Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 7619–7623, December 1982
Biochemistry

Characterization of the mRNA and cloned cDNA specifying the third component of mouse complement

(nucleotide sequence analysis/anaphylatoxin/internal thioester bond/proteolytic sites/precursor pro-C3)

HORST DOMDEY*, KARIN WIEBAUER†, MICHAEL KAZMAIER, VERENA MÜLLER, KAREL ODINK‡, AND GEORGE FEY§

Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges, Switzerland

Communicated by R. R. Porter, August 16, 1982

ABSTRACT **Eighteen cDNA clones containing inserts specific for the third component of complement (C3) have been derived from high molecular weight mouse liver mRNA. The inserts span 4,600 nucleotides of the C3 coding sequence, including the 3′ end of C3 mRNA. The length of C3 mRNA was determined to be 5,100 ± 200 nucleotides, including a poly(A)-containing tail of mean length 170 nucleotides. From cDNA sequence analysis of the 5′-proximal region of C3 mRNA, the NH_2-terminal amino acid sequence of the mature C3β chain was predicted to be Ile-Pro-Met-Tyr-Ser-Ile-Ile-Thr-Pro-Asn-Val-Leu-Arg-Leu-Glu. This sequence is in good agreement with the reported amino acid sequences of human and guinea pig C3β chains. These data position the C3β subunit to the NH_2-terminal portion of the precursor C3 molecule (pro-C3) and establish the order of subunits in pro-C3 to be NH_2–β–α–COOH. In addition, the cDNA sequence indicates that an NH_2-terminal extension peptide precedes the β chain in pro-C3. The amino acid sequence of the mouse C3a fragment and its flanking regions was determined. The data indicate the presence of four arginine residues located between the COOH terminus of the C3β and the NH_2 terminus of the C3α subunits in pro-C3. The coding sequences of the amino acids that constitute the internal thioester domain in C3 were determined. Unexpectedly, the glutamyl residue that has been shown to participate in the thioester bond in native C3 was found to be encoded as a glutamine.**

Complement is a group of interacting proteins, present in the blood of all vertebrates, that plays an essential part in the defense against microbial infections. Although the system is capable of direct killing and lysis of microorganisms in the absence of specific antibodies, complement also acts as an effector of the humoral immune response. The third component of complement, C3, is the central element in both the classical and the alternative pathways of complement activation. C3 contains specific sites of interaction for the classical pathway convertase $\overline{C4b,2a}$, the alternative pathway convertase $\overline{C3b,Bb}$, and the control proteins β1H and C3b inactivator. The protein is present in plasma as a two-chain molecule with disulfide-linked subunits α (M_r, 115,000) and β (M_r, 75,000) (for review, see refs. 1 and 2). C3 is synthesized as a single-chain precursor, pro-C3 (3, 4). Upon proteolytic activation by C3 convertases, C3 is cleaved into the fragments C3a (M_r, 9,000) and C3b (M_r, 180,000). C3a has anaphylactic activity and mediates inflammatory responses (5, 6). C3b has been shown to bind covalently (7) to the surfaces of foreign particles by means of a transacylation reaction (8) involving a unique structure in the C3 molecule, an internal thioester bond (9). Particle-bound C3b is cleaved by specific proteolysis into fragments C3c and C3d. The latter remains bound to the particle surface (10) and contains the thioester region (11). Clearance of C3b-coated particles by phagocytic cells involves interactions between specific cellular C3-receptor proteins and binding sites for these receptors on the C3 molecule (12–14). Thus, the C3 molecule carries sites for interaction with several complement components, control proteins, specific cellular receptors, and particle surfaces (1) and offers an excellent system for the study of separate functional and structural domains.

We have isolated and characterized several cDNA clones derived from mouse liver, covering almost the entire C3 mRNA coding region. Nucleotide sequence data from these clones were used to predict the amino acid sequence of selected domains of the C3 molecule.

MATERIALS AND METHODS

Isolation and Characterization of Cloned cDNA. Clones pMLC3/1–3 were isolated as described (15). Clones pMLC3/4–8 were identified by colony hybridization (16) with a nick-translated cDNA insert from clone pMLC3/1. Plasmids were propagated and plasmid DNAs were isolated as described (17). Restriction enzyme cleavage maps were established by partial hydrolysis of labeled fragments (18) and by total digestion with one or two of the restriction enzymes indicated.

