

Solvent Accessibility of Native and Hydrolyzed Human Complement Protein 3 Analyzed by Hydrogen/Deuterium Exchange and Mass Spectrometry¹

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Complement protein C3 is a 187-kDa (1641-aa) protein that plays a key role in complement activation and immune responses. Its hydrolyzed form, C3(H₂O), is responsible for the initiation of the activation of alternative complement pathway. Previous analyses using mAbs, anilinothalenesulfonate dyes, and functional studies have suggested that C3 is conformationally different from C3(H₂O). We have used amide hydrogen/deuterium exchange and MALDI-TOF mass spectrometry to identify and localize structural differences between native C3 and C3(H₂O). Both proteins were incubated in D₂O for varying amounts of time, digested with pepsin, and then subjected to mass-spectrometric analysis. Of 111 C3 peptides identified in the MALDI-TOF analysis, 31 had well-resolved isotopic mass envelopes in both C3 and C3(H₂O) spectra. Following the conversion of native C3 to C3(H₂O), 17 of these 31 peptides exhibited a change in deuterium incorporation, suggesting a conformational change in these regions. Among the identified peptides, hydrogen/deuterium exchange data were obtained for peptides 944–967, 1211–1228, 1211–1231, 1259–1270, 1259–1273, 1295–1318, and 1319–1330, which span the factor H binding site on C3d and factor I cleavage sites, and peptides 1034–1048, 1049–1058, 1069–1080, 1130–1143, 1130–1145, 1211–1228, 1211–1231, 1259–1270, and 1259–1273, spanning 30% of the C3d region of C3. Our results suggest that hydrolysis may produce a looser (more open) structure in the C3d region, in which some of the changes affect the conversion of helical segments into coil segments facilitating interactions with factors I and H. This study represents the first detailed study mapping the regions of C3 involved in conformational transition when hydrolyzed to C3(H₂O). *The Journal of Immunology*, 2005, 174: 3469–3474.

The third component of the complement system, C3, plays a key role in complement activation and host defense by interacting with >20 complement and noncomplement proteins (1, 2). This 187-kDa protein is composed of a 110-kDa α -chain and 75-kDa β -chain that are linked by a single disulfide bond and noncovalent forces (1, 2). During complement activation, the C3 molecule undergoes gross conformational changes because it is processed by the enzymes of the complement cascade. These alterations are evidenced by the ability of the generated fragments (C3a, C3b, iC3b, C3c, C3dg, C3d) to bind differentially to various complement components and receptors (1–3).

The changes in C3 conformation accompanying its activation events have been detected using an assortment of chemical probes, spectral and solution scattering techniques (4–8), and mAbs recognizing neoantigens that are specifically exposed in C3 fragments (1, 3, 9–16). In addition to the conformational changes that have been observed during the degradation of C3, conformational changes occur when C3 is hydrolyzed. Spontaneous hydrolysis of its internal thioester bond yields C3(H₂O) and is responsible for the formation of the convertase C3(H₂O)Bb and the initiation of the alternative pathway (2). Similar conformational changes are thought to occur in C3 when it is incubated with methylamine

(CH₃NH₂) (7). The reactivity of several mAbs with C3(H₂O) as well as C3b, but not with native C3 has suggested that conformational changes are induced when C3(H₂O) or C3b, is generated. This result is in agreement with the conformations observed spectroscopically, as well as with the similar functional activities expressed by these molecules: The C3(H₂O) product acquires “C3b-like activities” by expressing sites that are recognized by various C3b binding proteins (1, 2). The epitopes recognized by these Abs are located within different fragments of the C3 molecule, namely the C3c, C3dg, or C3a fragments (9). Despite the observed similarities between C3(H₂O) and C3b, epitopes expressed in the C3 degradation fragments C3a and C3b but not in C3(H₂O) have also been described (9), thus demonstrating that some conformational differences exist between these two molecules. Although the abovementioned techniques have been used to study the gross conformational changes (4–17) associated with C3 activation and degradation, the protein regions involved in this conformational transition are largely unknown; only a few regions have been identified using mAbs (9, 18, 19).

