Activation of C1 [and Discussion]

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Activation of C1

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The first component of complement, C1, is a calcium-dependent complex of two loosely interacting subunits: C1q, responsible for the binding of activators to C1; C1r2–C1s2, which supports the autoactivation potential of C1, together with the proteolytic activity of activated C̄1 on its two substrates, C4 and C2.

Isolated dimeric C1r2 is able to autoactivate through an intradimer cross-proteolysis; this capacity is lost when C1r2 is associated with two molecules of C1s inside the calcium-dependent C1r2–C1s2 subunit; this capacity is again observed in reconstituted C1.

A model for reconstituted soluble C1 is proposed, based on electron microscopy, neutron diffraction, ultra-centrifugation, various biochemical findings, as well as functional properties of C1 or of its subcomponents. The flexible rod-like structure of C1r2–C1s2 is folded around two arms of C1q, with the catalytic domains of C1r and C1s inserted inside the cone defined by the C1q stalks.

Activation of C1 which, *in vivo*, is controlled by C1 inhibitor, can be achieved by various activators, such as immune complexes; it appears to result from the suppression of a negative control and resides in a positive modulation of the intrinsic autocatalytic potential of C1r inside C1.

Activation of the classical pathway of complement is triggered at the level of C1, a calcium-dependent complex of subcomponents C1q, C1r and C1s. Recently several exhaustive reviews have been devoted partly or entirely to the activation of the classical pathway (Porter 1980; Reid & Porter 1981; Loos 1982; Reid 1983), which all reveal a great variety of activators. Immune complexes of IgG1, IgG2, IgG3, IgM and artificial polymers or aggregates of these same immunoglobulins have been known for a very long time as activators of C1 *par excellence*. More recently, it has clearly been shown that polyanions, such as monocatenary or bicatenary DNA, RNA, bacterial lipopolysaccharides, uric acid crystals, heparinoids, as well as small polysaccharides, extrinsic proteases such as activated factor XII fragment or even several viral membranes, are also able to activate C1. Activation of C1 can be distinguished experimentally into two phases: (i) binding of various activators to C1, which can be observed at 0 °C, is achieved by subcomponent C1q; (ii) subsequent activation of C1, a temperature-dependent process, is a function of subcomponents C1r and C1s: C1r acquires a proteolytic activity and subsequently activates C1s which assumes the proteolytic activity of C1 on its two natural substrates, C4 and C2, to contribute to the formation of the C3 convertase of the classical pathway. This dual binding and catalytic role of C1 corresponds to two distinct structural entities in this complex: C1q, on one hand, and C1r2–C1s2, on the other hand, as discussed previously (Arlaud *et al.* 1979). In fact in normal human serum approximately 25–30% of the C1 is believed to be present as free subunits (Ziccardi 1984) and dilution of C1 below its

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physiological concentration leads to its progressive dissociation into C1q and C1r2–C1s2 (Kilchherr *et al.* 1982). Association constants of between 2×10^7 and 7×10^7 M^{-1} have been evaluated for these subcomponents inside proenzyme C1 (Kilchherr *et al.* 1982; Hughes-Jones & Gorick 1982; Siegel & Schumaker 1983), with an increase of about 10 times when Cl is bound to activating immune complexes (Hughes-Jones & Gorick 1982; Siegel & Schumaker 1983) or incubated at low ionic strength (Ziccardi 1984). Direct evidence for the presence of C1q and C1r2–C1s2 subunits in C1 has also been provided by Villiers *et al.* (1982) from the dissociating effect of citrate on C1, and by Villiers *et al.* (1984), by a similar dissociating effect of diamino compounds.

Structure of the two subunits of C1

The C1q subunit

The knowledge of C1q structure has been based largely on early observations of the purified protein in the electron microscope, where this protein appears as a bunch of tulips, with six peripheral globular regions connected each by a fibrillar strand merging then into a common central bundle. Detailed biochemical analysis of C1q indicates that this large protein (molecular mass 459300 Da) consists of 18 polypeptide chains (6A, 6B, 6C), each of 225 amino acid residues, arranged in threes (A + B + C) and interconnected by a disulphide bridge (via the C chains). Complete sequence data on the polypeptide chains reveal that they consist of a collagen-like region of 78–81 amino acid residues, close to the N-terminal residue, whereas the rest of the chains has a non collagen-like structure. The collagen-like structure accounts for the fibrillar structure of the strands, arranged in classical triple helices with Gly, X, Y, repeating sequences, whereas the peripheral globular parts consist of non collagen-like sequences. At about half-way along the collagen-like region, the fibrillar triple helix strands diverge from the central bundle to lead to each globular region. This essential feature of C1 can be explained by the replacement of a glycine residue by an alanine residue at position 36 in the C chain and the insertion of a threonine residue between two Gly-X-Y triplets in the A chain at position 39. From electron microscopic observations on isolated C1q and crosslinked reconstituted C1, the fibrillar strands diverge from the main axis of the molecule with an average angle of 50° (Poon *et al.* 1983); a value of 45° has been calculated from neutron scattering of purified C1q in solution (Perkins *et al.* 1984).