Length Determination of C3 mRNA. Ten micrograms of polyadenylylated RNA (15) was coprecipitated with 25 μg of carrier *Escherichia coli* tRNA and 1.5 pmol of each of the cDNA fragments 4, 5, and 7 (Fig. 1), which were obtained by digestion of plasmid pMLC3/1 with *Pst* I and elution from agarose gels (19). The precipitate was dissolved in 2.5 μl of H_2O, 20 μl of formamide was added, and the mixture was incubated at 60°C for 10 min. After addition of 2.5 μl of 4 M NaCl/10 mM Na_2EDTA/0.1 M Pipes, pH 6.4 (20), the hybridization was carried out for 2 hr at 45°C, followed by precipitation with ethanol. The precipitate was dissolved in 200 μl of 10 mM Tris·HCl (pH 7.5). To this was added 22 μl of 0.1 M Tris·HCl, pH 7.5/1.3 M NH_4Cl/0.1 M $Mg(OAc)_2$/50% sucrose/1 unit of *E. coli* RNase H, followed by incubation of the mixture for 30 min at 37°C. If deadenylylation of the mRNA was desired, the incubation was carried out in the presence of 10 μg of oligo(dT). The sample was adjusted to 20 mM Na_2EDTA and 0.5% $NaDodSO_4$,

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: C3, C4, third and fourth components of complement; C3a, C3b, C3d, fragments of C3, abbreviated according to ref. 35; pro-C3, precursor polypeptide of C3; kb, kilobase pair(s).

* Present address: Department of Chemistry, University of California at San Diego, B017 La Jolla, CA 92093.
† Present address: Department of Human Genetics, University of Michigan Medical School, Ann Arbor, MI 48109.
‡ Present address: The Wellcome Research Laboratories, Beckenham, Kent BR3 3BS, United Kingdom.
§ To whom reprint requests should be addressed.

extracted with phenol/chloroform, and precipitated with ethanol. Glyoxal denaturation of the resulting RNA products, agarose gel electrophoresis, transfer to nitrocellulose filters, and hybridization with nick-translated fragment 2 or fragments 8 plus 9 (Fig. 1) were carried out as described (21, 22). *Hin*dIII and *Eco*RI fragments of phage λ DNA were denatured with glyoxal, 5′ end-labeled by established procedures (23), and used as molecular weight markers (21).

Synthesis of 5′ End-Labeled cDNA Representing the 5′ End of C3 mRNA. Primer DNA was produced by digesting clone pMLC3/7 with the restriction enzyme *Hin*dIII and by 5′ end-labeling the products. These were cleaved with *Eco*RI in order to generate a single end-labeled product because of the asymmetric location of the *Eco*RI site (Fig. 1). The resulting products were separated by polyacrylamide gel electrophoresis, and the 86-bp *Hin*dIII–*Eco*RI fragment 1 was eluted from the gel; 4 pmol (3×10^6 cpm) of this primer and 20 μg of gradient-purified high molecular weight (≥5,000 nucleotides) mouse liver mRNA (15) were dissolved in 45 μl of 90% formamide and heated for 10 min at 50°C. The NaCl/EDTA/Pipes buffer (5 μl) was added, and the sample was incubated for 90 min at 51°C. After precipitation with ethanol, the pellet was resuspended in 100 μl of 10 mM Tris·HCl, pH 8.3/7.5 mM KCl/7.5 mM $MgCl_2$/1 mM dithiothreitol/2 mM desoxyribonucleotide triphosphates/4 mM $Na_4P_2O_7$. Primer extension was performed by adding 50 units of avian myoblastosis virus reverse transcriptase (gift of J. Beard) and incubating the mixture for 90 min at 42°C. The reaction products were extracted with phenol, precipitated with ethanol, and resuspended in 50 μl of 0.1 M NaOH/1 mM Na_2EDTA. The 5′ end-labeled cDNA products were heated for 3 min at 42°C, quick-chilled on ice, and, after addition of 50 μl of 8 M urea, separated and purified by electrophoresis on a 0.35-mm thick 6% polyacrylamide gel containing urea.

DNA Sequence Analysis. Sequence determination of cloned cDNA fragments and single-stranded cDNA obtained by primer-extension experiments was performed by the procedure of Maxam and Gilbert (23).

RESULTS

Isolation of C3 cDNA Clones. Three C3 cDNA clones have been isolated previously from a library representing high molecular weight (≥5,000 nucleotides) mouse liver mRNA species (15). The cDNA insert of one of these (pMLC3/1, Fig. 1) was used to rescreen the collection of 312 cDNA clones, resulting in the identification of nine additional C3 clones. The restriction enzyme cleavage map of the eight unique cDNA inserts which were isolated is shown in Fig. 1. Four of the 12 clones had identical inserts. Further screening with cDNA fragments 2 and 8 (Fig. 1) as probes for colony hybridization resulted in the isolation of six additional C3 clones; all contained inserts already present in the first 12 clones. The cDNA inserts spanned a contiguous stretch of 4,600 nucleotides of the C3 mRNA sequence.

