Identification and Alignment of a Thiol Ester Site in the Third Component of Guinea Pig Complement†

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ABSTRACT: The third component of guinea pig complement (C3) was purified by poly(ethylene glycol) precipitation of ethylenediaminetetraacetic acid plasma, depletion of plasminogen on Sepharose–L-lysine, chromatography on DEAE-Sephacel, and gel filtration on Sepharose CL-6B. IgG was removed in a final step with protein A–Sepharose. The recovery of functional activity was 56% with a purification of 81-fold. An α- and β-chain structure with apparent molecular weights of 115 000 ± 12 000 and 65 000 ± 7000, respectively, was observed on sodium dodecyl sulfate ($NaDodSO_4$) gel electrophoresis. The guinea pig β chain, unlike human, does not stain for carbohydrate with periodic acid–Schiff's reagent. The α and β chains were separated, and the amino-terminal structure of each was determined by Edman degradation. The sequence of each chain was highly concordant with that of the corresponding chain in human C3. Incubation of guinea pig C3 with [^{14}C]methylamine resulted in a maximal uptake of 0.76 mol/mol into the α chain. This reaction was accompanied by a concomitant and stoichiometric (1:1) appearance of a free sulfhydryl group. Guinea pig [^{14}C]methylamine-inactivated C3 was immobilized on activated thiol–Sepharose and digested with porcine elastase. Bound material was then released with L-cysteine and analyzed by $NaDodSO_4$ gel electrophoresis, revealing a primary ^{14}C-labeled peptide band with a molecular weight of 33 000 (C3d). Prior to sequence analysis, the free sulfhydryl group in C3d was reacted with iodo[^{3}H]acetic acid. On Edman degradation, the ^{14}C label was quantitatively released at step 26 and corresponded to a modified glutamyl residue, γ-glutamyl[^{14}C]methylamide. The sulfhydryl residue observed on nucleophilic inactivation of C3 was recovered as *S*-([^{3}H]carboxymethyl)cysteine at position 23. Comparison of the amino-terminal structures of human and guinea pig C3d indicated 95% homology for the 37 residues identified. These results are consistent with the presence of an active ester, presumably a thiol ester, in the amino-terminal region of guinea pig C3d.

Complement proteins participate in immunosurveillance and inflammatory pathways; consequently, they fulfill an important role in host defense to bacterial and viral pathogens [for review see Stroud et al. (1979); Müller-Eberhard & Schreiber, 1980; Reid & Porter, 1981]. The third component of complement (C3) is a participant in both the classical and alternative complement pathways and is required for the activation of the late components which constitute the membrane attack (cytolytic) complex. Human C3 consists of two chains, α and β, and is activated in vivo by either the classical or alternative pathway C3 convertase ($\overline{C4b2a}$ or $\overline{C3bBb}$, respectively), which cleaves the α chain to generate two fragments, C3a and C3b. C3a (M_r 9000) is derived from the amino-terminal end of the α chain (Hugli, 1975; Tack et al., 1979) and has anaphylactic properties (Da Silva & Lepow, 1967). The macromolecular C3b fragment, having an $\alpha'\beta$ chain structure, is able to bind covalently to cell surface membrane components (Law & Levine, 1977; Law et al., 1979a). An internal thiol ester bond has been identified in native human C3 (Tack et al., 1980; Janatova et al., 1980) which, following proteolytic cleavage of the molecule, is capable of donating its acyl group in a transesterification reaction with small acceptor molecules (Hostetter et al., 1982). A similar reaction is believed to pertain to the covalent interaction of C3b with cell surface membrane components (Law et al., 1980). Subsequent interaction of bound C3b with specific receptors on the macrophage and neutrophil cell surface is the basis for the opsonic property of this protein (Gigli & Nelson, 1968; Ross et al., 1973; Ehlenberger & Nussenzweig, 1977). Surface-bound C3b is subject to cleavage in the α' chain by C3b inactivator, generating C3bi. This cleavage reaction is dependent on the presence of a second protein, β_1H globulin (Whaley & Ruddy, 1976). Additional serum proteases, acting at specific cleavage sites in C3bi, produce the C3c and C3d fragments. C3c consists of two α' chain derived fragments and the β chain. C3d (M_r 33 000) is derived from an interior segment of the α' chain and contains the thiol ester site (Thomas et al., 1982).

