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Immunology

Sulfation of tyrosine residues increases activity of the fourth component of complement

(complement activation/sulfates/plasma proteins)

GLEN L. HORTIN*, TIMOTHY C. FARRIES†, JAMES P. GRAHAM*, AND JOHN P. ATKINSON†

*Department of Pediatrics and †Howard Hughes Medical Institute, Washington University School of Medicine, Saint Louis, MO 63110

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ABSTRACT **Sulfation of tyrosine residues recently has been recognized as a biosynthetic modification of many plasma proteins and other secretory proteins. Effects of this site-specific modification on protein function are not known, but the activity of several peptides such as cholecystokinin is greatly augmented by sulfation. Here, we examine the role of sulfation in the processing and activity of C4 (the fourth component of complement), one of the few proteins in which sites and stoichiometry of tyrosine sulfation have been characterized. Our results, with C4 as a paradigm, suggest that sulfation of tyrosine residues can have major effects on the activity of proteins participating in protein–protein interactions. Sulfation of C4 synthesized by Hep G2 cells was blocked by incubating the cells with $NaClO_3$ and guaiacol. These sulfation inhibitors did not alter secretion or other steps in the processing of C4. However, hemolytic activity of C4 was decreased more than 50%. The inhibitors' effect on C4 activity was prevented by adding Na_2SO_4 to restore sulfation of C4. Activity of C3, a complement component homologous to C4 but lacking tyrosine sulfate residues, was minimally reduced (19%) by the inhibitors. Decreased hemolytic activity of nonsulfated C4 apparently resulted from impaired interaction with complement subcomponent $C\overline{1}s$ (EC 3.4.21.42), the protease that physiologically activates C4. Purified $C\overline{1}s$ was able to cleave nonsulfated C4, but ≈10-fold higher concentrations of $C\overline{1}s$ were required for that cleavage than to yield equivalent cleavage of sulfated C4. Our results suggest that activation of C4, a central component in the classical pathway of complement activation, is influenced by the level of sulfation of the protein. Thus, sulfation of C4 provides a potential locus for physiological or pharmacological modulation of complement-mediated opsonization and inflammation.**

A growing roster of proteins is recognized to contain tyrosine sulfate, including physiologically important plasma proteins such as C4 (the fourth component of complement) (1, 2), fibrinogen (3), fibronectin (3), α_2-antiplasmin (4), laminin (5), heparin cofactor II (6), and coagulation factor VIII (7). This posttranslational modification of proteins occurs along the secretory pathway, probably in the Golgi apparatus (8, 9). The sulfotransferase acting on tyrosine residues is highly selective, so that it efficiently transfers sulfate from the sulfate donor 3′-phosphoadenosyl 5′-phosphosulfate to one or a few specific tyrosine residues of substrate proteins (10). The function of this highly organized modification of proteins and its effects on the activity of the many proteins thus modified are not known, but foregoing studies show that sulfation dramatically enhances the activity of several bioactive peptides including cholecystokinin (11), leucosulfakinin (12), and phyllokinin (13).

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Complement component C4 is one of the few proteins in which the sites and stoichiometry of sulfation have been analyzed in detail (1). This central component of the classical pathway of complement activation is a complex molecule of about M_r 200,000 composed of three disulfide-linked peptide chains, α, β, and γ, of about M_r 93,000, M_r 78,000, and M_r 33,000, respectively (14–16). The site of sulfation is a short segment at the C-terminal end of the α chain. Three tyrosine residues are tightly grouped at this site, and two or three of these residues are modified by sulfation (1). The α chain contains major known functional and antigenic sites of C4—the thioester (17); the site of cleavage by complement subcomponent $C\overline{1}s$ (EC 3.4.21.42), which activates C4 and releases the C4a peptide (14, 15); two points of cleavage by factor I (a C4 inactivator) (18, 19); and several polymorphic sites that distinguish the *C4A* and *C4B* gene products (20, 21). The segment of the α chain containing tyrosine sulfate residues is well separated from these known functional sites in terms of the protein's linear amino acid sequence (1), but the true proximity in the native protein is not known, as there is little information regarding the three-dimensional structure of C4. The site of sulfation of C4 must be exposed at the surface of the protein to permit access to a sulfotransferase. Further evidence for surface localization of this segment is proteolytic cleavage of the α chain only a few residues distal from the site of sulfation (22, 23). Sulfation may well influence the susceptibility of C4 to this cleavage, which occurs after secretion of C4 into the blood circulation, but the function of this cleavage, like that of sulfation, is unknown (24, 25). There is also the possibility that sulfation may influence other interactions of C4. Exposure on the surface of C4 implicates the site of sulfation as a prime candidate site for participating in one or more of the many protein–protein interactions of C4 (14–16, 26)—with $C\overline{1}s$, C2, C4-binding protein, factor I, complement receptors, and other proteins.