Length of C3 mRNA. The length of C3 mRNA was measured by agarose gel electrophoresis in two ways: (*i*) direct analysis of C3 mRNA and (*ii*) RNase H treatment of mRNA·cDNA hybrids. Polyadenylylated mRNA from mouse liver was hybridized with the internal cDNA *Pst* I fragments 4, 5, and 7 (Fig. 1), followed by treatment with ribonuclease H. After gel electrophoresis and transfer of the RNase H-resistant fraction to nitrocellulose filters, nick-translated cDNA fragments representing the end regions of the C3 mRNA were used as probes for hybridization analysis. Hybridization with the 5′-proximal fragment 2 revealed a predominant band of 1.95 kb (Fig. 2*A*, track c). From these data we concluded that the 5′ end of C3 mRNA is located 1.95 kb upstream of the 5′ end of the cDNA fragment 4—i.e., 350 nucleotides upstream of the unique *Eco*RI site at the boundary of fragment 1 (Fig. 1)—and that the 5′ end of C3 mRNA is not represented by the cloned cDNA. Two fainter bands at 2.6 and 3.5 kb were interpreted to be due to incomplete hybridization of the internal cDNA fragments with C3 mRNA prior to treatment with RNase H, thereby generating longer RNase H-resistant RNA fragments. Hybridization of the gel blot with the 3′-proximal cDNA fragments 8 and 9 gave a diffuse band with an intensity maximum at 440 nucleotides (Fig. 2*B*, track g). When oligo(dT) was included in the hybridization reaction of mRNA with internal cDNA fragments, a 270-nucleotide

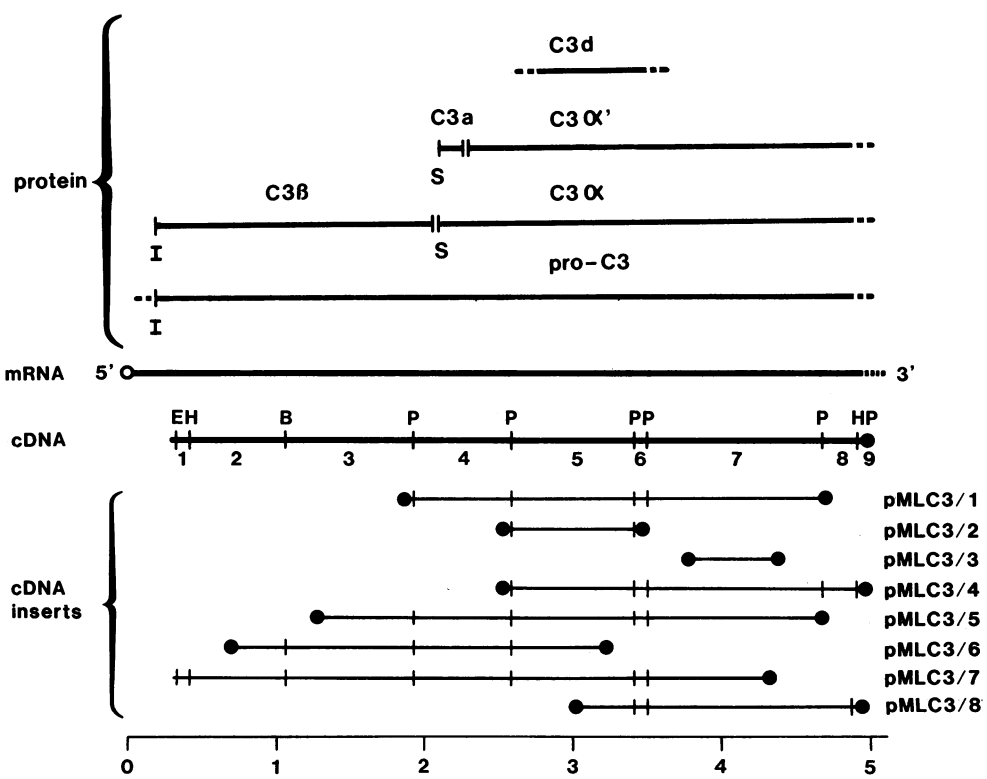

FIG. 1. (*Lower*) Restriction map of the cDNA inserts contained in clones pMLC3/1–8 and their location with respect to C3 mRNA. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; P, *Pst* I. ●——●, cDNA inserts, where end circles symbolize *Pst* I sites generated by the cloning procedure. The scale in the bottom line is in kilobase pairs (kb). (*Upper*) Position of the principal subunits and peptide fragments contained in the pro-C3 molecule, with respect to the C3 mRNA. I, isoleucine residue at the NH_2 terminus of pro-C3 and mature C3β. Dots preceding I indicate the amino-terminal extension peptide in the initial translation product. S, serine residue at the NH_2 terminus of C3α and C3a.