Shin & Mayer (1968) were the first to obtain guinea pig C3 in a biochemically pure state, although the protein had been previously prepared in a functionally pure form (Nelson et al., 1966). Further procedures for the isolation of guinea pig C3 have been published; however, yields based on quantitative functional and antigenic analyses were not reported (Bitter-Suermann et al., 1970; Kawachi-Takahashi et al., 1973; Okuda, 1973; Meuer et al., 1978). In this paper we (1) describe a significantly improved method for the isolation of guinea pig C3, (2) present the amino-terminal structures of several subdomain structures of the protein, (3) identify the presence of a thiol ester site in the native protein, and (4) position this site within the amino-terminal region of C3d.

Experimental Procedures

Materials. Sheep red blood cells, hemolysin, and guinea pig C2 and C5-9 were obtained from Cordis Laboratories. Hydrazine, methylamine, periodic acid, and basic fuchsin were from Eastman Chemicals. Protein standards (molecular

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weight markers) and chromatographic media were from Pharmacia. Anti-guinea pig whole serum was from Capel Laboratories. Dithiothreitol, iodoacetamide, 2-mercaptoethanol, stannous chloride, and the $NaDodSO_4$[1] used for gel filtration were from Pierce Chemical Co. Ultrex HCl was from J. T. Baker Co. PMSF was from Sigma Chemicals Co. Benzamidine was from Aldrich. Analyzer buffers and sequencer reagents were from Beckman Instruments. Sequencer solvents were from Burdick & Jackson. [^{14}C]Methylamine, iodo[1-^{14}C]acetamide, and iodo[^{3}H]acetic acid were from Amersham and New England Nuclear. Biofluor was from New England Nuclear. Porcine elastase was purchased from Worthington and further purified (Narayanan & Anwar, 1969). Anti-guinea pig C3 was prepared by immunizing New Zealand white rabbits with the purified antigen emulsified in complete Freund's adjuvant.

Immunological Techniques. A quantitative hemolytic assay for guinea pig C3 was prepared with Cordis components according to the manufacturer's instructions. C3 titers were determined as previously described (Hammer et al., 1981). For determination of the elution position of C3 from chromatographic steps, a qualitative hemolytic assay was prepared by combining a plasma depleted of C3 and C7 by affinity chromatography with a plasma depleted of C3 and C4 activity by treatment with hydrazine. This combined reagent was diluted 1:200 and was devoid of C3 activity. The assay consisted of 5 μL of column fraction combined with 0.5 mL of C3-deficient reagent, 0.1 μL of complement-coated cellular intermediate $EAC\overline{14b}$ (1.5×10^8 cells/mL), and 0.4 mL of $GVB.^{2+}$ The mixture was incubated at 37 °C for 1 h and the reaction terminated by the addition of 1 mL of cold isotonic saline. The extent of lysis was determined by measuring the absorbance at 415 nm.

Radial immunodiffusion, two-dimensional immunodiffusion, and immunoelectrophoresis were performed as described in the *Handbook of Experimental Immunology* (Ouchterlony & Nilsson, 1978).

$NaDodSO_4$–Polyacrylamide Gel Electrophoresis. $NaDodSO_4$–polyacrylamide slab gel electrophoresis was performed by using the $NaDodSO_4$/Tris–glycine buffer system (Laemmli, 1970). The resolving gel was made 7.5% acrylamide with an acrylamide to bis(acrylamide) ratio of 22:1. Gels were stained for protein with Coomassie brilliant blue R250 and, where applicable, prepared for autofluorography (Bonner & Laskey, 1974). Samples analyzed for the presence of carbohydrate were electrophoresed in tube gels (Weber & Osborn, 1969) and stained with periodic acid–Schiff's reagent (Fairbanks et al., 1971).