The dual structure of C1q has been studied by selective proteolysis of the molecule and functional analysis of the residual fragments: digestion of C1q by pepsin leads to a collagen-like fragment which retains the capacity of native C1q for binding to the C1r2–C1s2 subcomponent (Siegel & Schumaker 1983), to fibronectin (Reid 1983) and also to cellular C1q receptors (Arvieux *et al.* 1984); digestion of C1q by collagenase leaves the six globular regions intact with a capacity to compete with total C1q for the binding to several activators of C1, such as immune complexes. The model shown in figure 1 takes into consideration the different structural and functional data available on isolated C1q or C1q incorporated in C1.

The C1r2–C1s2 subunit

This subunit of C1, which comes apart very easily from C1q, consists of two different proteins C1r and C1s, associated in the presence of calcium; this association is easily disrupted in the presence of calcium chelators, such as EDTA and the dissociation yields one C1r2 dimer and two molecules of C1s.
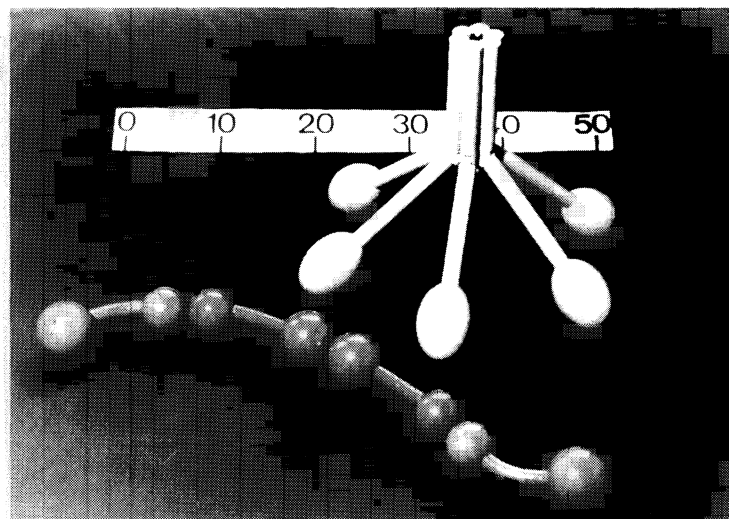
FIGURE 1. Models of C1q and C1r2–C1s2. The proposed models of human C1q (right) and C1r2–C1s2 (left) are shown. The ruler indicates 50 nm.

Dimeric proenzyme C1r2 is an association of two polypeptide chains (total molecular mass 180000 Da); this dimer is stable even in the presence of EDTA and dissociates only at acid pH (Arlaud *et al.* 1980). Preliminary electron microscopic observations and data from limited proteolytic attack of C1r are consistent with the presence, in each monomer of C1r, of two globular domains, one slightly larger than the other, with an interdomain linker. Isolated proenzyme C1s is a monochain molecule (molecular mass 86000 Da) able to form artificial dimers, in the presence of calcium. Preliminary electron microscope observations also indicate a two-domain structure in C1s. The calcium-dependent C1r2–C1s2 association has been studied by electron microscopy and ultracentrifugation (Tschopp *et al.* 1980) as well as by neutron diffraction (Boyd *et al.* 1983). All observations show that the isolated subunit of C1 has a very elongated shape which can be roughly assimilated to a cylinder of 2–3 nm in diameter and 50–60 nm in length. Different biochemical data, based mainly on the proteolysis of $\overline{\text{C1r}}$ and functional analysis of proenzyme and activated C1r, are all consistent with the model proposed in figure 1 for isolated C1r2–C1s2. The slightly inverted **S**-shaped association consists of a central part made of the two monomers of C1r, in mutual contact through their globular domain (catalytic domain), while a molecule of C1s is attached at both ends of C1r2; C1r and C1s interaction is at the level of distinct domains (interaction domains). C1r2–C1s2 appears thus to consist of several domains connected by inter-domain linkers and is likely to be very flexible, to fulfil the functional requirements of C1 activation, as will be described later. The proposed model may also account for the involvement of calcium in C1r2–C1s2 subunit, as C1r, C1s and C1r2–C1s2 all bind calcium (Villiers *et al.* 1980).

