distribution of radioactivity in the different proteins and chains was analysed by SDS/polyacrylamide-gel electrophoresis.

Iodination of proenzyme C1 bound to IgG–ovalbumin aggregates. (IgG–ovalbumin)–C1 complexes were prepared in the presence of proteinase inhibitors as described previously (Arlaud *et al.*, 1980*b*). Iodination was as for the individual subcomponents, but in the presence of 1mM-*p*-nitrophenyl *p'*-guanidinobenzoate and led to an average labelling of 0.02 atom of iodine per protein molecule. The absence of any significant activation at the end of iodination was verified by SDS/polyacrylamide-gel electrophoresis.

After centrifugation for 5min at 10000*g* ($r_{av.}$ 8.7cm), labelled (IgG–ovalbumin)–C1 complexes were incubated three times in 5ml portions of 120mM-NaCl/5mM-$CaCl_2$/20mM-Tris/HCl, pH7.0, for 30min at 37°C with centrifugation after each incubation.

The treatment led to the activation of $\underline{C}1$ bound to IgG–ovalbumin aggregates. Activated C1r and C1s, then C1q, were extracted and analysed as described for (IgG–ovalbumin)–$\bar{C}1$ complexes.

Iodination of C1q bound to IgG–ovalbumin aggregates. C1r and C1s were removed from (IgG–ovalbumin)–$\bar{C}1$ complexes by washing with 20mM-EDTA / 60mM-NaCl / 50mM-Tris / HCl, pH7.0, as described by Arlaud *et al.* (1979). Iodination of the resulting (IgG–ovalbumin)–C1q complexes was as described by Arlaud *et al.* (1980*a*). Labelled C1q (0.02 atom of iodine per protein molecule) was extracted as described by Arlaud *et al.* (1979) and analysed by SDS/polyacrylamide-gel electrophoresis.

C1s esterase activity. $\bar{C}1s$ esterase activity was measured from the hydrolysis of *p*-tosyl-L-arginine methyl ester as described by Arlaud *et al.* (1980*a*).

Results

Iodination of C1q

Iodination of C1q in the fluid phase resulted in the preferential labelling of the C-chain, whereas the A-

Table 1. *Distribution of ^{125}I radioactivity in C1q*

Iodinated protein	Radioactivity (%) A-chain	B-chain	C-chain
Soluble C1q	8.3	14.0	77.7
(IgG–ovalbumin)–C1q	5.2	6.5	88.3
(IgG–ovalbumin)–C1 } (IgG–ovalbumin)–C1 }	17.8	15.6	66.6

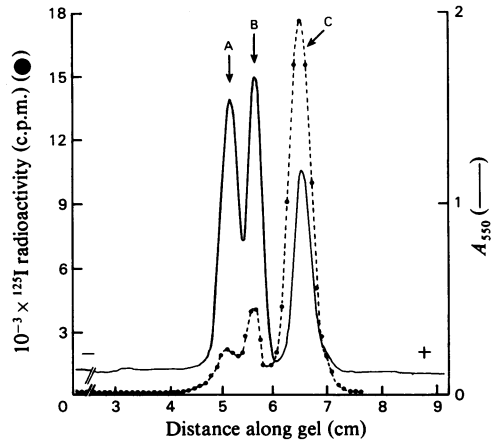

Fig. 1. *Distribution of radioactivity on purified C1q iodinated in the presence of EDTA*

Reduced sample was run on an SDS/12%-polyacrylamide gel. ——, Protein; ●, ^{125}I.

and B-chains took together only 22% of the bound label (Table 1 and Fig. 1). No difference was observed whether the labelling was performed in the presence of calcium or of EDTA.

When iodination was performed on C1q bound to IgG–ovalbumin aggregates, the A- and B-chains on one hand, the C-chain on the other hand, took respectively less and more label than in soluble C1q (Table 1).