Isolation of Guinea Pig C3. Guinea pig C3 was isolated in a manner similar to that reported for the isolation of human C3 (Hammer et al., 1981). Fasted Hartly guinea pigs were bled weekly. The blood was diluted with $^1/_{10}$ volume of a 10× inhibitor solution [0.1 M Na_2EDTA and 0.1 M benzamidine hydrochloride (pH 7.5)]. The plasma was separated by centrifugation (600*g*, 20 min), adjusted to 1 mM PMSF, frozen on dry ice, and stored at −70 °C. Upon thawing, 500 mL of plasma was pooled, again adjusting the PMSF concentration to 1 mM, and precipitated with 5% PEG (w/v). The supernatant was further precipitated with 15% PEG (w/v). The 15% PEG precipitate was dissolved in 0.1 M potassium/sodium phosphate (pH 7.6) containing 0.15 M NaCl, 10 mM Na_2EDTA, and 10 mM benzamidine hydrochloride and then chromatographed on L-lysine–Sepharose. The plasminogen-depleted pool was diluted 3–4-fold with 5 mM potassium/sodium phosphate buffer (pH 7.6, 1.4 mS/cm) containing 7 mM benzamidine hydrochloride, 7 mM Na_2EDTA, and 3.5 mM EACA and then concentrated to the initial plasma volume with a Pelicon ultrafiltration system (Millipore). When this last step was repeated 2–3 times, the conductivity of the protein solution was decreased to 2.24 mS/cm. The protein sample was then applied to a column of DEAE-Sephacel (5 × 150 cm, equilibrated in the above buffer) at a flow rate of 96 mL/h. The column was washed with 1.5 L of buffer and the protein eluted with an 8-L linear salt gradient of 0–300 mM NaCl. The absorbance (280 nm) of column fractions was determined on aliquots of each diluted 10-fold with phosphate-buffered saline. Conductivity measurements were performed at 0 °C. The C3-containing fractions were pooled and made 15% (w/v) in PEG. The precipitate was dissolved in 0.1 M potassium/sodium phosphate buffer (pH 7.6) containing 0.15 M NaCl and 10 mM Na_2EDTA and applied to a Sepharose CL-6B column (5 × 150 cm) at a flow rate of 100 mL/h. The C3 pool was concentrated to 5.7 mg/mL by ultrafiltration on an XM 50 A membrane (Amicon) and depleted of IgG by affinity chromatography in two batches on a 3.5-mL column of protein A–Sepharose. Between batches, the column was washed with 10 mL of 1 M acetic acid and then reequilibrated. Hemolytic and radial immunodiffusion assays were used to quantitate C3 at each step in the purification. In control studies, benzamidine did not have an inhibitory effect on functional assays. The final product was frozen in 50-μL drops in liquid nitrogen and stored at −70 °C.

Separation of C3 Polypeptide Chains. Guinea pig C3 (46 mg) was reduced and alkylated in 1% $NaDodSO_4$ in a manner otherwise identical with that previously described for human C3 (Tack et al., 1979) and applied to a Sepharose CL-4B column (2.5 × 200 cm) equilibrated in 0.1 M ammonium bicarbonate (pH 8.0) containing 0.2% $NaDodSO_4$. The flow rate was 10 mL/h. Fractions containing α and β chains were pooled and lyophilized.

Incorporation of Methylamine and the Titration of a Thiol Group. C3 was inactivated by incubating the protein with 50 mM methylamine for 6 h at 37 °C under nitrogen in 0.1 M Tris-HCl buffer (pH 8.0) containing 10 mM Na_2EDTA. When [^{14}C]methylamine was used, the specific activity was reduced to 0.5–5 mCi/mmol with [^{12}C]methylamine from a stock 1 M solution in Tris-HCl buffer (pH 7.5). Alkylation of thiol groups was performed with either iodo[1-^{14}C]acetamide or iodo[^{3}H]acetic acid under nitrogen, using a 10-fold molar excess over protein. Samples were desalted either by chromatography on Sephadex G-25M or by dialysis against 0.1 M Tris-HCl buffer (pH 8.0) containing 10 mM Na_2EDTA. Incorporations of radiolabeled methylamine and iodoacetamide were determined as previously described (Janatova et al., 1980).