Recent studies have identified inhibitors of sulfation that effectively block biosynthetic sulfation of proteins by cells in culture (1, 27, 28). This permits comparison of the properties of sulfated and nonsulfated forms of a protein, providing a simple experimental approach to examine the biological significance of the sulfation of proteins. In the present study, we have used sulfation inhibitors to examine the role of sulfation in the processing and activity of human C4 synthesized by Hep G2 cells in culture. We observed that sulfation contributes to activity of C4 by enhancing its interaction with $C\overline{1}s$.

MATERIALS AND METHODS

Materials. [^{3}H]Leucine and [^{35}S]sulfate were purchased from ICN Radiochemicals. Antiserum to C4 was from ICN Immunobiologicals. Sheep erythrocytes, rabbit antiserum against these cells, and human C2 were obtained from Cordis (Miami). Purified $C\overline{1}s$ (29) was generously provided by D. H. Bing (Boston, MA). Previously described methods were used to purify properdin and factors B and D (30). The Hep G2 cell line was established by Barbara Knowles (31) (Wistar Insti-

tute, Philadelphia). $NaClO_3$ was bought from EM Science. Other chemicals were obtained from Sigma.

Cell Culture. Hep G2 cells were grown in 75-cm^2 flasks in Earle's minimal essential medium supplemented with 10% (vol/vol) fetal calf serum and antibiotics. Cells were used for experiments after reaching confluence as in previous studies (1). Serum-free Earle's medium deficient in sulfate and supplemented with 10 mM Hepes (pH 7.4) was used for experimental incubations. The same medium, except deficient in leucine, was used for incubations with labeled leucine. [^{3}H]Leucine, [^{35}S]sulfate, and other compounds were added to incubations as indicated in figure legends. Labeled C4 was isolated by immunoprecipitation (1, 32) and analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (33).

Complement Activity Assays. C4 activity was assayed by measuring lysis of antibody-coated sheep erythrocytes in the presence of C4-deficient guinea pig serum (34), which was diluted 1:15. Activity of C4 secreted by HepG2 cells was determined by assaying serial 1:2 dilutions of culture medium and calculating the dilution that yielded 50% lysis of the erythrocytes. Cell culture medium was concentrated 10-fold by using Centricon 30 concentrators (Amicon) prior to assay.

C3 activity was measured by using a modification of a procedure described by Harrison and Lachmann (35). A 2% suspension of antibody-coated sheep erythrocytes was incubated 20 min at 37°C with 10 CH_{50} units of guinea pig C1 per ml in 4 mM sodium barbital, pH 7.2/145 mM NaCl/0.8 mM $MgCl_2$/0.25 mM $CaCl_2$/0.1% gelatin. (One CH_{50} unit is the amount of a complement component required to support 50% lysis of a suspension of erythrocytes.) Cells were pelleted and resuspended at 2% in the same buffer containing 70 CH_{50} units of human C2 per ml and 30 μg of human C4 per ml. After 5 min at 37°C, the erythrocytes coated with C1/C4b/C2a were pelleted and resuspended. Aliquots (50 μl) of cells were mixed with 50 μl of serially diluted culture medium. After 30 min at 37°C, 50 μl of buffer containing 250 μg of factor B, 10 μg of factor D, and 6 μg of properdin per ml was added to each sample. Five min later, 850 μl of 1% normal guinea pig serum in 140 mM NaCl/10 mM EDTA/10 mM NaH_2PO_4, pH 7.4, was added. Lysis was measured after 30 min by pelleting cells and measuring absorbance at 415 nm.