Over the past decade, hydrogen/deuterium exchange (HDX)³ in combination with mass spectrometry has become a widely used technique to study protein structure and dynamics (20–26). Although nuclear magnetic resonance spectroscopy has been the traditional method for the measurement of hydrogen exchange rates of specific amide protons (27), mass spectrometry has emerged as a powerful technique to measure the exchange rates of amide hydrogens within segments of proteins. The exchange rate of amide

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³ Abbreviations used in this paper: HDX, hydrogen/deuterium exchange; TFA, trifluoroacetic acid; MS/MS, tandem mass spectrometry.

hydrogens in unstructured peptides at neutral pH occurs very rapidly (k_{ch} , 10^1 – 10^3 sec⁻¹). In contrast the exchange rates of backbone amide hydrogens can occur more slowly with half-lives ranging from milliseconds to years (28, 29). Hydrogen bonding and solvent accessibility are directly related to protein structure, and as a result, the exchange rates of amide hydrogens within a protein can be used as probes to follow structural changes. In this study, we have analyzed the amide hydrogen exchange rates of native and hydrolyzed C3 (C3(H₂O)) using MALDI-TOF mass spectrometry and have identified regions of C3 that undergo conformational changes upon its hydrolysis.

Materials and Methods

Materials

Human plasma was obtained from the University of Pennsylvania Blood Bank. For buffer exchange, PD10 columns were purchased from Amersham Biosciences, and Ultrafree-MC centrifugal filter devices were obtained from Amicon. Deuterium oxide (99.9%) was obtained from Cambridge Isotope Laboratories, and immobilized pepsin was purchased from Pierce. Trifluoroacetic acid (TFA) was attained from Applied Biosystems. Acetonitrile was obtained from Fisher. α -Cyano-4-hydroxycinnamic acid, methanol, angiotensin, and adrenocorticotrophic hormone, fragment 18–39 were purchased from Sigma-Aldrich.

Protein preparation and purification

C3 was isolated from platelet-free EDTA human plasma using methods previously described (30–32), with the following modifications: C3 samples were further purified using Mono-Q HR10 columns, and C3(H₂O) was separated from native C3 using Mono-S ion exchange chromatography. Chromatography was performed using a flow rate of 1 ml/min at 4°C as previously described (33, 34).

H/D exchange experiments

Native C3 and C3(H₂O) aliquots were deuterated by the addition of 100 μ l of D₂O (pH 6.5) to 5 μ l of protein (4 mg/ml in PBS buffer (pH 7.4)). After various periods of time (30 s, 5 min, 10 min, 30 min, 1 h, 2 h, and 10 h) in deuterium, 10- μ l aliquots of the protein-deuterium mixture were added to 10 μ l of 0.15% TFA (pH 2.6; 0°C), quenching the amide exchange reaction, and the samples were immediately placed into liquid nitrogen. Each frozen aliquot was quickly thawed and mixed with 40 μ l of immobilized pepsin (0.15% TFA (pH 2.6), 0°C) for 5 min. The pepsin was quickly removed by centrifuging the sample for 30 s at 12,000 \times *g* and 4°C. Immediately following centrifugation, 2 μ l of the peptide mixture was mixed with a 4- μ l aliquot of matrix solution, and 1.5 μ l of this mixture was quickly spotted onto a prechilled MALDI target plate. The matrix solution consisted of a saturated 20 mg/ml α -cyano-4-hydroxycinnamic acid in 1:1 acetonitrile, 0.1% TFA. The analyte-matrix mixture was dried under moderate vacuum for 1 min, and then analyzed by MALDI-TOF mass spectrometry using a Micromass Tof Spec 2E. Repeated measures were taken to ensure that the time between each step following the addition of the quenching/pepsin solution was the same for each time point. Each spec-

trum was an average of 80 shots from the laser. Data were acquired at a 2-GHz sampling rate and 20-kV operating voltage. The mass spectrometric instrument was externally calibrated using angiotensin and adrenocorticotrophic hormone, fragment 18–39.