AUTOACTIVATION POTENTIAL OF ISOLATED C1r

The above-described C1r2–C1s2 subunit does not activate upon prolonged incubation at 37 °C. In contrast, after treatment of the subunit by EDTA, the isolated C1r dimer is able to autoactivate spontaneously at 37 °C and to activate subsequently the accompanying C1s. Similarly, when C1q is incubated with the C1r2–C1s2 subunit, in the presence of calcium, the

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resulting reconstituted C1 is also able to autoactivate spontaneously at 37 °C. These observations, which are summarized in figure 2, support the hypothesis that the autocatalytic potential of C1r is similar in C1 or in the isolated C1r2 dimer. Thus the isolated C1r2 dimer is a valid model to study the activation of C1.
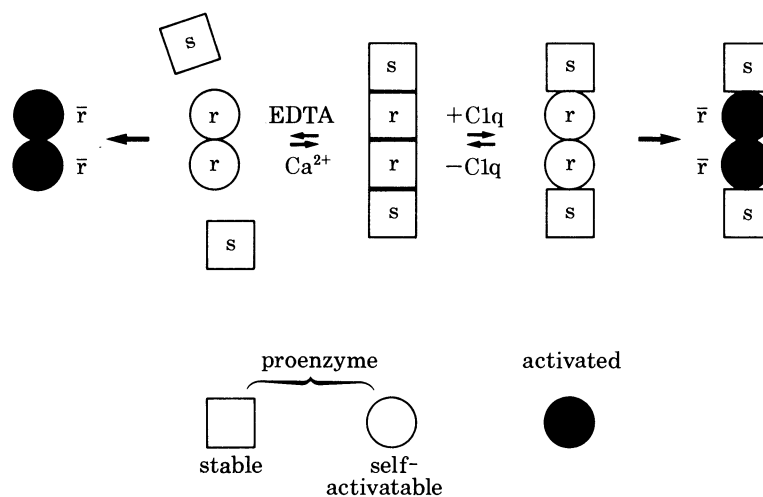

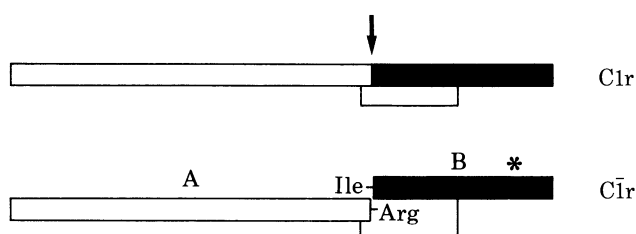FIGURE 2. Activatibility of C1r as a function of its state of association.

FIGURE 3. Proteolytic cleavage of human C1r upon activation. The relative positions of the interchain disulphide bridge and of the active serine (*) are indicated. The overall scheme is also valid for human C1s.

Activation of C1r, and this is also valid for the activation of C1s, proceeds with the proteolytic cleavage of a single Arg–Ile bond in the monochain proenzyme (figure 3); it results in the formation of an active protease consisting of two chains, A and B. Kinetic studies, based on the appearance of these two chains, show that C1r spontaneous autoactivation proceeds according to a sigmoid, comparable to the sigmoid observed for the activation of C1 (figure 4); these studies also show that low concentrations of diamino compounds, which have been shown to dissociate C1 into C1q and C1r2–C1s2 (Villiers et al. 1984), almost completely abolish C1 activation. The sigmoid shape of the activation process can be explained by the superimposition of two different reactions: (i) the initial autoactivation process, which has been shown to be an intra-dimer reaction (Arlaud et al. 1980) and represents the key event in the activation of C1; (ii) an additional contribution of activated C̄1r generated during the activation process, this interdimer activation being demonstrable from the action of exogenous activated C̄1r on C1r autoactivation (Villiers et al. 1982).

Detailed intra-dimer activation

Activation of isolated C1r can be monitored, after reduction of the interchain disulphide bridge (figure 3), by quantification of the characteristic A and B chains of activated C$\bar{1}$r (figure 5). The activation process is also associated with and correlated to an increase in the intrinsic fluorescence of the protein (figure 5, Villiers *et al.* 1983). However, the crucial question is to know whether this fluorescence increase, mainly attributable to tryptophan residues, precedes or follows the cleavage of the Arg–Ile bond generating the active two-chain structure. On the basis of the amino acid sequence of human C1r B chain (Arlaud & Gagnon 1983), and of
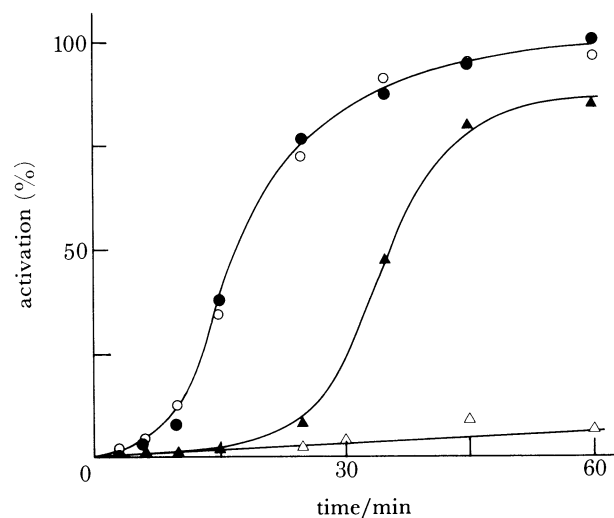