RESULTS

Incubation of Hep G2 cells overnight with a combination of sulfation inhibitors in sulfate-deficient culture medium markedly inhibited the sulfation of C4. In multiple experiments, incorporation of [^{35}S]sulfate into C4 was decreased more than 90% by the combination of 2 mM $NaClO_3$ and 0.2 mM guaiacol. Results of a representative experiment are shown in Table 1. In this experiment cells were preincubated for 4 hr with or without the inhibitors to permit secretion of most of the preformed C4 from the cells. Fresh medium was added, and incubation was continued for 8 hr. [^{35}S]Sulfate incorporation into C4 over this time was decreased 91% by the combination of inhibitors. These results agree with the previous observation that this combination of inhibitors can block a number of sulfation reactions, including sulfation of C4 (27). Also, as previously noted (36), incubation with the sulfation inhibitors has little quantitative effect on the synthesis and secretion of C4. [^{3}H]Leucine incorporation into secreted C4 typically was reduced only 10–20% by 12- to 16-hr incubation with the inhibitors.

Although quantitative assessments of C4 indicated that the sulfation inhibitors had only a slight effect on C4 synthesis and secretion, the inhibitors reduced by more than 50% the C4 hemolytic activity secreted from Hep G2 cells (Table 1). The effect of the sulfation inhibitors on C4 activity was prevented by adding 2 mM Na_2SO_4 together with the inhibitors. The sulfation inhibitors are presumed to act by depleting intracellular stores of sulfate and 3′-phosphoadenosine 5′-phosphosulfate, the sulfate donor for sulfation of proteins and other substrates (27). Consequently, effects of the inhibitors on sulfation should be overcome by adding high concentrations of sulfate. This was a particularly important control because a number of compounds and metabolites inactivate C4 by chemical reaction with its thioester (37, 38). This possibility was ruled out because the inhibitors were present throughout the course of the incubation, and only sulfation reactions should be influenced by the addition of sulfate. Restoration of C4 activity by addition of sulfate indicates that effect of the inhibitors was, in fact, due to inhibition of its sulfation rather than due to direct interaction with C4 or the assay. Direct effects of the inhibitors on the hemolytic assay were excluded by adding the compounds to the assay and observing no effect on hemolysis (not shown) and by the fact that 50% lysis of target erythrocytes was achieved at high dilutions of culture medium, 1:100 and greater.

Table 1. Effect of sulfation inhibitors on C4's hemolytic activity and incorporation of [^{35}S]sulfate

Additions to culture medium	[^{35}S]Sulfate incorporation,* dpm	C4 activity, % of control†
No additions (control)	369	100 ± 8
2 mM $NaClO_3$ + 0.2 mM guaiacol	33	46 ± 7
2 mM Na_2SO_4 + 2 mM $NaClO_3$ + 0.2 mM guaiacol	(background)	103 ± 15

*After a 4-hr preincubation, cells were incubated 8 hr with 10 ml of medium containing 200 μCi (1 μCi = 37 kBq) of [^{35}S]sulfate. Labeled C4 was isolated by immunoprecipitation, and duplicate aliquots were assayed. Incubation with 2 mM Na_2SO_4 plus the inhibitors was used to estimate background values (36 dpm) due to nonspecific binding of label and to background radiation. The background was subtracted from other values.

†C4 hemolytic activity was determined on triplicate flasks. Limits of variation are standard deviations.