Mass spectrometric identification of pepsin-generated C3 fragments

Fragments generated from the pepsin digestion of native C3 were identified using an ESI-LC-Q-Duo mass spectrometer (Thermo Finnigan). Two separation steps were used to obtain maximum sequence coverage. Native C3 (80 μ g) was digested with pepsin (pH 2.5), and the generated peptide fragments were separated on a reversed-phase C-18 column (inner diameter, 1.0 mm). The peaks collected were then injected onto a second C-18 column (inner diameter, 0.3 mm) online with the mass spectrometer. The data set generated was then analyzed by the SEQUEST software to obtain sequence information and identify the sequence of the peptides.

Data analysis and controls

The program MAG-TRAN, written by Dr. Z. Zhang (Amgen, Inc., Thousand Oaks, CA) and available as freeware at (www.ionsource.com), was used to calculate the centroid of the isotopic envelopes. Centroid values were computed by selecting the left-most edge of the isotopic peak to the right-most edge of the highest observable peak. Back-exchange experiments were performed as previously described (26). Although these calculations were not incorporated into the data presented here, the average percent back-exchange ranged from 15 to 37% per peptide. The results reported here are from three different experiments with each individual experiment showing similar results.

Results

Identification of C3 pepsin fragments

The identities of the pepsin-generated C3 fragments were determined by tandem mass spectrometry. A total of 354 peptides were identified, covering 80% of the total sequence of native C3 (Fig. 1). The peptides were identified using the SEQUEST software to search a C3 peptide database. An accepted SEQUEST identification had to have an Xcorr (cross-correlation) score >2.0 and a Δ Cn value of at least 0.1. Mass spectrometry/mass spectrometry (MS/MS) spectra for peptides in Tables I and II were also manually inspected. The MS/MS-generated map in Fig. 1 was used to assign identities to the *m/z* values observed in the MALDI-TOF mass spectrometric analysis. The regions that were not identified in the MS/MS analysis included the two glycosylation sites (residues 63 and 917) and peptide segments that participate in disulfide bonding.

Hydrogen/deuterium exchange

The level of deuterium incorporation for each peptide was followed as a function of time at room temperature and quenched with a low-pH buffer to reduce the extent of back-exchange during

FIGURE 1. Mass spectrometric map of the peptic digest of complement protein C3. Fragments were generated by pepsin digestion of native C3 and separated on a reversed-phase C-18 column. Ninety-six fractions were then injected onto a second C-18 column and analyzed online with an ESI-LC-Q-Duo mass spectrometer. The data set generated was then analyzed by the SEQUEST software that identified the sequence of the peptide ions. Black lines indicate the coverage of peptides identified by MS/MS analysis. Residues represented in bold indicate cysteines that are involved in disulfide linkages and amino acids that have *N*-linked carbohydrate moieties attached.



Table I. List of native C3 and C3(H₂O) peptides for which HDX were obtained: α -chain peptides

Observed Mass (M+H)	α -Chain	<i>n</i> ^a	Isotopic Envelope Shift upon C3 Hydrolysis ^b
1227.6	852–861	8	0
2703.5	944–967	20	+2
1427.8	978–990	11	+2
1326.8	979–990	10	+2
1690.9	1034–1047	13	0
1804.1	1034–1048	14	0
1149.7	1049–1058	9	+1
1376.8	1069–1080	10	0
1544.8	1130–1143	13	-2
1717.1	1130–1145	15	-2
2163.2	1211–1228	16	+4
2505.4	1211–1231	19	+5
1336.7	1259–1270	11	+6
1655.7	1259–1273	14	+4
2747.7	1295–1318	22	0
1438.7	1319–1330	12	-1

^a *n* represents the number of exchangeable amide hydrogens.

^b A positive (+) number represents higher deuterium levels in C3(H₂O), whereas a negative (-) number represents higher deuterium levels in native C3. Zero denotes no change.

the analysis. MALDI-TOF mass spectrometry was used to measure the extent of deuterium incorporation for each native C3 and C3(H₂O) fragment. A typical MALDI-TOF mass spectrum profile generated from the pepsin digestion of native C3 and C3(H₂O) samples is shown in Fig. 2. In all, 111 peptides were identified in the native C3 and C3(H₂O) MALDI-TOF MS spectra, respectively. Obtaining quantitative exchange data for each peptide was not possible, given the complexity of the mixture. Overlapping peaks and inconsistent signal intensities were common problems. HDX data were obtained for 31 peptides present in both native C3 and C3(H₂O), covering 22% of the C3 sequence listed in Tables I and II.