Iodination of C1r–C1s associations

In the presence of 5mM-EDTA, C1r (7.1S) and C1s (4.6S) associated into a complex sedimenting at 7.6S on sucrose-gradient ultracentrifugation. This association is likely a C1r–C1r–C1s complex, equivalent to the trimeric complex described previously for the activated subcomponents by Arlaud *et al.* (1980*a*). Proenzymic C1r and C1s incorporated equivalent amounts of ^{125}I upon individual labelling in the presence of EDTA. Under the same conditions, iodination of a mixture of equimolar amounts of C1r and C1s led to a label distribution of 64 and 36% in each protein respectively.

In the presence of 5mM-Ca^{2+}, a tetrameric $C1r_2$–$C1s_2$ complex sedimented at 9.1S, corresponding to the association described by Ziccardi & Cooper (1976) and by Tschopp *et al.* (1980). Again, C1r and C1s labelled individually in the presence of Ca^{2+} incorporated equivalent amounts of ^{125}I, whereas a mixture of equimolar amounts of C1r and C1s shared respectively 70 and 30% of the total bound label.

Table 2. *Distribution of ^{125}I radioactivity in C1r and C1s after labelling of $C1r_2$–$C1s_2$ and of C1 complexes bound to IgG–ovalbumin aggregates*

Iodinated protein	Radioactivity (%) $C\bar{1}r$ a-chain	$C\bar{1}r$ b-chain	$C\bar{1}s$ a-chain	$C\bar{1}s$ b-chain
$C1r_2$–$C1s_2$	19	81	84	16
$C\bar{1}$	19	81	85	15
$C1r_2$–$C1s_2$	2	98	77	23
$C\bar{1}$	7	93	74	26

The distribution of ^{125}I label on the chains of each component of proenzymic $C1r_2$–$C1s_2$ or activated $C\bar{1}r_2$–$C\bar{1}s_2$ complex is shown in Table 2. In each case, the b-chain moiety of C1r and the a-chain moiety of C1s were predominantly labelled. In addition, the relative amount of label on the a-chains was decreased in the case of the activated $C\bar{1}r_2$–$C\bar{1}s_2$ complexes.

Iodination of proenzyme or activated C1 bound to IgG–ovalbumin aggregates

C1 complex sedimenting at 15.2S was reconstructed, in the presence of Ca^{2+}, from a mixture of C1q and proenzymic C1r and C1s in a 1:2:2 molar ratio (Fig. 2*a*). The contribution of C1q, C1r and C1s to the complex was established from individual labelling of the subcomponents. When iodinated C1r was used, C1 reconstruction was nearly completely inhibited, whereas assembly of the $C1r_2$–$C1s_2$ complex was not affected. A mixture of C1q and activated subcomponents $C\bar{1}r$ and $C\bar{1}s$, in the presence of Ca^{2+}, did not lead to $C\bar{1}$ formation (Fig. 2*b*), whereas using proenzyme C1r together with C1q and activated C1s allowed the reconstruction of 'hybrid' $C\bar{1}$ (Fig. 1*c*). Reconstructed C1 was able to bind to IgG–ovalbumin aggregates and to undergo activation upon incubation of the (IgG–ovalbumin)–C1 complex for 30min at 37°C.

As the fluid-phase reconstruction of C1 was never a complete process, (IgG–ovalbumin)–C1 was found to be more reliable for studying the distribution of ^{125}I in C1 after surface labelling. In these conditions, the radioactivity bound to C1 was distributed equally between C1q and $C1r_2$–$C1s_2$. On C1q itself, the A- and B-chain on one hand, the C-chain on the other hand were respectively more and less heavily iodinated than in C1q bound to