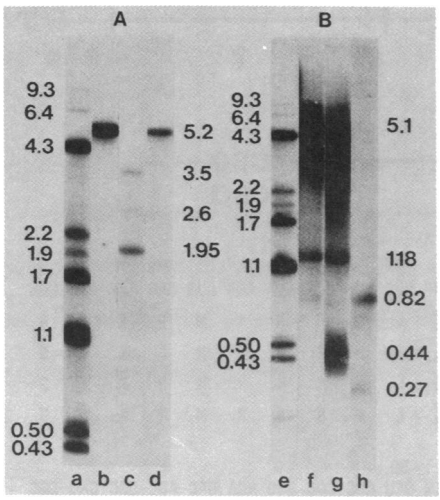

FIG. 2. Determination of the length of C3 mRNA by electrophoresis in agarose gels. Lanes: b–d and f–h, polyadenylylated mouse liver RNA treated prior to electrophoresis in different ways; c, g, and h, hybridization with the internal cDNA fragments 4, 5, and 7 (see Fig. 1), followed by incubation with RNase H; h, oligo(dT) present during the hybridization; d and f, RNase H treatment in the presence of cDNA fragments 4, 5, and 7 but without prior hybridization; b, no treatment; a and e, restriction fragments of phage λ DNA 5′ end-labeled with ^{32}P as molecular weight markers (shown in kilobases). Before electrophoresis on a 1.2% (*A*) or a 1.6% (*B*) agarose gel, all samples (lanes a–h) were denatured with dimethyl sulfoxide and glyoxal. After transfer to nitrocellulose filters, hybridizations were performed by using the nick-translated cDNA fragments 2 (*A*) and 8 plus 9 (*B*) as probes. The bands at positions 0.82 and 1.18 kb in lanes f–h are due to a contamination of the nick-translated probe with fragments 5 and 7.

RNase H-resistant RNA fragment was generated (Fig. 2*B*, track h). The 820- and 1,180-nucleotide fragments visible in tracks f, g, and h of Fig. 2*B* were due to a contamination of the radioactive hybridization probe with these fragments. These data suggest that the 3′ end of C3 mRNA is represented by the cloned cDNA and that C3 mRNA contains a poly(A)-containing tail with an average length of 170 nucleotides. In control experiments in which no hybridization with internal fragments was performed prior to RNase H treatment (Fig. 2, tracks d and f), or where the RNA was not exposed to RNase (Fig. 2*A*, track b), the length of C3 mRNA was determined to be 5,100–5,200 nucleotides. From the map positions of these cDNA fragments (Fig. 1) and the size of the RNase H-resistant RNA fragments, the length of C3 mRNA also was determined to be 5,100 ± 200 nucleotides.

Sequence Analysis of the 3′ End of C3 mRNA. Sequence analysis was performed on cDNA fragments 8 and 9 in both directions from the 3′-proximal *Hin*dIII site (Fig. 1), and a sequence of 257 base pairs was obtained (unpublished data; ref. 24). This sequence contains a cluster of seven consecutive adenosine residues. At 16 nucleotides upstream, a polyadenylylation signal A-A-U-A-A-A (25) was identified. Translation termination codons occur in this sequence in all three possible reading frames. It was not possible to determine the exact location of the COOH terminus of pro-C3 because corresponding amino acid sequences are not known for mouse C3. The reported COOH terminus of human C3 (26) does not resemble the sequences preceding the translation termination codons contained in any of the three reading frames determined for mouse C3 cDNA. However, from the nucleotide sequence and the position of these fragments relative to the cDNA map, we conclude that this cDNA region represents the 3′-end of C3 mRNA.