Peptides that exhibited changes in deuterium incorporation following native C3 hydrolysis included 11 α -chain peptides: 944–967 (*m/z* 2703.5), 978–990 (*m/z* 1427.8), 979–990 (*m/z* 1326.8), 1049–1058 (*m/z* 1149.7), 1130–1143 (*m/z* 1544.8), 1130–1145 (*m/z* 1717.1), 1211–1228 (*m/z* 2163.2), 1211–1231 (*m/z* 2505.4), 1259–1270 (*m/z* 1336.6), 1259–1273 (1655.7), and 1319–1330 (*m/z* 1438.7); and six β -chain peptides: 257–270 (*m/z* 1586.9), 281–299 (*m/z* 2096.2), 375–395 (*m/z* 2218.3), 376–395 (*m/z* 2071), 535–548 (*m/z* 1463.9), and 564–580 (*m/z* 1839). Fourteen peptides did not show any change in deuterium incorporation following hydrolysis (Tables I and II). These were α - and β -chain peptides: 23–35 (*m/z* 1617.9), 27–37 (*m/z* 1139.7), 43–69 (*m/z* 2884.7), 45–69 (*m/z* 2672.4), 165–180 (*m/z* 1751.9), 462–471 (*m/z* 1171.6), 476–489 (*m/z* 1772), 493(4)–513(4) (*m/z* 2379.3), 584–595 (*m/z* 1293.7), 852–861 (*m/z* 1227.6), 1034–1047 (*m/z* 1690.9), 1034–1048 (*m/z* 1804.1), 1069–1080 (*m/z* 1376.8), and 1295–1318 (*m/z* 2747.7).

The values in the far right-hand columns in Tables I and II represent the largest observed shift in the corresponding peptide's isotopic mass envelope (Fig. 2) following C3 hydrolysis. A zero value represents no change in deuterium incorporation for a peptide over the entire 30-s to 10-h D₂O incubation time range. A positive (+) number represents higher deuterium levels in C3(H₂O), whereas a negative number (-) represents higher deuterium levels in native C3. To be designated a positive or negative value, the difference in deuterium incorporation between C3 and C3(H₂O) peptides had to be a >1-mass unit shift in the isotopic envelope (Fig. 2).

Table II. List of native C3 and C3(H₂O) peptides for which HDX were obtained: β -chain peptides

Observed Mass (M+H)	β -Chain	<i>n</i> ^a	Isotopic Envelope Shift upon C3 Hydrolysis ^b
1617.9	23–35	11	0
1139.7	27–37	9	0
2884.7	43–69	24	0
2672.4	45–69	22	0
1751.9	165–180	13	0
1586.9	257–270	13	-4
2096.2	281–299	19	-1
2218.3	375–395	17	-1
2071	376–395	16	-1
1171.6	462–471	8	0
1772	476–489	13	0
2379.3	493(4)–513(4)	19	0
1463.9	535–548	13	+1
1839	564–580	14	-2
1293.7	584–595	10	0

^a *n* represents the number of exchangeable amide hydrogens.

^b A positive (+) number represents higher deuterium levels in C3(H₂O), whereas a negative (-) number represents higher deuterium levels in native C3. Zero denotes no change.

β -Chain peptides showing solvent accessibility differences between C3 and C3(H₂O)

Fig. 3 shows deuterium incorporation for two peptides residing in the β -chain. A decrease of isotopic envelope shifts of 2–4 mass units following C3 hydrolysis was observed for peptides 257–270 and 564–580. These peptides are more solvent accessible in native C3 following its hydrolysis, suggesting conformational changes in these regions. Peptides 281–299, 375–395, and 376–395 exhibited similar changes in deuterium incorporation, 1-mass unit increases in the isotopic envelope shifts in native C3. This also suggests a conformational change in these regions following the conversion of native C3 to C3(H₂O).