Analyses of C4 by sodium dodecyl sulfate/polyacrylamide gel electrophoresis indicated that the sulfation inhibitors had no effect on the other steps in the processing of C4, as noted previously with a less-effective sulfation inhibitor (1). Little qualitative or quantitative effect of the sulfation inhibitors was noted in comparing [^{3}H]leucine-labeled C4 synthesized in the absence (Fig. 1, lane 1) or presence (lane 2) of 2 mM $NaClO_3$ and 0.2 mM guaiacol. Analogous comparison of [^{35}S]sulfate-labeled C4 (lanes 3 and 4) demonstrates the profound inhibition of sulfation of C4 in this same experiment. The multiple posttranslational modifications of C4 besides sulfation, including proteolytic cleavages of a single-chain precursor (39–41) and addition and selective maturation of several oligosaccharide chains (16, 42, 43), were not affected by inhibiting sulfation as judged by the lack of change in the electrophoretic mobility of products. A number of compounds impair the normal proteolytic processing of C4 (32), but the sulfation inhibitors used here did not. The relative amounts of incompletely processed precursors of C4 were similar with and without sulfation inhibitors. These precursor forms included proC4 and C4 in which the cleavage between β and α chains or between α and γ chains failed to occur. The higher molecular weight polypeptides derived from incomplete processing of C4 are indicated in Fig. 1. Also, autolytic cleavage of C4 (2, 37) was assessed to determine whether blocking sulfation had influenced the formation of the internal thioester in C4, and the inhibitors were noted to have no effect (not shown). Results of the electrophoretic analyses of C4 indicate that the diminished activity of C4 synthesized in the presence of sulfation inhibitors did not result from blocking other steps in the processing of C4.

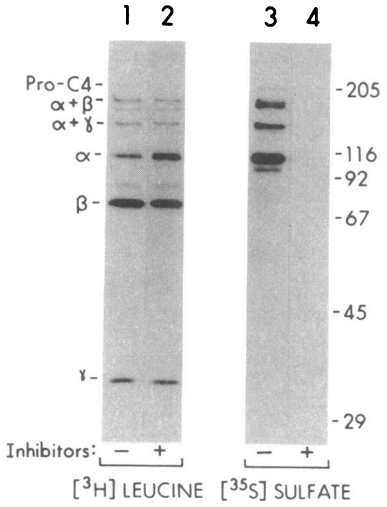

FIG. 1. Analysis by sodium dodecyl sulfate/polyacrylamide gel electrophoresis of the effect of sulfation inhibitors on secretion and processing of C4. Hep G2 cells were labeled for 12 hr with either [^{3}H]leucine (lanes 1 and 2) or [^{35}S]sulfate (lanes 3 and 4). Sulfation inhibitors, 2 mM $NaClO_3$ and 0.2 mM guaiacol, were added to the medium for lanes 2 and 4. Labeled C4 secreted by the cells was isolated by immunoprecipitation and analyzed by polyacrylamide gel electrophoresis under reducing conditions. The molecular weight markers ($\times 10^{-3}$) and the identities of C4 polypeptide chains are indicated.

Several combinations of sulfation inhibitors decreased the activity of C4 secreted by Hep G2 cells (Table 2). Effects of these compounds on C4 activity were completely or partially blocked by addition of Na_2SO_4. The combination of $NaClO_3$ and guaiacol caused the greatest decrease in C4 activity in this experiment, consistent with the observation in previous studies, that this combination is the most effective among these at inhibiting sulfation (27). Although other compounds had a lesser effect on C4 activity than the combination of chlorate and guaiacol, this experiment illustrates that the effect is not unique to that combination but rather depends upon the efficacy of inhibiting sulfation.

Activity of C3 in culture medium was examined as a further control for specific action of the sulfation inhibitors. C3 is a homologous protein to C4 (44) and contains an internal thioester, but, unlike C4, it contains no tyrosine sulfate (1). If the inhibitors affected C4 specifically by inhibiting sulfation, there should be little corresponding effect on C3 activity. This expectation was confirmed experimentally (Table 3). Activity of C3 secreted into culture medium was decreased only 19% by 2 mM $NaClO_3$ and 0.2 mM guaiacol. In the same samples, C4 activity was decreased by 70%. This experiment provides further evidence that the effect of