FIGURE 4. Autoactivation of C1r and C1. Effect of lysine. Activation of purified proenzyme C1r was measured at 37 °C in 145 mM NaCl, 5 mM triethanolamine (pH 7.4), in the absence (●) or in the presence (○) of 20 mM lysine. Activation of C1 was measured at 37 °C in 5 mM CaCl$_2$, 145 mM NaCl, 5 mM triethanolamine (pH 7.4), in the absence (▲) or in the presence (△) of 20 mM lysine.
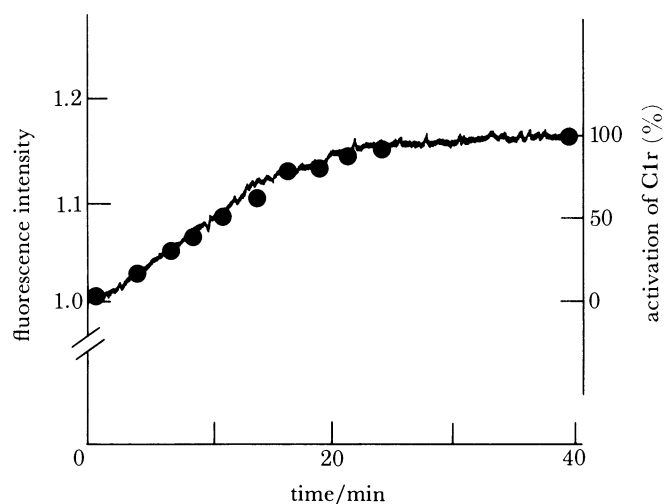

FIGURE 5. Intrinsic-fluorescence increase upon activation of proenzyme C1r. C1r was incubated at 37 °C in 145 mM NaCl, 5 mM triethanolamine (pH 7.4). The intrinsic-fluorescence emission (continuous line) was measured (excitation at 289 nm, emission at 333 nm), as well as the percentage of C1r activation (●).
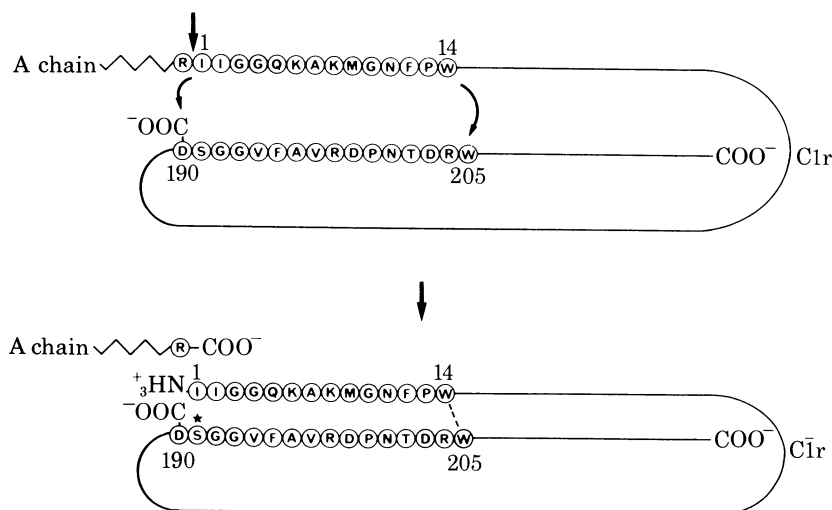
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FIGURE 6. Tentative explanation for the increase of C1r intrinsic fluorescence upon activation. Amino acid residues 1–14 and 190–205 (C$\bar{1}$r B chain numbering) are indicated. Other features, such as disulphide bridges, are not depicted.