Sequence Analysis of the 5′-Proximal Region of C3 mRNA. Because the 5′-terminal part of the C3 mRNA was not represented in the cloned C3 cDNA, a 5′ end-labeled product, generated by reverse transcription of this region, was synthesized by using C3 mRNA as template and the 5′ end-labeled *Hin*dIII–*Eco*RI fragment 1 (Fig. 1) as primer. A 430-nucleotide cDNA product was obtained, and the sequence of its first 300 nucleotides, starting from the 5′-proximal *Hin*dIII site and extending toward the 5′ end of C3 mRNA, was determined. However, it was not possible to evaluate the nucleotide sequence of the extreme 5′ end (i.e., the first 130 nucleotides of the C3 mRNA). Aided by comparison with the known NH_2-terminal sequences of guinea pig (27) and human C3 chains (26), we predict the NH_2-terminal amino acid sequence of mouse C3β to be: Ile- Pro- Met- Tyr- Ser- Ile- Ile- Thr- Pro- Asn- Val- Leu- Arg- Leu- Glu (Fig. 3). Furthermore, within the same 430-nucleotide primer-extended cDNA product, sequences that covered the next 10 nucleotides upstream from the triplet coding for the NH_2-terminal Ile-residue of the mature C3β subunit were determined (data not shown). No methionine codon was detected in this region. Therefore, we conclude that the translation of the pro-C3 molecule probably does not start with the NH_2 terminus of mature C3β, but contains an NH_2-terminal extension peptide.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	AUC	CCC	AUG	UAU	UCC	AUC	AUU	ACU	CCC	AAU	GUC	CUA	CGG	CUG	GAG
MOUSE	I	P	M	Y	S	I	I	T	P	N	V	L	R	L	E
GUINEA-PIG	D	P	M	Y	S	I	I	T	P	N	I	L	R	L	E
HUMAN	S	P	M	Y	S	I	G	T	P	B					

FIG. 3. Comparison of the NH_2-terminal amino acid sequences of C3β from mouse, guinea pig (27, 28), and man (26). The top row shows the mRNA sequence derived from the cDNA sequence.

Sequence Analysis of the Region Coding for C3a. From the known structure of C3, the region containing the anaphylactic peptide, C3a, is predicted to be contained in cDNA fragment 4 (Fig. 1). Therefore, nucleotide sequences of a portion of this fragment were determined; the corresponding amino acid sequence of C3a and its flanking regions was derived (Fig. 4) and compared with the known C3a amino acid sequences of other species (29–31). These results demonstrate that mouse C3a shares 72, 52, and 50 residues, respectively, out of 78 with rat and out of 77 with human and porcine C3a. The region of this cDNA fragment that corresponds to the COOH terminus of the mouse C3β subunit (Fig. 4) specifies the amino acid sequence Pro-Ala-Ala-Arg-Arg-Arg-Arg. The Pro-Ala-Ala sequence corresponds to the known COOH terminus of human C3β (26); the four consecutive arginines appear to be present only in the precursor pro-C3 molecule.

Sequence Analysis of the Internal Thioester Bond Region. A major portion of the C3d fragment, known to contain the thioester site, was predicted to be encoded by cDNA fragment 5. In Fig. 5, the nucleotide sequence encoding this site is shown and compared with the amino acid sequence of the corresponding region of human C3 (11). Thirty-five out of 41 residues were identical. In particular, all 10 residues that are held in common among human C3d, C4d, and α2-macroglobulin were identical (refs. 32 and 33; boxed in Fig. 5). The cDNA sequence indicates that the second glutamyl residue (residue 26 in Fig. 5), which is involved in the formation of the thioester bond, is encoded as a glutamine.

DISCUSSION

We have isolated and characterized eight cDNA clones (pMLC3/1–8) containing inserts representing mouse C3 mRNA sequences. We had reported previously that the plasmids pMLC3/1–3 contained cDNA sequences capable of retaining mRNA that