Table 2. Effect of sulfation inhibitors on hemolytic activity of C4

Additions to culture medium	Hemolytic activity (relative to control), %
2 mM Na_2SO_4 (control)	100
2 mM Na_2MoO_4 + 0.5 mM guaiacol	76
2 mM Na_2SO_4 + 2 mM Na_2MoO_4 + 0.5 mM guaiacol	101
2 mM $NaClO_3$ + 0.5 mM salicylamide	29
2 mM Na_2SO_4 + 2 mM $NaClO_3$ + 0.5 mM salicylamide	80
2 mM $NaClO_3$ + 0.5 mM guaiacol	22
2 mM Na_2SO_4 + 2 mM $NaClO_3$ + 0.5 mM guaiacol	79

Table 3. Effect of sulfation inhibitors on C3 and C4 hemolytic activity

Additions to culture medium	C4 activity, % of control	C3 activity, % of control
No additions (control)	100	100
2 mM $NaClO_3$ + 0.2 mM guaiacol	30	81
2 mM Na_2SO_4 + 2 mM $NaClO_3$ + 0.2 mM guaiacol	82	80

sulfation inhibitors on C4 activity resulted solely from blocking sulfation.

The mechanism by which sulfation affects the hemolytic activity of C4 was analyzed further by examining the interaction of C4 with purified $C\overline{1}s$, the physiological activator of C4. $C\overline{1}s$ activates C4 by cleavage between Arg-77 and Ala-78 in the α chain, excising the C4a peptide of 77 amino acid residues (45). Various concentrations of $C\overline{1}s$ were added to [^{3}H]leucine-labeled products secreted by Hep G2 cells, and cleavage of C4 was assessed by polyacrylamide gel electrophoresis. Cleavage of C4 by $C\overline{1}s$ resulted in a shift of the α chain to a form (α' chain) with an electrophoretic mobility appropriate for a peptide with a M_r about 7,000 less than that of the intact α chain (Fig. 2). Comparing the cleavage by $C\overline{1}s$ of C4 synthesized in the presence (lanes 1, 3, 5, 7, 9, and 11) and absence of the sulfation inhibitors (lanes 2, 4, 6, 8, 10, and 12) revealed that approximately 10-fold higher concentrations of $C\overline{1}s$ were required to cleave C4 synthesized in the presence of the inhibitors to the same extent as C4 synthesized in the control incubation. Although cleavage of the nonsulfated C4 was less efficient, at high concentrations of $C\overline{1}s$, most of it was susceptible to cleavage by $C\overline{1}s$. This suggests that sulfation of the C4 was not essential for cleavage by $C\overline{1}s$ but that it greatly augments the efficiency of this process. C4 in which the thioester was inactivated by reaction with methylamine was not cleaved appreciably by $C\overline{1}s$ at the highest concentration used here (not shown), providing further evidence that the thioester was intact in nonsulfated C4.

An additional observation in this experiment was that $C\overline{1}s$ very inefficiently cleaved partially processed C4 in which the

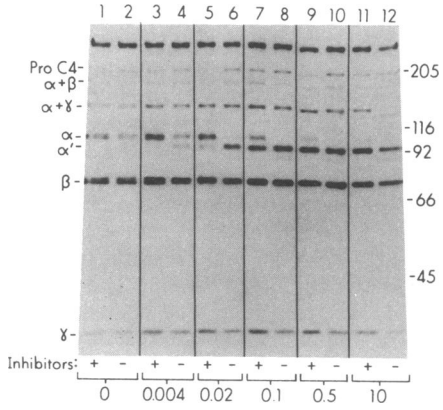

FIG. 2. Cleavage of normal and nonsulfated C4 by $C\overline{1}s$. [^{3}H]Leucine-labeled C4 synthesized by Hep G2 cells in the presence (lanes 1, 3, 5, 7, 9, and 11) or absence (lanes 2, 4, 6, 8, 10, and 12) of sulfation inhibitors was incubated for 2 hr at 37°C with indicated concentrations of $C\overline{1}s$. The C4 was isolated by immunoprecipitation and analyzed by polyacrylamide gel electrophoresis. The molecular weight markers ($\times 10^{-3}$) and identities of C4 polypeptide chains are indicated. The slightly smaller α chain resulting from cleavage by $C\overline{1}s$ is designated α'. The band near the top of the gel probably represents molecular aggregates containing C4; various amounts are observed in different experiments.