the mechanism of active site formation in serine proteinases, a tentative explanation has been proposed (Villiers *et al.* 1983). As shown schematically in figure 6, it is likely that the formation of C$\bar{1}$r active site involves an ion pair between the amino group of the newly formed N-terminal isoleucine residue of C1r B chain and the carboxyl group of the aspartic acid residue at position 190 of this chain (Arlaud & Gagnon 1983). This interaction could bring closer the tryptophan residues located at positions 14 and 205 in the C$\bar{1}$r B chain (figure 6), thus accounting for the fluorescence increase observed during C1r activation. A plot of the hydrophilicity profile of C$\bar{1}$r B chain according to the method of Hopp & Woods (1981) shows that tryptophan residues at positions 14 and 205 are both located in areas of comparable hydrophobicity levels, thus reinforcing the likelihood of the above hypothesis. Also consistent is the observation that C1s intrinsic fluorescence is stable on activation, and that tryptophan 205 is not found at homologous position in C$\bar{1}$s B chain, whereas isoleucine 1, tryptophan 14 and aspartic acid 190 are all present at identical or equivalent positions in C$\bar{1}$s (Sim *et al.* 1977; Carter *et al.* 1983).

How are the cleavages mediated?

Thus, if it is likely that once the Arg–Ile bond is cleaved in each monomer of the C1r2 dimer, the formation of the active sites follows the mechanism common to other serine proteinases, the question of how the cleavages are mediated arises. Obviously, as the activation is an intradimer process, two mechanisms are possible *a priori*: either each monomer cleaves itself, or one monomer cleaves the other, and conversely (cross-activation). Based upon its homology with the amino acid sequence and the three-dimensional structure of trypsin and chymotrypsin, a computer-generated three-dimensional model of the catalytic chain of C$\bar{1}$r has been prepared (Caporale *et al.* 1983). Such a model allows the precise location of the active site and of the peptide bond cleaved during activation, showing that the two sites are distant from each other, in such a way that, inside a catalytic chain, a contact between them is physically not possible.

This indicates, therefore, that a monomer cannot cleave itself, which would lead to the conclusion that activation of the C1r2 dimer is a cross-activation. This is in agreement with earlier observations (Arlaud *et al.* 1980) that C1r self-activation requires the dimeric C1r2 structure: activation of isolated C1r is inhibited in the acid pH range (4.5–6.5) which coincides with the dissociation of the C1r2 dimer, whereas no inhibition occurs in the alkaline pH range (7.0–10.0) where the C1r2 dimer is stable.
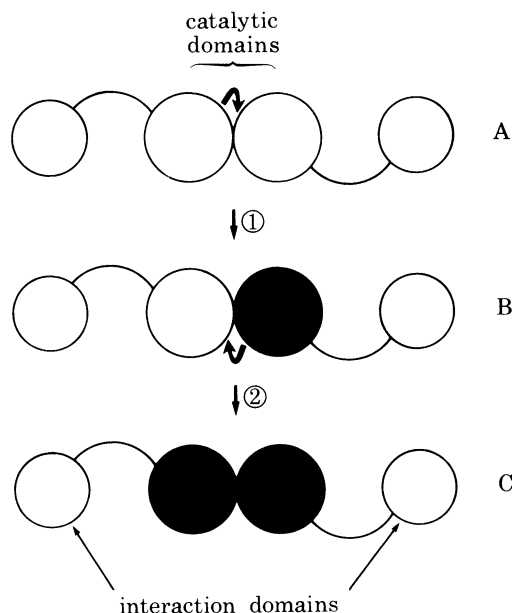

FIGURE 7. Proposed structure and intrinsic activation mechanism of C1r2 dimer. The cleavage reactions are symbolized by arrows. The activation state of the central catalytic domains is represented: (○) proenzyme state; (●) activated state.

The putative structure of the C1r2 dimer is shown schematically in figure 7. Each monomer comprises one catalytic domain and one interaction domain, connected by an inter-domain. The catalytic domains are centrally located, whereas the interaction domains, located at both ends of the dimer, are thought to mediate C1r–C1s interactions. Again, two alternative activation mechanisms are possible (figure 7). First, an asymmetrical mechanism, which would require two steps: a first step where one monomer cleaves the second one (a reaction mediated by a pro-site); a second step where the second monomer cleaves in turn the first one (a reaction then mediated by an active site). Secondly, a symmetrical mechanism, where both reactions would be concomitant, and mediated by the two pro-sites. The last mechanism would lead directly from state A (entirely proenzyme C1r) to state C (entirely activated C̄1r) (figure 7). Symmetrical and asymmetrical mechanisms seem equally possible. In both cases, the cleavages would be mediated by the B chain moieties located in the central catalytic domains.