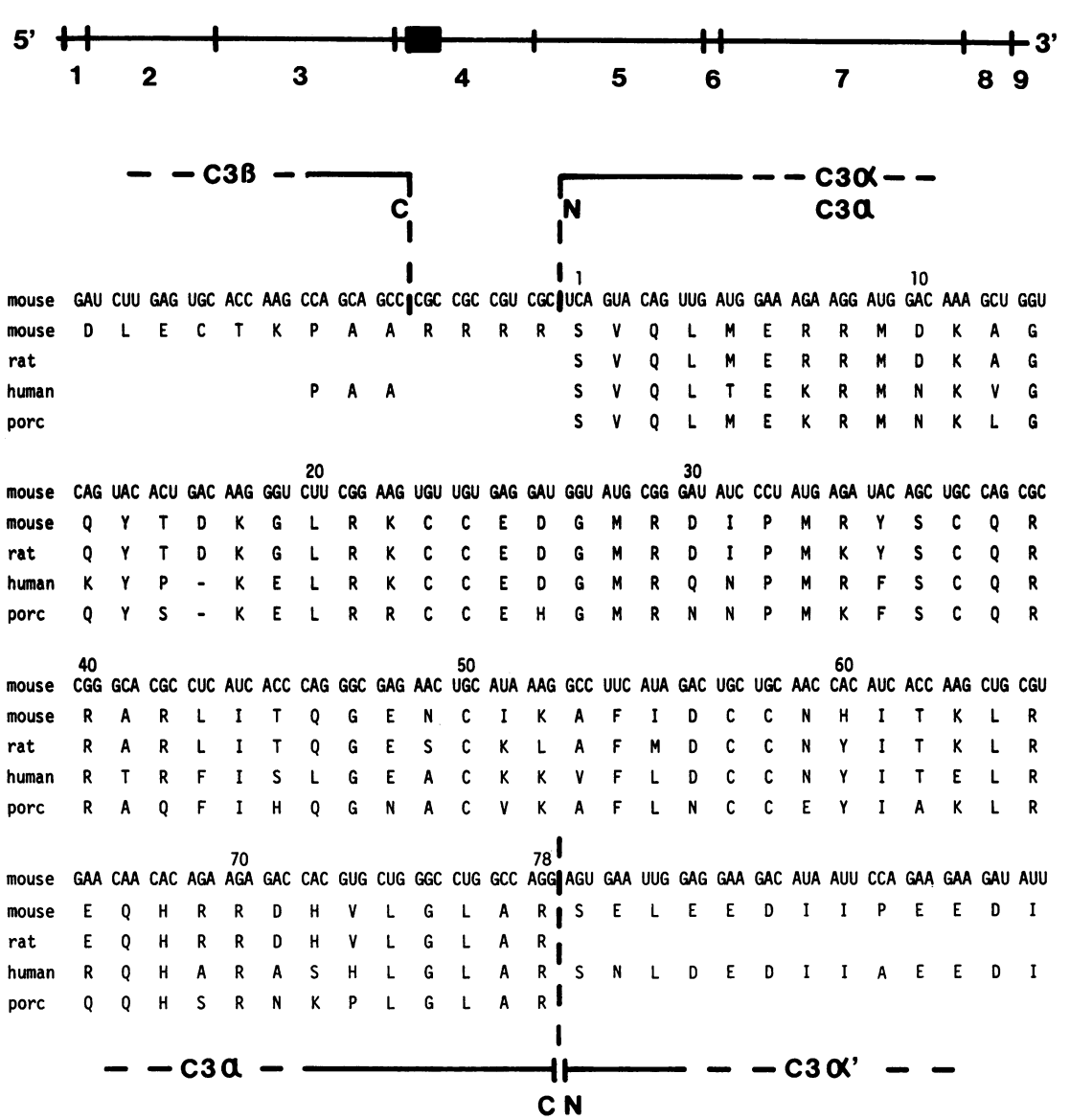

FIG. 4. Comparison of the amino acid sequences of C3a and its flanking regions. (*Top*) ■, Portion of fragment 4 whose sequence was determined. The positions of the COOH terminus (C) of the C3β subunit and the NH_2 terminus (N) of the C3α subunit and the C3a fragment are shown. (*Middle*) Amino acid sequences. The mouse mRNA sequence was derived from cDNA sequence. Rat, human, and porcine amino acid sequences were taken from Jacobs *et al.* (31), Hugli (29), and Corbin and Hugli (30), respectively. (*Bottom*) Positions of the COOH terminus (C) of C3a and the NH_2 terminus (N) of C3α'.

could be translated *in vitro* into immunoprecipitable C3-reactive polypeptides (15). In the present study, we compared our cloned DNA sequence data with known amino acid sequences for selected regions of guinea pig, rat, human, and porcine C3. We conclude that these clones are specific for mouse C3 because of the high degree of sequence homology—for example, 92% homology with rat C3a and 85% homology with the thioester site in human C3d. Furthermore, from the known structure of C3, we can predict the location of the C3 subunit and the C3a and C3d fragments with respect to the cDNA map. From cDNA sequence data, we find an exact correspondence with the location of these subdomains in C3. No protein sequence data are available for mouse C3 and, thus, direct identification of cloned mouse cDNA sequences is not possible.

Using three different methods, we have obtained a value of 5,100 ± 200 nucleotides for the length of C3 mRNA: (*i*) direct size measurement by agarose gel electrophoresis of RNA denatured with glyoxal; (*ii*) size measurement and map position of cloned cDNA fragments giving a minimum value of 4,600 nucleotides; subsequent determination by RNase H analysis of the distance from the 5' terminus of C3 mRNA to known restriction sites; (*iii*) sizing of the 5'-terminal mRNA portion, not represented in cDNA clones, by electrophoresis of primer-extended cDNA in polyacrylamide gels. Previously, we reported a size of 7,500 nucleotides (15), using another procedure. We feel the present value, which includes an analysis of double-stranded C3 cDNA, is more reliable. From the abundance of C3 clones in the cDNA collection (18 of 312) and a 20-fold enrichment of C3 mRNA by size selection (15), we calculated the frequency of C3 mRNA in the total mouse liver mRNA population to be on the order of 0.2–0.3%.