α and γ chains still comprised a single peptide chain of about 125 kDa. Cleavage of this form of C4 was noted only at the highest $\overline{C1s}$ concentration of 10 μg/ml (lane 12). The corresponding nonsulfated form of C4 with joined α and γ chains was not cleaved significantly by this concentration of $\overline{C1s}$ (lane 11). Thus, sulfation also affected cleavage of this incompletely processed form of C4.

DISCUSSION

Although a considerable number of proteins are modified by sulfation of tyrosine residues, little is known regarding the physiological role of this modification or its effect on the activity of proteins. Huttner (8) has suggested that tyrosine sulfate may serve as an intracellular sorting signal, directing proteins along the secretory pathway. There is a correlation of sulfation with directed intracellular segregation in the case of pituitary gonadotropins (reviewed in ref. 46), but in that case sulfate is added to N-linked oligosaccharides rather than to tyrosine residues. Recent studies with sulfation inhibitors provide evidence that sulfation of tyrosine residues does not serve a role in protein targeting. Secretion of two tyrosine sulfate-containing proteins, C4 and α_2-antiplasmin, was not affected by inhibiting their sulfation (36), just as, in the present study, sulfation inhibitors did not reduce secretion of C4. Thus, the physiological function of the sulfation of tyrosine residues remains an enigma. It is a widely distributed process among multicellular organisms (8), requiring a specific sulfotransferase and considerable expenditure of energy to activate sulfate. Such a process should not arise and remain conserved through evolution unless it confers adaptive value. A structural role for tyrosine sulfate is suggested by distribution among proteins with widely disparate functions. Two potential roles for this modification are: (*i*) to protect tyrosine residues from oxidants and other chemical reactants or (*ii*) to stabilize tyrosine residues in hydrophilic environments. These roles are consistent with localization of tyrosine sulfate residues to very hydrophilic sites in proteins (10) and occurrence of this modification on secretory proteins that are exposed to the oxidizing extracellular environment. Chemical modification of surface-exposed tyrosine residues in proteins has demonstrated that significant stabilization of protein structure occurs when prosthetic groups are added to render tyrosine residues more hydrophilic (47).

Data presented here indicate that sulfation of C4 markedly increases its activity, measured either as hemolytic activity or as ability to serve as a substrate for fluid-phase $\overline{C1s}$. These results provide the first known example in which sulfation of a protein influences its activity, extending the observation that sulfation dramatically affects the activity of some bioactive peptides (11–13). Sulfation increased the hemolytic activity of C4 about 2- or 3-fold and the rate of cleavage by $\overline{C1s}$ about 10-fold. The different magnitude of the effect of sulfation in the two types of assay is not surprising considering that one is a surface-activated process (hemolytic assay) and the other is a fluid-phase reaction (cleavage by $\overline{C1s}$). The effect of sulfation on C4 activity raises the possibility of effects on activity of the many other proteins that are modified by sulfation of tyrosine residues (3–8). In particular, sulfation might be expected to affect the activity of proteins that, like C4, participate in protein–protein interactions. Tyrosine residues that are acceptor sites for sulfation are by necessity located at the surface of these proteins because the sulfate is added in the Golgi apparatus (9) after folding of the proteins occurs. The highly charged and hydrophilic peptide segments containing tyrosine sulfate should be highly accessible (10) and are good candidates for contact sites with other proteins.

