Phylogeny of the Third Complement Component, C3, and Conservation of C3-Ligand Interactions[a]

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Of the 30 distinct complement proteins recognized to date, C3 is probably the most versatile and multifunctional molecule known, interacting with at least 20 different proteins.[1,2] It plays a critical role in both pathways of complement activation and participates in phagocytic and immunoregulatory processes.[1,2] The study of C3 molecules from different species gives insight into the structural elements involved in its different functions, the structural features constrained by selection, and the evolutionary history of C3 and complement in general. C3-like activity has been reported in a variety of species, including invertebrates, yet thus far, C3 has been purified only from chordates and has been found to be present in representatives of each of the seven living classes of vertebrates.[1,2] The complete primary structures have been deduced for C3 of human, guinea pig, mouse, rat, hagfish, lamprey, and cobra; and partial primary structures have been determined for C3 of rabbit and *Xenopus* (for review see Lambris[2]).

In this study we purified C3 from different species, analyzed the conservation of its structural and functional features, and obtained the cDNA sequence of frog *(Xenopus gilli),* trout *(Salmo gairdneri),* and chicken C3. C3 was purified from swine (Po), rabbit (Rb), mouse (Mo), cobra (Co), *Xenopus (Xe),* axolotl (Ax), chicken (Ch), and trout (Tr) serum.[3] All C3s tested were composed of two chains (α/β-chain) and contain a thioester bond within the α-chain. The two N-linked high-mannose carbohydrates found in human C3 were only conserved in Rb C3. In contrast, Xe, Ax, and Tr C3 have this moiety only in the β-chain and Po and Mo C3 only the α-chain. Co C3, in contrast to cobra venom factor (CVF), lacks Con A binding carbohydrates in either chain. The NH_2-termini of the *Xe* and Ax C3 β-chains were found to be blocked. TABLE 1 summarizes the features of C3 from different species characterized in this and other studies (for review see Lambris[2]).

The cDNA sequences of Tr, *Xe,* and Ch C3s were obtained and the amino acid sequence similarity between the different C3s is shown in TABLE 2. Several C3 regions have been found to be highly conserved including that of the thioester bond, which is crucial to the function of C3. The thioester site, GCGEE, is 100% conserved in all species, and the amino acid sequences of the surrounding regions are highly similar. This conservation of the thioester bond and its surrounding hydrophobic amino acids emphasizes the functional importance of this region in maintaining,

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TABLE 1. Amino Acid Sequence Conservation between Trout C3 and Other Related Proteins

	Hu C3	Rb C3[a]	Ra C3	Mo C3	GP C3	Ch C3[a]	*Xe* C3[a]	Co C3	Tr C3	La C3	Ha C3	Hu C4	Mo C5	Hu α_2M
Human C3	100/100	79.6/87.0	78.2/85.5	77.1/85.3	78.1/86.3	56.3/70	52.2/66.3	51.3/66.1	44.1/58.9	32.3/48.1	30.9/46.8	28.4/43.3	28.0/45.0	21.3/35.4
Rabbit C3[a]		100/100	79.8/87.5	78.5/86.1	78.4/87.5	53.1/67.1	50.7/65.3	49.7/66.0*	43.1/58.3	33.3/48.2	28.4/44.8	26.7/41.7	26.3/45.4	18.0/35.5
Rat C3			100/100	90.1/94.1	81.0/87.6	57.3/70.6	53.1/67.6	52.2/66.7	44.2/58.5	32.7/48.3	28.7/45.1*	27.4/42.4	28.6/45.4	21.4/35.7
Mouse C3				100/100	79.8/87.1	57.2/70.5	53.5/67.6	51.5/66.3	43.3/59.6	32.2/48.0	29.1/45.2*	27.4/42.6	28.3/44.9	21.0/35.2
Guinea Pig C3					100/100	55.6/70.2	53/66.9	52.5/67.4	44.9/59.7	33.1/49.0*	30.2/46.4	30.4/46.0	29.0/45.7	24.8/39.8
Chicken C3[a]						100/100	55.9/68.5	58.4/70.3	46.3/60.7	34.5/52.0	32.5/48.4	31/46	28.1/44.9	22.7/37.7
Xenopus C3[a]							100/100	54.8/68.2	46.7/60.0	35.2/51.6	32.3/49.5	30.2/45.8	28.5/45.3	23.9/39.5
Cobra C3								100/100	43.8/60.2	32.3/47.0	30.6/45.6	27.3/42.1	27.5/43.4	21.2/35.4
Trout C3									100/100	33.1/50.2	29.9/46.1	27.5/42.0*	28.2/44.7	22.9/37.2
Lamprey C3										100/100	32.8/48.4	29.0/44.6	29.1/44.8	21.6/35.2
Hagfish C3											100/100	27.9/42.2	28.3/43.3	20.6/35.3
Human C4												100/100	27.3/42.2	21.7/34.9
Human C5													100/100	11.4/26.8
Human α_2M														100/100

ABBREVIATIONS: Hu, human; Mo, mouse; Ra, rat; Rb, rabbit; *Xe, Xenopus;* Ax, axolotl; Co, cobra; GP, guinea pig; Tr, trout; Po, pig; Ch, chicken; La, lamprey; Ha, hagfish.