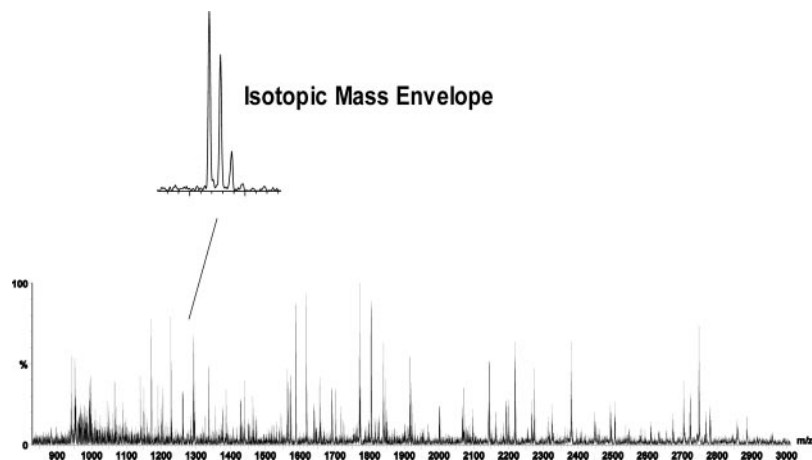
Factor I cleavage sites

Fig. 3 also shows the deuterium incorporation for peptides spanning the factor I cleavage sites. The peptide 1295–1318, containing the factor I cleavage site Arg¹³⁰³-Ser¹³⁰⁴, did not exhibit significant changes in deuterium incorporation following native C3 hydrolysis. However, peptide 944–967, containing the factor I cleavage, Arg⁹⁵⁴-Glu⁹⁵⁵, showed higher levels of deuterium incorporation in C3(H₂O) than in native C3. After 30-s and 1-min D₂O incubations, an increase of 2 mass units in the isotopic envelope shift was observed for peptide 944–967 following native C3 hydrolysis. Peptide 944–967 appears to be more solvent accessible in C3(H₂O) than in native C3, suggesting a conformational change in this region following the conversion of native C3 to C3(H₂O). Peptide 1319–1330, containing the Arg¹³²⁰-Ser¹³²¹ factor I cleavage site, showed only 1 mass unit shift in the isotopic envelope following C3 hydrolysis, thus suggesting a small conformational change in this region.

C3d fragment and factor H binding site

HDX data were obtained for several C3d peptides. These peptides cover 30% of the total C3d sequence and include the following peptides: 1034–1047, 1034–1048, 1049–1058, 1069–1080, 1130–1143, 1130–1145, 1211–1228, 1211–1231, 1259–1270, and 1259–1273. Seven of the 10 C3d peptides (Tables I and II) exhibited differences in deuterium levels in native C3 compared with C3(H₂O). C3d peptides 1049–1058, 1130–43, 1130–45, 1211–1228, 1211–1231, 1259–70, and 1259–1273 exhibited changes in

FIGURE 2. MALDI-TOF spectrum from a digestion of C3 with pepsin at pH 2.6, generating over 100 peptides. Centroid values were determined from the mass envelopes of each peptide. This was done by selecting the *left-most edge* of the monoisotopic peak and the *right-most edge* of the highest observable peak.



deuterium incorporation following the conversion of native C3 to C3(H₂O). The largest change in deuterium incorporation following native C3 hydrolysis was observed for peptides 1211–1228, 1211–1231, 1259–1270, and 1259–1273, suggesting a significant conformational changes in these regions; these segments have been previously shown to be involved in factor H binding to C3d (35). The deuterium incorporation at different time points for peptides 1211–1231 and 1259–1270 are shown in Fig. 4. For peptide 1211–1231, the shift in the isotopic mass envelopes increased from 3 to 5 mass units in C3(H₂O) following 30-s and 1-min D₂O incubation, respectively. The higher deuterium levels for this peptide in C3(H₂O) remained for up to 10-h D₂O incubation. Peptide 1259–1270 exhibited an increase in deuterium incorporation corresponding to a 5- to 6-mass unit difference in the C3(H₂O) isotopic envelope shift compared with native C3. As shown in Fig. 4, the higher level deuterium incorporation in C3(H₂O) continued through the 10-h D₂O incubation for this peptide. Peptides 1049–1058, 1130–1143, and 1130–1145 exhibited minimal differences

(1-mass unit shifts in isotopic envelopes), in deuterium incorporation (Fig. 3). Peptides 1034–1047, 1034–1048, and 1069–1080 did not show any difference in deuterium incorporation between native C3 and C3(H₂O), thus suggesting that these regions do not appear to undergo any significant conformational change upon conversion of C3 to C3(H₂O).