STRUCTURE AND ACTIVATION OF C1

A model of human C1 has been built, which takes into account the known physicochemical and structural properties of C1q, C1r2–C1s2 and C1, as well as the biological functions of C1, namely activation, expression of activity, and control of both processes. The models used for

the isolated subunits C1q and C1r2–C1s2 are shown in figure 1. C1q was built according to the model proposed by Reid & Porter (1976). In the case of C1r2–C1s2, the model is mainly an interpretation of the electron microscope pictures obtained by Tschopp *et al.* (1980), as described above.
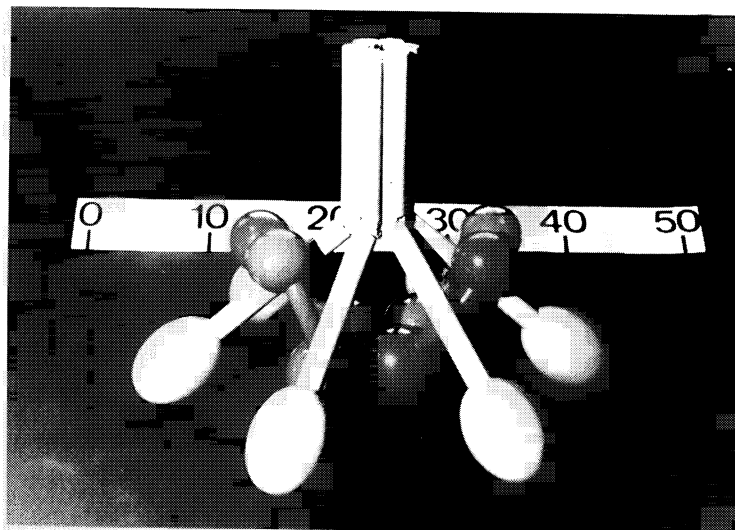

FIGURE 8. Model of C1. C1 is assembled from C1q and C1r2–C1s2 (see figure 1) as described in the text. The ruler indicates 50 nm.

Assembly of C1 can be realized by placing the rod-like, inverted **S**-shaped C1r2–C1s2 complex underneath C1q across the C1q arms, then bending each end of the C1r2–C1s2 complex around two opposite C1q arms so that both C1s catalytic domains (located at both ends of C1r2–C1s2) come into contact with the centrally located catalytic domains of C1r (figure 8). In the resulting model, the overall shape of the C1r2–C1s2 complex would be that of a distorted **8**, with a clear separation between two groups of domains, associated with two specific functions: the interaction domains, located on the outside part of the C1q stalks, would mediate the interactions between the C1r2–C1s2 subunit and C1q; the catalytic domains, located inside the cone defined by the C1q stalks, would mediate the whole catalytic function of C1, that is, self-activation and proteolytic activity.

The proposed model is compatible with electron microscope observations of C1 (Poon *et al.* 1983) and neutron diffraction data (Perkins *et al.* 1984) which both agree with a rather compact model, in which part of the C1r2–C1s2 tetramer would be centrally located, some of it lying outside. The close vicinity of the four catalytic domains of C1r and C1s is also compatible with the C1 activation function: it allows autoactivation of the C1r catalytic domains, which, once activated, can in turn activate the corresponding domains of C1s. The model would also allow accessibility of the $\overline{C1s}$ active site (which could be located on the outside part of the C1s catalytic domain) to C4 and C2, the natural substrates of C1. In the same way, it is possible to envisage that C1 inhibitor, which is a highly elongated molecule (Odermatt *et al.* 1981), would have access to both $\overline{C1r}$ and $\overline{C1s}$ active sites, to fulfil its function of control of $\overline{C1}$ activity.

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Conclusion

Activation of C1 can be visualized as directed by the autocatalytic potential inherent in the C1r2 dimer. This potential, associated with the energy level of the molecule (Arlaud *et al.* 1980; Ziccardi 1982) appears to be dependent on the association states of C1r, as well as on the nature of the effectors acting on C1 (figure 9). Thus, the isolated C1r2 dimer is activatable, whereas formation of the calcium-dependent C1r2–C1s2 complex abolishes the activation potential,
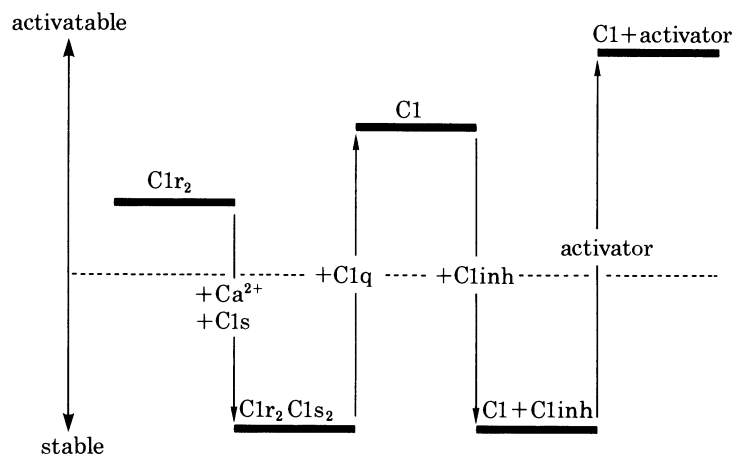