The extreme 5'-terminal sequence of C3 mRNA was not represented in our cDNA collection. However, its length has been determined (350 nucleotides), and approximately two-thirds (220/350) of its nucleotide sequence was obtained by primer-extension experiments. The NH_2 terminus of the mature mouse C3β subunit was identified and positioned in primer-extended cDNA. Thus, the order of subunits in pro-C3 was determined to be NH_2–β–α-COOH, in agreement with protein sequence studies of Goldberger *et al.* (27). Furthermore, our data indicate

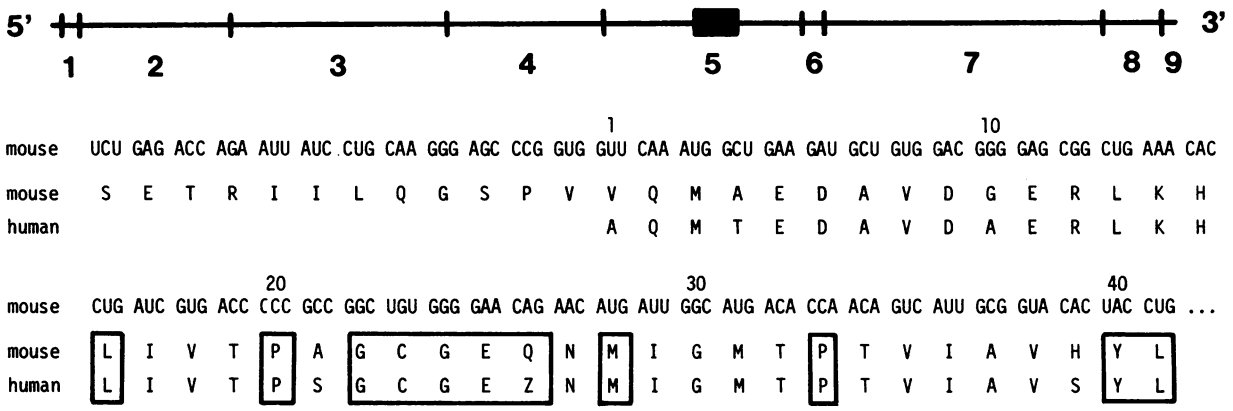

FIG. 5. Comparison of the amino acid sequences encompassing the internal thioester site from murine (predicted from the nucleotide sequence) and human (11) C3d. (*Upper*) ■, Portion of fragment 5 whose sequence was determined. (*Lower*) Numbering of amino acid residues in the C3d fragment is from Thomas *et al.* (11). The boxes indicate conserved amino acid residues that are identical in human C3d, C4d, and α2-macroglobulin (32, 33).

that additional coding sequences preceding those specifying the NH_2 terminus of mature C3β are present in C3 cDNA in the same open reading frame. Because C3 is a secreted serum glycoprotein, the initial translation product may well contain a signal peptide (34).

The derived sequence for mouse C3a strongly resembles known rat, human, and porcine C3a anaphylatoxins. The sequence that is COOH-terminal to mouse C3a constitutes the NH_2-terminal region of the C3α′ fragment (Fig. 1) and shows identity in 10 out of 13 residues with human C3α′ (ref. 26; Fig. 4). The COOH terminus of C3a and the NH_2 terminus of the α′ chain are encoded by contiguous triplets of arginine-serine; thus, the proteolytic removal of C3a involves cleavage of a single peptide bond, confirming previous evaluations of amino acid sequence in the region (6, 26). The cDNA data indicate the existence of four consecutive arginine residues in pro-C3 located between the COOH terminus of C3β and the NH_2 terminus of C3α (C3a).

A high degree of homology was observed for the C3d region containing the internal thioester site on comparing the mouse cDNA and human protein sequence data. The second glutamyl residue (residue 26 in Fig. 5) which participates in linkage to the cysteinyl sulfhydryl group (residue 23 in Fig. 5), forming the thioester, has been shown to appear as glutamic acid after hydrolysis of the bond (9). However, because this highly reactive ester represents a secondary protein modification, it is not possible to decide from protein sequence data whether this residue was initially encoded as glutamic acid or as glutamine. The nucleotide sequence data clearly indicate a glutamine.