Discussion

Several studies have indicated that C3 undergoes conformational changes upon its conversion to C3(H₂O), but the regions involved in these changes are largely unknown (1, 3, 5–16). Using HDX and mass spectrometry, we have localized changes in solvent accessibility between native C3 and C3(H₂O). Following incubation in D₂O, pepsin digestion, and mass spectrometric analysis, 17 of 31 peptide fragments exhibited different deuterium levels in C3(H₂O) in comparison to native C3, and these are localized in both the α - and β -chains. Our data suggest that there is a structural change that occurs in both the α - and β -chains following hydrolysis of native

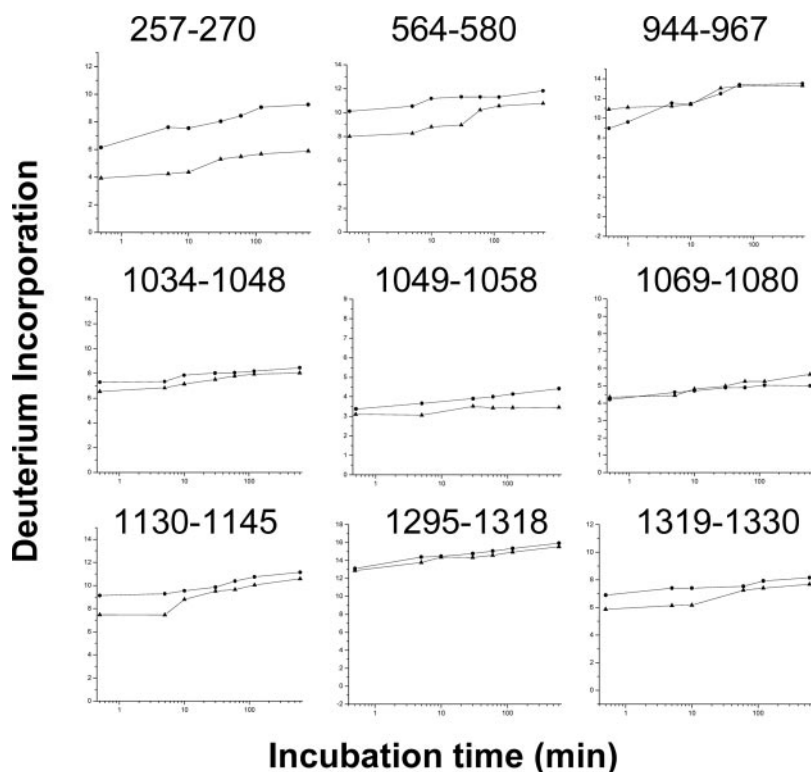
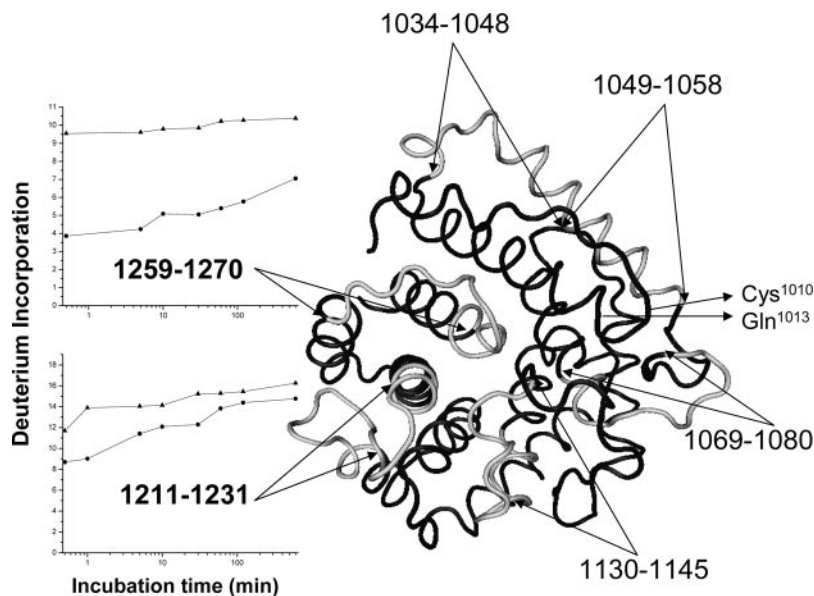