C3d Isolation. C3d was isolated by two different methods. In the first procedure, [^{14}C]methylamine-treated C3 (containing a free SH group) was immobilized on activated thiol–Sepharose by incubating for 2 h at 37 °C under nitrogen in 0.1 Tris-HCl buffer (pH 8.0) containing 10 mM Na_2EDTA. Prior to coupling of the protein, potential free thiol groups on

[1] Abbreviations: DEAE, diethylaminoethyl; EACA, ε-aminocaproic acid; EDTA, ethylenediaminetetraacetic acid; HPLC, high-performance liquid chromatography; $NaDodSO_4$, sodium dodecyl sulfate; PAS, periodic acid–Schiff's reagent; PEG, poly(ethylene glycol); PMSF, phenylmethanesulfonyl fluoride; PTH, phenylthiohydantoin; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; SH, sulfhydryl; GVB^{2+}, 5 mM sodium veronal buffer (pH 7.4) containing 0.14 M NaCl, 1 mM $MgCl_2$, 0.15 mM $CaCl_2$, 5% (w/v) glucose, and 0.1% (w/v) gelatin; complement nomenclature is that recommended by the World Health Organization (1970).

the gel matrix were blocked by treatment with 10 mM iodoacetamide. After coupling, the beads were washed extensively with 0.1 M Tris-HCl (pH 8.0) containing 1 mM $MgCl_2$ and 1 mM $CaCl_2$ to remove unbound protein. The beads were then resuspended in this buffer, and bound protein was digested with 4% (w/w) elastase for 2 h. With a gentle vacuum applied, the beads were washed on a sintered-glass funnel with 10–20 volumes of the Tris–EDTA buffer to remove protein released during the course of digestion. The remaining bound protein was then released by incubating the beads with 10 mM L-cysteine for 30 min at 37 °C and collecting the eluate by filtration. The elution with L-cysteine was repeated, and both eluates were pooled and dialyzed against 0.1 M Tris-HCl buffer (pH 8.0) containing 10 mM Na_2EDTA and 1 mM L-cysteine. The dialysate was alkylated by treating with 23 mM iodo[^{3}H]acetic acid for 1 h at 37 °C. Alternatively, C3d was genrated by digesting C3 with 0.5% (w/w) elastase for 3 h at 37 °C in 0.1 M sodium/potassium phosphate buffer (pH 7.6) containing 0.15 M NaCl and 10 mM Na_2EDTA. The digestion was stopped by the addition of PMSF to 1 mM. C3d was separated from C3c by gel filtration on a column of Sephadex G-100 SF (2.5 × 180 cm) equilibrated in 50 mM sodium acetate (pH 5.6) containing 1 M NaCl.

Amino Acid Analysis. Samples for amino acid analysis were dialyzed exhaustively against distilled H_2O or 0.1 M acetic acid and lyophilized in hydrolysis tubes. They were redissolved in 0.5 mL of 5.7 N HCl (Ultrex) containing 5 mM phenol and 0.1% 2-mercaptoethanol. The tubes were swirled in dry ice–methanol, evacuated to a pressure of 25 μm or less, and sealed under vacuum. Samples were hydrolyzed for either 24, 48, or 72 h at 110 °C in a heating block. Following hydrolysis, the tubes were opened and dried in vacuo over NaOH. The dried hydrolysates were resuspended in Beckman sample dilution buffer and analyzed on a Beckman 121 MB analyzer. Each amino acid was averaged over the three time points with the following exceptions: Ser, Thr, and Trp were extrapolated to time zero; Ile and Val values were those obtained for the 72-h time point. Chain compositions were determined by using apparent molecular weights derived from $NaDodSO_4$–polyacrylamide gel electrophoresis.

Amino-Terminal Sequence Analysis. Samples (30–50 nmol) were prepared for sequence analysis by exhaustive dialysis against distilled H_2O or 1 M acetic acid. A Beckman 890 spinning cup sequencer modified with cold trap was used for automated Edman degradation employing a 0.1 M Quadrol buffer (Brauer et al., 1975). In later sequence analysis of C3d, a P-6 autoconverter (Sequemat) was used for the conversion step. PTH-amino acid derivatives were identified by HPLC by using a Waters instrument with a Zorbax ODS-C18 column (Du Pont) equilibrated with 20% acetonitrile in 0.01 M sodium acetate (pH 4.5) (Zimmerman & Pisano, 1977). Some residues were identified after back-hydrolysis in constant boiling HCl containing 0.1% stannous chloride at 150 °C for 4 h in vacuo (Mendez & Lai, 1975). Identification was made by using a Beckman 121 MB amino acid analyzer. Initial yields for sequence analysis were routinely between 40 and 50% with repetitive yields between 92 and 95%. All sequence data presented are the results of at least two analyses. A representative recovery of PTH-amino acids is given in Table I of the supplementary material (see paragraph at end of paper regarding supplementary material).