We thank H. Diggelmann, O. Hagenbüchle, U. Schibler, N. Fasel, L. and B. Tack, and B. Hirt for discussions and support; M. Thomas for communication of unpublished results; and S. Cherpillod for typing the manuscript. This work was supported by a grant from the Swiss National Science Foundation. H.D. and K.W. were recipients of postdoctoral fellowships from the Deutsche Forschungsgemeinschaft and the European Molecular Biology Organization, respectively. K.O. was supported by fellowships from the Swiss Confederation and the Emil Barell Foundation.

1. Müller-Eberhard, H. J. & Schreiber, R. D. (1980) *Adv. Immunol.* **29**, 1–53.
2. Reid, K. B. M. & Porter, R. R. (1981) *Annu. Rev. Biochem.* **50**, 433–464.
3. Brade, V., Hall, R. E. & Colten, H. R. (1977) *J. Exp. Med.* **146**, 759–765.
4. Hall, R.. E. & Colten, H. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1707–1710.
5. Hugli, T. E. & Müller-Eberhard, H. J. (1979) *Adv. Immunol.* **26**, 1–53.
6. Hugli, T. E. (1981) *CRC Crit. Rev. Immunol.* **1**, 321–366.
7. Law, S. K. & Levine, R. P. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2701–2705.
8. Hostetter, M. K., Thomas, M. L., Rosen, F. S. & Tack, B. F. (1982) *Nature (London)* **298**, 72–75.
9. Tack, B. F., Harrison, R. A., Janatova, J., Thomas, M. L. & Prahl, J. W. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5764–5768.
10. Law, S. K., Fearon, D. T. & Levine, R. P. (1979) *J. Immunol.* **122**, 759–765.
11. Thomas, M. L., Janatova, J., Gray, W. R. & Tack, B. F. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1054–1058.
12. Fearon, D. T. (1980) *J. Exp. Med.* **152**, 20–30.
13. Lambris, J. D., Dobson, N. J. & Ross, G. D. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1828–1832.
14. Perlman, H., Perlman, P., Schreiber, R. D. & Müller-Eberhard, J. J. (1981) *J. Exp. Med.* **153**, 1592–1603.
15. Odink, K. G., Fey, G., Wiebauer, K. & Diggelmann, H. (1981) *J. Biol. Chem.* **256**, 1453–1458.
16. Grunstein, M. & Hogness, D. S. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3961–3965.
17. Clewell, D. B. & Helinski, D. R. (1970) *Biochemistry* **9**, 4428–4440.
18. Smith, H. O. & Birnstiel, M. L. (1976) *Nucleic Acids Res.* **3**, 2387–2398.
19. McMaster, G. K., Beard, P., Engers, H. D. & Hirt, B. (1981) *J. Virol.* **38**, 317–326.
20. Casey, J. & Davidson, N. (1977) *Nucleic Acids Res.* **4**, 1539–1552.
21. McMaster, G. K. & Carmichael, G. G. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4835–4838.
22. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5201–5205.
23. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–560.
24. Wiebauer, K., Domdey, H., Diggelmann, H. & Fey, G. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7077–7081.
25. Proudfoot, N. & Brownlee, G. G. (1976) *Nature (London)* **263**, 211–214.
26. Tack, B. F., Morris, S. C. & Prahl, J. W. (1979) *Biochemistry* **18**, 1497–1503.
27. Goldberger, G., Thomas, M. L., Tack, B. F., Williams, J., Colten, H. R. & Abraham, G. N. (1981) *J. Biol. Chem.* **256**, 12617–12619.
28. Thomas, M. L. & Tack, B. F. (1982) *Biochemistry*, in press.
29. Hugli, T. E. (1975) *J. Biol. Chem.* **250**, 8293–8301.
30. Corbin, N. C. & Hugli, T. E. (1976) *J. Immunol.* **117**, 990–995.
31. Jacobs, J. W., Rubin, J. S., Hugli, T. E., Bogardt, R. A., Mariz, I. K., Daniels, J. S., Daughaday, W. H. & Bradshaw, R. A. (1978) *Biochemistry* **17**, 5031–5038.
32. Harrison, R. A., Thomas, M. L. & Tack, B. F. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 7388–7392.
33. Campbell, R. D., Gagnon, J. & Porter, R. R. (1981) *Biochem. J.* **199**, 359–370.
34. Blobel, G. & Dobberstein, B. (1975) *J. Cell Biol.* **67**, 852–862.
35. (1968) *Bull. W. H. O.* **39**, 935.