FIGURE 3. Deuterium incorporation vs incubation time plotted for β -chain peptides 257–270 (*m/z* 1586.9) and 564–580 (*m/z* 1839); peptides containing factor I cleavage sites 944–967 (*m/z* 2703.5), 1295–1318 (*m/z* 2747.7), and 1319–1330 (*m/z* 1438.7); C3d peptides 1034–1048 (*m/z* 1804.1), 1049–1058 (*m/z* 1149.7), 1069–1080 (*m/z* 1376.8), and 1130–1145 (*m/z* 1717.1). The trend lines (●) and (▲) represent native C3 and C3(H₂O), respectively.

FIGURE 4. Diagram of the C3d crystal structure with peptide sequences 1034–1048 (m/z 1804.1), 1049–1058 (m/z 1149.7), 1069–1080 (m/z 1376.8), 1130–1145 (m/z 1717.1), 1211–1231 (m/z 2505.4), and 1259–1270 (m/z 1336.6) represented in light gray. C3d sequence numbering for each native C3 and C3(H₂O) peptide listed above; 41–54, 55–65, 76–87, 137–152, 218–238, and 266–277, respectively. The deuterium incorporation vs incubation time plots are linked to sequences 1211–1231 (m/z 2505.4) and 1259–1270 (m/z 1336.6) with black arrows. The trend lines (●) and (▲) represent native C3 and C3(H₂O), respectively. Peptide 1259–1270 contains Tyr²⁷³ (Tyr¹²⁶⁶), which is thought to be in close proximity to the thioester bond and participate in a conserved surface patch shielding the thioester in native C3. Amino acids involved in the thioester bond in native C3 are labeled Cys¹⁰¹⁰ and Gln¹⁰¹³.



C3. It is interesting that the α -chain, specifically the C3d region, appears to exhibit the largest changes in solvent accessibility following the conversion of native C3 to C3(H₂O). The relation of the regions in β -chain of C3 undergoing structural changes to its functional sites is at the present unclear. Previous studies have shown that mAbs to the β -chain of C3 inhibit factor H, CR1, and factor B binding to C3b (9). It is conceivable that the epitopes recognized by these Abs are within the segments that change in solvent accessibility upon hydrolysis of C3. Such correlations will be possible with the improvement of the mass spectrometric analysis to obtain peptides covering the entire C3 sequence as well as with the mapping of the functional and antigenic sites.

During complement activation, C3(H₂O) and C3b are converted, by factor I, to iC31/iC32/C3ac/C3dg and iC3b1/iC3b2./C3c/C3dg, respectively (36, 37). This conversion occurs in three cleavage steps: first, between Arg¹³⁰³ and Ser¹³⁰⁴ on the α -chain; second, between Arg¹³²⁰ and Ser¹³²¹, liberating C3f and yielding iC3b; and third, between Arg⁹⁵⁴ and Glu⁹⁵⁵, generating C3dg and C3c. For this cleavage, factor I requires the presence of various cofactor molecules (37–40) that are thought to induce a conformational change in C3(H₂O) and C3b, thus making the cleavage sites accessible to factor I (37, 41, 42). However, it is not known what are the exact residues on C3b and C3(H₂O) that interact with factor H and where on C3b and C3(H₂O) the structural change occurs once bound to factor H. Using mAbs and synthetic peptides, three different regions of C3 have been found to be involved in H binding. These included the N terminus of the α -chain, the C3d, and the β -chain (3, 9, 43). The C3d site includes peptides 1211–1228, 1211–1231, 1259–1270, and 1259–1273, which showed the largest increases in deuterium incorporation following the conversion of native C3 to C3(H₂O) (Fig. 4). This suggests a significant increase in solvent accessibility in these regions following C3 hydrolysis, perhaps making this region more available for factor H binding. This conclusion is based on the fact that native C3 is not known to be cleaved by factors H and I. From the C3 regions that contain the factor I cleavage site, only the region (peptide 944–967) containing the site Arg⁹⁵⁴-Glu⁹⁵⁵, involved in the C3b₂ cleavage to C3c and C3dg, exhibits significant differences in deuterium levels between native C3 and C3(H₂O). This suggests that this region is more solvent accessible in C3(H₂O) than in native C3. The structural changes observed for peptides 944–967, 1211–1228, 1211–1231, 1259–1270, and 1259–1273