Figure 9. Modulations of C1r autocatalytic potential. The autocatalytic potential of C1r, associated with its energy level, is depicted as a function of the association states of C1r, and of the effectors acting on C1.

which is restored upon interaction with C1q leading to the formation of C1 (figure 9). The natural tendency of C1 can be considered as to self-activate, this function being submitted *in vivo* to a dual control: a negative control by C1-inhibitor, which again abolishes the autocatalytic potential (Ziccardi 1982); a positive control by activators, able to release the control exerted by C1 inhibitor (figure 9). Thus, activation of C1 can be considered as the result of the suppression of a negative control.

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Discussion

Linda A. Fothergill. (*Department of Biochemistry, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, U.K.*). Could Professor Colomb tell us where the calcium atoms are located in his model? How many calcium atoms are there in the C1 complex?

M. G. Colomb. Calcium binding sites have been demonstrated in the interaction domain of C1r and are likely to be present also in the corresponding domain of C1s. Calcium binding to C1q has also been shown. No data on calcium binding to the whole C1 complex has yet been obtained. The only available data relate to the isolated subcomponents and the C1r2–C1s2 subunit, which all bind calcium.
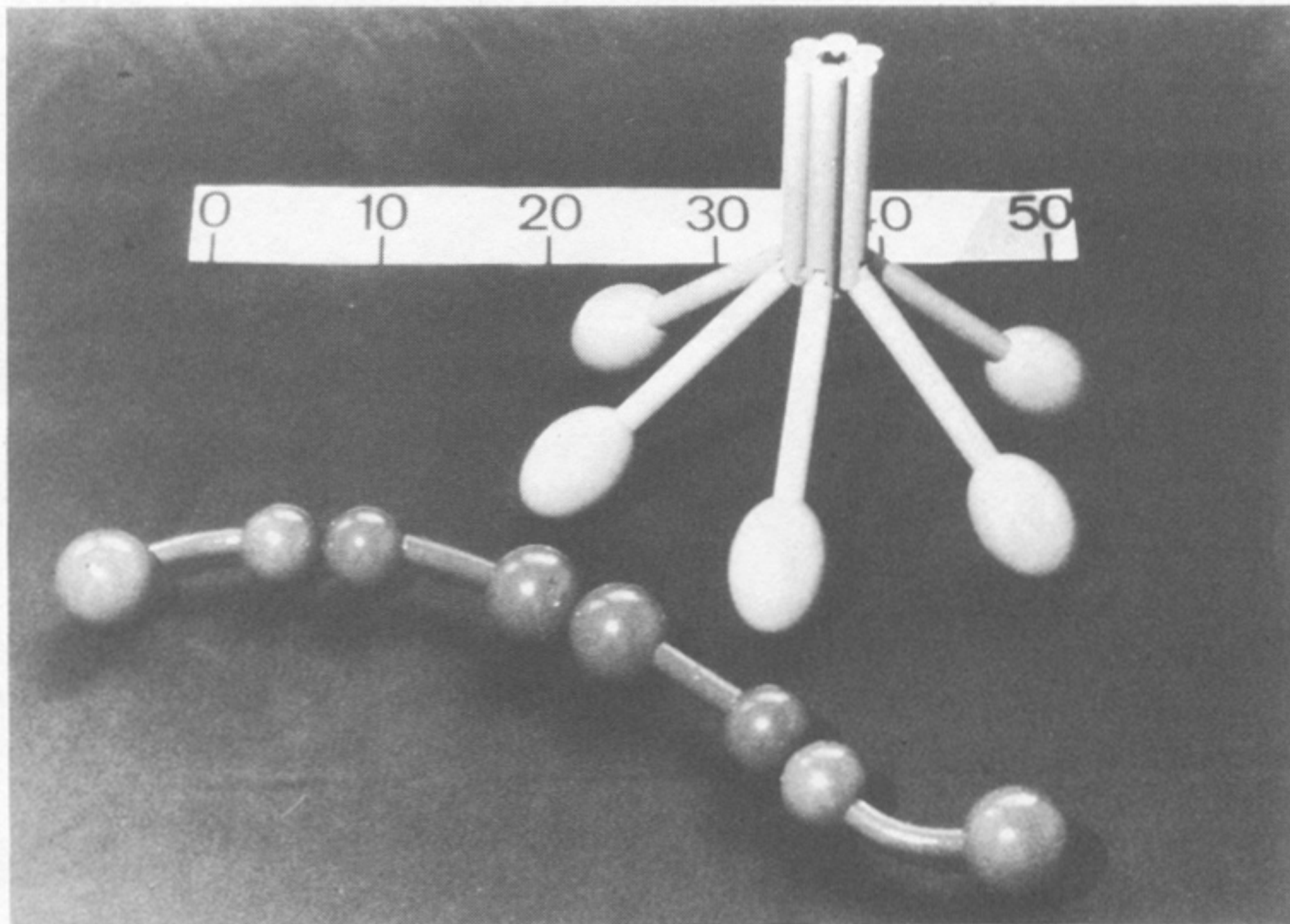
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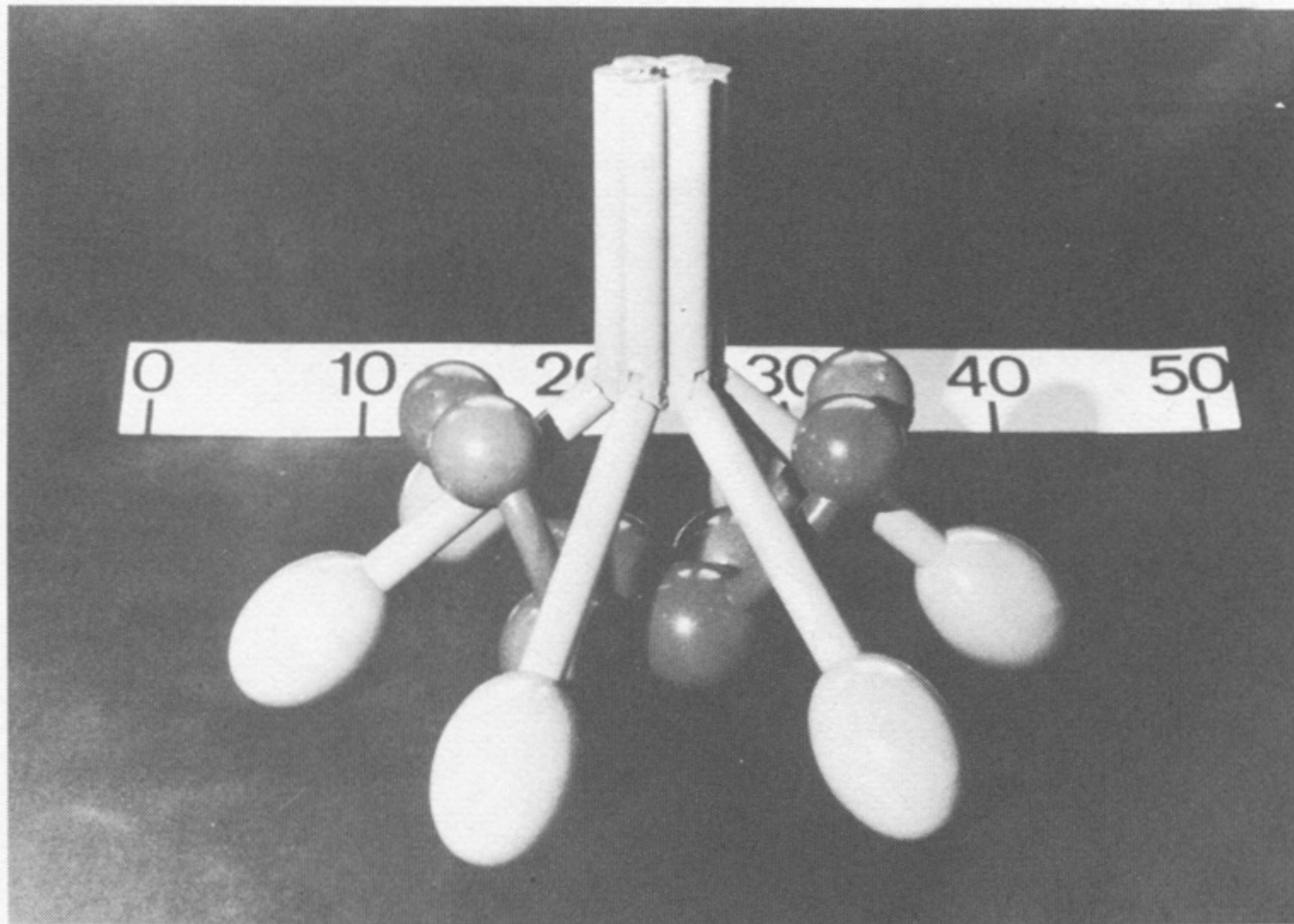
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