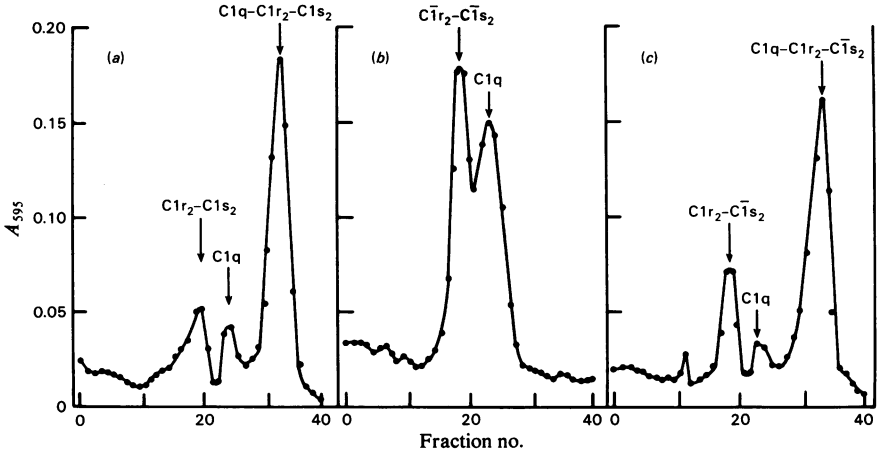

Fig. 2. *Sucrose gradient ultracentrifugation of C1 reassembled from its purified subcomponents*
C1q (60 μg), C$\bar{1}$r or C1r (35 μg) and C1s or C$\bar{1}$s (35 μg) in 50 μl of 5 mM-triethanolamine/145 mM-NaCl/5 mM-$CaCl_2$, pH 7.4, were incubated for 30 min at 0°C before centrifugation. Fractions (120 μl) were collected from top of the gradient and proteins were determined by the technique of Bradford (1976). (*a*) C1q + C1r + C1s; (*b*) C1q + C$\bar{1}$r + C$\bar{1}$s; (*c*) C1q + C1r + C$\bar{1}$s.

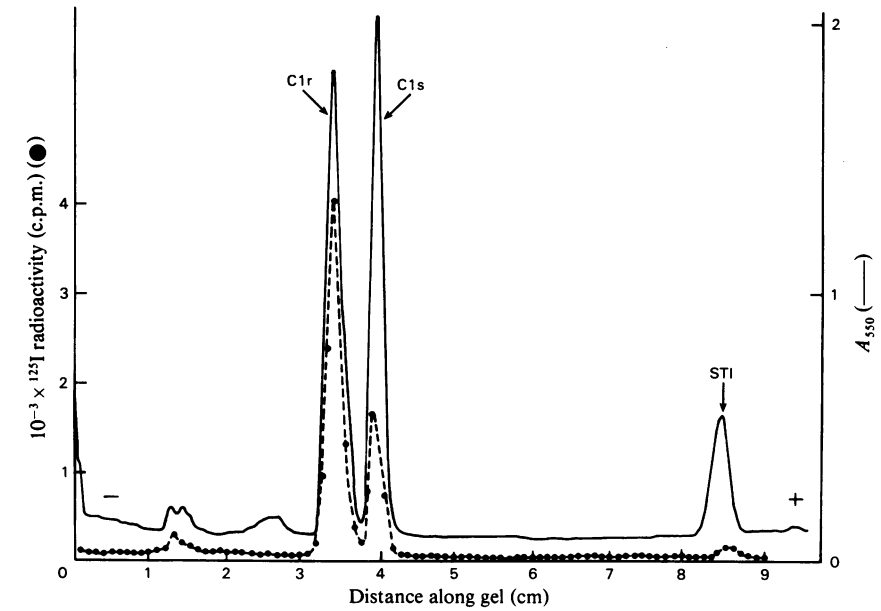

Fig. 3. *Distribution of ^{125}I in C1r and C1s after surface iodination of C1 bound to IgG–ovalbumin aggregates*
Unreduced sample was run on an SDS/6%-polyacrylamide gel. ——, Protein; ●, ^{125}I. Abbreviation used: STI, soya-bean trypsin inhibitor.

IgG–ovalbumin aggregates (Table 1). Within $C1r_2$–$C1s_2$, C1r took 74% of the label compared with 26% in C1s (Fig. 3). Within each proenzymic component, a predominant iodination was observed in the a-chain moiety of C1s and in the b-chain moiety of C1r (Table 2).