could be required for the initial binding of factor H and sequential conformational changes and cleavage by factor I.

Differences in HDX rates were observed for seven peptides spanning C3d, a fragment of native C3 for which the x-ray crystal structure is available (44). This molecule also contains the thioester site (labeled Cys¹⁰¹⁰ and Gln¹⁰¹³ in Fig. 4). Although Cys¹⁰¹⁰ involved in the thioester formation has been mutated to an Ala residue in the crystallized C3d protein, it does represent a model to better interpret and validate the HDX changes obtained in this study for native C3 following the hydrolysis of its internal thioester. As such, native C3 and C3(H₂O) peptides covering the C3d region have been mapped (light gray) onto the C3d crystal structure in Fig. 4. It appears from our data that the C-terminal region of C3d is significantly affected by the hydrolysis of native C3 to C3(H₂O). Peptides 1034–1047, 1034–1048, 1049–1058, 1069–1080, 1130–1143, and 1130–1145 deuterium incorporation were the least affected by C3 hydrolysis. There are a number of surface-exposed nonpolar residues in the C3d crystal structure, including Ile²³, Phe⁶⁶, Arg⁷⁶, Ile¹³², and Tyr²⁷³ (corresponding to Ile¹⁰¹⁶, Phe¹⁰⁵⁹, Arg¹⁰⁶⁹, Ile¹¹²⁶, and Tyr¹²⁶⁶ in native C3). Given the conservation of this region among different species, it is thought that these amino acids play an important structural role in native C3, acting as a surface domain to protect the thioester from hydrolysis. This region is also thought to provide some of the strain energy that holds native C3 in a conformational “closed” structure. However, this possibility has not been conclusively established because the C3d crystal structure lacks an intact thioester and there is no crystal structure for the protein with an intact thioester. We observed the largest increase in amide hydrogen exchange following the hydrolysis of native C3 in peptides 1259–1270 and 1259–1273. These peptides contain Tyr²⁷³ (Tyr¹²⁶⁶), which is thought to be in very close proximity to the thioester bond and participate in the conserved surface patch mentioned above (44). This arrangement suggests an increased solvent accessibility in these regions, implying a conformational rearrangement, and may be the result of the release of some of the strain energy required for the molecule. Because both C3d and C3(H₂O) lack the thioester, it is plausible that the regions spanning peptides 1211–1231 and 1259–1273 could be more buried inside the native C3 molecule (less solvent accessible). It is also possible that these regions could contain more amino acids involved in higher ordered structures in native C3 like helices. Although the alignment of fragments 1211–1331

and 1259–1273 in Fig. 4 does show these fragments span helical regions, they also contain amino acids that occupy random coiled structures (Fig. 4). It is possible that hydrolysis produces a looser (more open) structure, in which most changes affect the conversion of helical segments in C3d into coil segment in hydrolyzed C3d, possibly by fraying at the termini. These changes may affect the overall packing of the elements of secondary structure during the C3 to C3(H₂O) conversion. These structural changes may also be required for the recognition and subsequent cleavage by factors H and I.

In conclusion, this study represents the first detailed analysis mapping the regions of C3 involved in conformational transition when hydrolyzed to C3(H₂O) and sets the groundwork for future studies to increase the coverage map, identify the conformational changes of C3 during its degradation, and study its interactions with its various ligands.

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Disclosures

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