NOTE: Percent identity/identity plus similarity. All alignments were performed using open gap cost 7 and unit gap cost 2 except the ones indicated with an asterisk for which open gap cost 15 was used.

[a]Partial amino acid sequence.

TABLE 2. Summary of Structural and Functional Properties of C3 from Different Species

Species	Hu	Po	Rb	Ra	GP	Mo	Ch	*Xe*	Co	Ax	Tr	Ha	La
Number of residues	1663	nd	nd	1663	1666	1661	nd	nd	1651	nd	1640[c]	1620	1660
% Identity/similarity to human C3	100/100	nd	80/87[a]	78/86	78/86	77/85	56/70[a]	52/66[a]	51/66	nd	44/59	31/47	32/48
Chain structure	α/β	α/β	α/β	α/β	α/β	α/β	α/β	α/β	α/β	α/β	α/β	α/β	α/β/γ
M_r α/β × 10^3	115/75	112/68	119/72	115/65	115/65	113/66	118/68	112/83	107/64	110/73	112/70	115/77	84/74/32
Thioester in	α	α	α	α	α	α	α	α	α	α	α	α	α
Con A binding to	α/β	α	α/β	nd	α	α	α	β	–	β	β	nd	nd
Human CR1 binding	+	+	+	nd	+	+	–	+	–	–	–	nd	nd
Human CR2 binding	+	+	+	+	nd	+	–	+	–	–	–	nd	nd
Human C5 binding	+	nd	+	+	nd	+	+	+	nd	+	+	nd	nd
Human H binding	+	+	+	+	+	+	+	–	–	+	+	nd	nd
Human B binding	+	+	nd	nd	+/–	nd	nd	–	nd	nd	nd	nd	nd
Human P binding	+	nd	+	+	nd	+	nd	+	nd	nd	–	nd	nd
Human MCP binding	+	nd	+	nd	–	nd	nd	nd	nd	nd	nd	nd	nd
Classical pathway	+	+	+	+	+	+	+	+	+	nd	+	–	–
Alternative pathway	+	+	+	+	+	+	+	+	+	nd	+	+	+[b]

[a]Based on partial sequence.
[b]Functioning only in opsonization.
[c]Without the signal peptide.

throughout evolution, the capacity of C3 to attach to surfaces.

Binding studies using the different C3s and Hu C3-ligands showed that H binds to Hu, Rb, Po, Ch, and Tr iC3; CR1 to Hu, Rb, Po, and *Xe* iC3; CR2 to Hu, Rb, Mo, and *Xe* iC3; and B to Hu and Po C3b (see TABLE 1). These data suggest that the conservation of the sequences comprising the binding sites for H, P, B, CR1, and CR2 is important in maintaining binding to these molecules.[1,2] Hu C3 was recently found by our laboratory to have multiple interaction sites for human C3b/C4b binding proteins CR2, H, and B.[1,2] The differential binding of the human factor H, factor B, CR1, and CR2 to different C3s made possible the conclusion that although these four molecules bind to the same domain in human C3, the exact binding sites are different.[1,2]

The interactions of C3 from different species with autologous C3-binding proteins and the C3 convertase and factor I cleavage specificities were investigated by analyzing electrophoretically the C3 fragments fixed on zymosan after activation of Tr, Ax, and *Xe* complement. We found that the degradation pattern is similar to that observed for human C3. NH_2-terminal amino acid sequence of the 68-kDa and 43-kDa fragments showed that these fragments are analogues of human C3 fragments generated by C3 convertase and factors I and H. Although similar fragments were found in the presence or absence of EGTA (which inhibits classical complement pathway), no fragments were detected when the activation of the different sera was performed in the presence of EDTA (which inhibits both complement pathways). These data suggest that the species so far tested possess proteins with functions similar to those of human factors B, D, I, and H. The C3 convertase cleavage site is conserved within all the C3s tested, and their C3 convertases have the same specificity (Arg-Ser) as those of human complement. In relation to the factor I cleavage sites, not all are conserved in all species. The factor I cleavage specificity is Arg-X.

REFERENCES

1. LAMBRIS, J. D. 1990. The Third Component of Complement: Chemistry and Biology. Springer-Verlang. Berlin.
2. LAMBRIS, J. D. 1993. Chemistry, biology and phylogeny of C3. *Complement Profiles* **1:** 16–45.
3. ALSENZ, J., D. AVILA, H. HUEMER, I. ESPARZA, D. BECHERER, T. KINOSHITA, Y. WANG, S. OPPERMANN & J. D. LAMBRIS. 1992. Phylogeny of the third component of complement, C3: Analysis of the conservation of human CR1, CR2, H and B binding sites, Con A binding sites and thiolester bond in the C3 from different species. Dev. Comp. Immunol. **16:** 63–76.