After iodination of activated C$\bar{1}$ bound to IgG–ovalbumin aggregates, the radioactivity bound

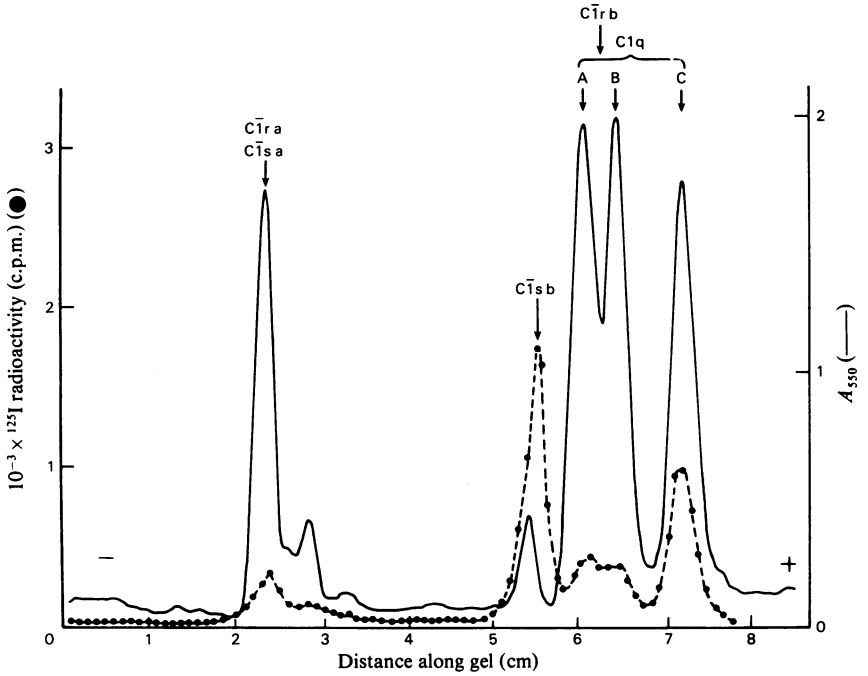

Fig. 4. *Distribution of ^{125}I in C1q, C1̄r and C1̄s after surface iodination of C1̄ bound to IgG–ovalbumin aggregates*
The reduced sample was run on an SDS/12%-polyacrylamide gel. ——, Protein; ●, ^{125}I.

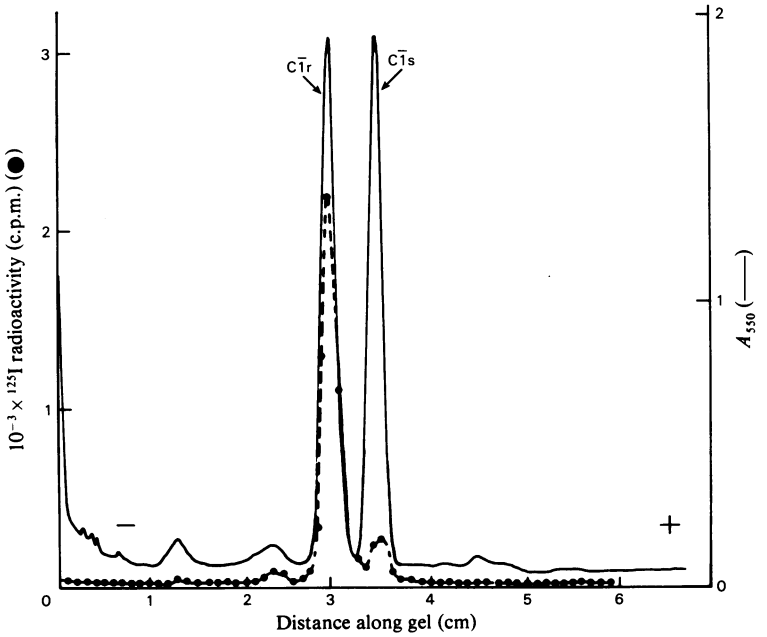

Fig. 5. *Distribution of radioactivity in C1r and C1s after surface iodination of C1 bound to IgG–ovalbumin aggregates*
The unreduced sample was run on an SDS/6%-polyacrylamide gel. ——, Protein; ●, ^{125}I.

to C1̄ was shared equally between C1q and $C1r_2$–$C1s_2$ (Fig. 4). The distribution of ^{125}I in the chains of C1q was the same as that measured after labelling of this subcomponent in proenzymic C1 bound to IgG–ovalbumin aggregates. Within the $C1r_2$–$C1s_2$ complex itself, 90% of the label was

found on $\bar{C}1\bar{r}$ and 10% on $\bar{C}1\bar{s}$ (Fig. 5); the a- and b-chains of C1r took respectively 7 and 93% of the radioactivity bound to the whole molecule, whereas the corresponding distribution within C1s was 74 and 26%.