Results of the present study suggest that the site of sulfation of C4 may have a functional role in the interaction of C4 with $\overline{C1s}$. This was unexpected in that the major site of interaction of $\overline{C1s}$ with C4 has been thought to be the C4a peptide (45, 48), which is located at the opposite end of the α chain from the site of sulfation (1). Synthetic peptides corresponding to the C-terminal end of C4a peptide interact with $\overline{C1s}$ with moderate affinity (48). However, it is not clear whether binding to the C4a region accounts entirely for the specificity of the interaction of $\overline{C1s}$ with C4. C4a has considerable amino acid sequence homology with the C3a and C5a peptides of C3 and C5, particularly adjoining their sites of excision from parent proteins (45). $\overline{C1s}$ specifically cleaves C4 but not C3 or C5, so that it must distinguish among these related proteins. It may discriminate between these proteins by binding to unique segments of the C4a region not immediately adjacent to the cleavage site or by binding to other unique segments in the C4 molecule. The site of sulfation of C4 is one such region that shows no homologous segment in C3 or C5 (44, 49). One possible explanation of the observed effect of sulfation on the cleavage of C4 by $\overline{C1s}$ is that the site of sulfation comprises a binding site for $\overline{C1s}$. Such an accessory binding site could increase the specificity and the rate of cleavage of the C4a peptide. A second possibility is that sulfation of tyrosine residues may alter the conformation of the C4 molecule within the C4a region. In either event, the site of sulfation probably folds into close proximity with the C4a segment, although these two sites are located at opposite ends of the α chain, separated by more than 600 amino acid residues.

A schematic diagram of known structural features of C4 is presented as Fig. 3. The C4 molecule consists of three disulfide-linked peptide chains. The α chain is modified by the occurrence of three asparagine-linked oligosaccharides (43), the thioester (17), 2 or 3 sulfate groups linked to tyrosine residues (1), and by cleavage at its C terminus by a metalloprotease in blood (22–25). The site of cleavage by $\overline{C1s}$ to release the C4a anaphyllatoxin peptide (47) is indicated. There is little information about three-dimensional structure of C4, but our results suggest that, in the native protein, the site of sulfation and the junction of the α and γ chains are located close to the site of cleavage by $\overline{C1s}$ as diagrammed. An intriguing possibility is that evolutionary divergence of C4 from its homologues C3 and C5 has been directed to promote accessibility of the C-terminal end of the α chain. Exposure of this site is promoted by two proteolytic cleavages that are unique to C4 relative to C3 and C5. The first occurs intracellularly, cleaving between α and γ chains (40, 41). The second cleavage, mediated by a metalloprotease in blood, removes about 20 residues immediately distal to the site of sulfation of the α chain (22–25).

C4 is one of the limiting components of complement activity, rendering individuals with low C4 activity more susceptible to developing immune complex-related disease

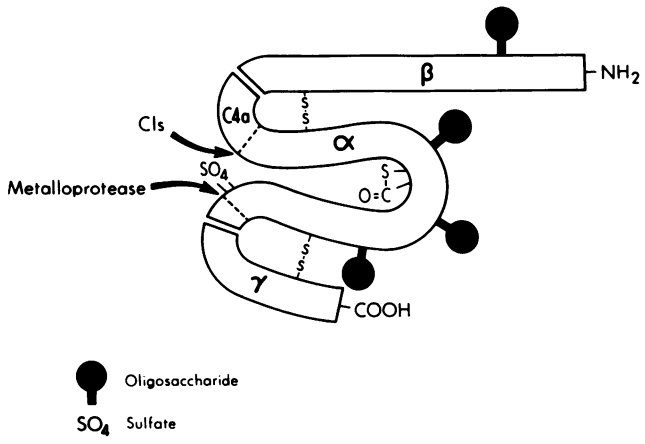

FIG. 3. Structure of C4.

such as systemic lupus erythematosus (26, 50–53). There are two genetic loci, *C4A* and *C4B*, both expressing functional C4 protein. Products of the two genes are activated similarly by $\overline{C1s}$ (54, 55) but differ considerably in the reaction specificity of their thioesters (54–56). The experimental system, Hep G2 cells, used for the present studies expresses both *C4A* and *C4B* gene products (57). Each of the C4 loci are highly polymorphic (58), and the multiple genetic forms express varying levels of activity (54–56). Our finding that activity of C4 is affected by its level of sulfation adds another potential source of functional variability to be considered. It is not known whether there is pathological or physiological variation in the sulfation of C4 or other proteins as has been noted for the peptide hormone gastrin (59, 60). This becomes an important issue to resolve now that there is evidence that full activity of C4 depends on its sulfation. Alteration of the sulfation of C4 could serve as either a physiological or a pharmacological mechanism for modulating complement activity with attendent changes in opsonization of immune complexes and in inflammatory responses.

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