Discussion

Lactoperoxidase-catalysed iodination of isolated C1q led to a predominant labelling of the C-chain, a result obtained with the same technique by others (Heusser *et al.*, 1973; Lin & Fletcher, 1978; Tenner *et al.*, 1981). Ca^{2+} did not affect either the distribution of radioactivity in the C1q chains or the total amount of label bound to the whole molecule. This last result contrasts with the observation of Bauer & Valet (1981) on C1q iodination by the chloramine-T method, showing that Ca^{2+} is responsible for a 3-fold decrease of the total label. Proenzymic C1r and C1s associated, in the presence of Ca^{2+}, into a tetrameric $C1r_2$–$C1s_2$ complex, whereas the formation of a trimeric $C1r_2$–C1s association in the presence of EDTA was established from surface iodination and sucrose-gradient-ultracentrifugation results; an homologous trimeric association was obtained with activated subcomponents (Arlaud *et al.*, 1980*a*).

Surface iodination of the proenzymic $C1r_2$–$C1s_2$ complex showed a predominant labelling of C1r, although to a less extent than in the case of the activated complex (Arlaud *et al.*, 1980*a*). These results suggest that C1s is relatively buried in the $C1r_2$–$C1s_2$ association, which is in apparent contradiction with the structure proposed by Tschopp *et al.* (1980), in which C1s is located at both ends of a rod-like complex. The distribution of ^{125}I label on the chains of C1r or C1s was comparable whether the iodination was performed on the proenzymic $C1\bar{r}_2$–$C1\bar{s}_2$ complex or on the activated $\bar{C}1\bar{r}_2$–$\bar{C}1\bar{s}_2$ complex, which suggests that the overall organization of both types of complexes is similar, although not identical.

When C1q was labelled after binding to IgG–ovalbumin aggregates, the iodination pattern of the A-, B- and C-chains was modified in the same way as described previously by Lin & Fletcher (1978). When iodination was performed on proenzymic or activated C1 bound to IgG–ovalbumin aggregates, the distribution of the label in C1 was about 50% on C1q and 50% on $C1r_2$–$C1s_2$, a result that differs significantly from that of Folkerd *et al.* (1980).

The overall labelling of $C1r_2$–$C1s_2$ was not modified by the association with C1q, as the label distribution inside the proenzymic $C1r_2$–$C1s_2$ complex was the same whether iodination was performed in the fluid phase or on C1 bound to IgG–ovalbumin aggregates; this was also valid for the activated $C1r_2$–$C1s_2$ complex. These findings add to previous observations that C1q and $C1r_2$–$C1s_2$ behave in C1 as loosely interacting entities (Nagasawa *et al.*, 1974; Gigli *et al.*, 1976; Arlaud *et al.*, 1979). However, surface iodination discriminated between two apparently different structural states, corresponding to the proenzymic and the activated forms of $C1r_2$–$C1s_2$.

Soluble C1 could be reassembled from C1q and proenzymic C1r and C1s in the presence of Ca^{2+}, as reported previously (Valet & Cooper, 1974; Assimeh & Painter, 1975; Ziccardi & Cooper, 1976). Iodination of C1q and of Ca^{2+}-dependent dimeric C1s was without effect on C1 reconstruction, whereas iodination of C1s in its monomeric form, which also prevents Ca^{2+}-dependent dimerization of this subcomponent (Chesne *et al.*, 1982), partially inhibited this reconstruction. In the case of C1r, iodination almost completely prevented C1 reassembly, whereas the Ca^{2+}-dependent $C1r_2$–$C1\bar{s}_2$ formation was not affected. Soluble activated $\bar{C}1$ could not be reconstructed from C1q and activated $\bar{C}1\bar{r}$ and $\bar{C}1\bar{s}$ in the presence of Ca^{2+}, but reassembly of hybrid C1q–$C1r_2$–$C1\bar{s}_2$ complex was obtained from C1q, proenzyme C1r and activated $\bar{C}1\bar{s}$. The last observations tend to indicate that C1r is located at the C1q–($C1r_2$–$C1s_2$) interface, although it was not possible to show any C1q–C1r interaction in a mixture of these two subcomponents.

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References

Arlaud, G. J., Sim, R. B., Duplaa, A.-M. & Colomb, M. G. (1979) *Mol. Immunol.* **16**, 445–450

Arlaud, G. J., Chesne, S., Villiers, C. L. & Colomb, M. G. (1980*a*) *Biochim. Biophys. Acta* **616**, 105–115

Arlaud, G. J., Villiers, C. L., Chesne, S. & Colomb, M. G. (1980*b*) *Biochim. Biophys. Acta* **616**, 116–129

Assimeh, S. N. & Painter, R. H. (1975) *J. Immunol.* **115**, 488–494

Barkas, T., Scott, G. K. & Fothergill, J. E. (1973) *Biochem. Soc. Trans.* **1**, 1219–1220

Bauer, J. & Valet, G. (1981) *Biochim. Biophys. Acta* **670**, 129–133

Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254

Chesne, S., Villiers, C. L., Arlaud, G. J., Lacroix, M. B. & Colomb, M. G. (1982) *Biochem. J.* **201**, 61–70

Fairbanks, G., Stech, T. L. & Wallach, D. F. H. (1971) *Biochemistry* **10**, 2606–2617

Folkerd, E. J., Gardner, B. & Hughes-Jones, N. C. (1980) *Immunology* **41**, 179–185

Gigli, I., Porter, R. R. & Sim, R. B. (1976) *Biochem. J.* **157**, 541–548

Heusser, C., Boesman, M., Nordin, J. H. & Isliker, H. (1973) *J. Immunol.* **110**, 820–828

Lin, T. Y. & Fletcher, D. S. (1978) *Immunochemistry* **15**, 107–117
Martin, R. G. & Ames, B. N. (1961) *J. Biol. Chem.* **236**, 1372–1379
Müller-Eberhard, H. J. (1979) in *Modulation of Protein Function* (Atkinson, D. E. & Fox, C. F., eds.), pp. 219–232, Academic Press, New York and London
Nagasawa, S., Takahashi, K. & Koyama, J. (1974) *FEBS Lett.* **41**, 280–282
Reid, K. B. M., Lowe, D. M. & Porter, R. R. (1972) *Biochem. J.* **130**, 749–763
Reid, K. B. M. & Porter, R. R. (1981) *Annu. Rev. Biochem.* **50**, 433–464
Sim, R. B. & Porter, R. R. (1976) *Biochem. Soc. Trans.* **4**, 127–129
Sim, R. B., Porter, R. R., Reid, K. B. M. & Gigli, I. (1977) *Biochem. J.* **163**, 219–227
Sim, R. B. (1981) *Methods Enzymol.* **80**, in the press
Takahashi, K., Nagasawa, S. & Koyama, J. (1975) *FEBS Lett.* **55**, 156–160
Tenner, A. J., Lesavre, P. H. & Cooper, N. R. (1981) *J. Immunol.* **127**, 648–653
Tschopp, J., Villiger, W., Fuchs, H., Kilchherr, E. & Engel, J. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 7014–7018
Valet, G. & Cooper, N. R. (1974) *J. Immunol.* **112**, 1667–1673
Villiers, C. L., Duplaa, A.-M., Arlaud, G. J. & Colomb, M. G. (1982) *Biochem. Biophys. Acta* **700**, 118–126
World Health Organisation (1968) *Bull. W.H.O.* **39**, 935–936
Ziccardi, R. J. & Cooper, N. R. (1976) *J. Immunol.* **116**, 496–503