Results and Discussion

Purification. Guinea pig C3 was purified by using the method of Hammer et al. (1981), with analogous results observed. One noticeable difference, however, was that guinea pig C5 eluted at 7.8 mS/cm (after C3) upon DEAE-Sephacel chromatography. Human C5 eluted from this column at 5.5 mS/cm, immediately before elution of C3. Ceruloplasmin, a major contaminant in most human C3 preparations, was resolved from guinea pig C3 but coeluted with C5. The final specific activity of guinea pig C3 was 2.35×10^4 CH_{63} units/unit of absorbance at 280 nm, corresponding to an 81-fold purification. This material gave a single immunoprecipitation line against anti-guinea pig whole serum and exhibited a line of identity with anti-guinea pig C3 (data not shown). The final yield of C3 based on both functional and antigenic assays was 56% and 40%, respectively. The difference between the two values could be ascribed to the presence of the C3 inhibitors, C3b INA and β1H, in plasma which could depress the initial functional values. The amount of C3 in the starting plasma was calculated to be 0.85 mg/mL by radial immunodiffusion, assuming an extinction coefficient ($E^{1\%}_{1cm}$) of 10.0 for the purified protein.

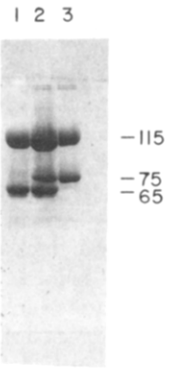

FIGURE 1: $NaDodSO_4$–polyacrylamide gel electrophoresis of guinea pig and human C3. (Track 1) 6 μg of guinea pig C3; (track 2) 6 μg of guinea pig C3 plus 6 μg of human C3; (track 3) 6 μg of human C3.

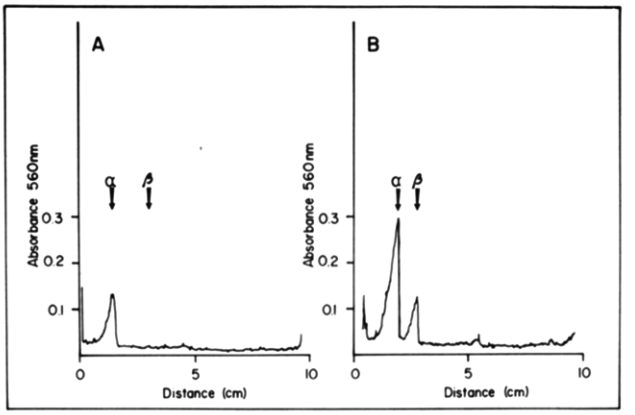

FIGURE 2: PAS stain of 150 μg of guinea pig and human C3 following electrophoresis on $NaDodSO_4$–polyacrylamide gels. (A) is a scan of guinea pig C3 and (B) a scan of human C3.

$NaDodSO_4$–Polyacrylamide Gel Analysis. Both guinea pig C3 and human C3 were electrophoresed on polyacrylamide gels under reducing conditions (Figure 1). While the α chains were similar in size, the guinea pig β chain was smaller by 10000 in apparent molecular weight. This difference in mobility could be partially accounted for by the lack of detectable carbohydrate on the guinea pig β chain. Figure 2 shows a scan at 560 nm of an acrylamide gel containing guinea pig and human C3 which had been stained with PAS. Both the human α and β chains and the guinea pig α chain reacted positively for carbohydrate, while the guinea pig β chain was surprisingly unreactive. In addition, the human α chain stained more intensely with the PAS reagent than did the guinea pig

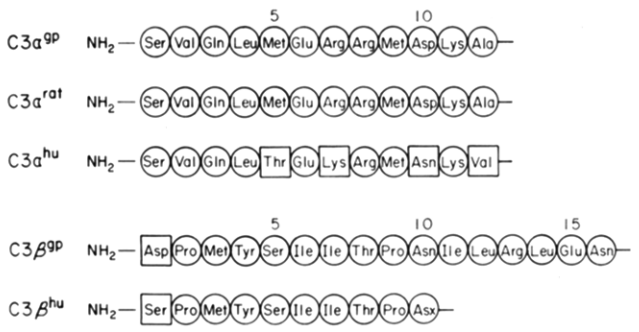

FIGURE 3: Amino-terminal structures of guinea pig C3 α and β chains compared with rat C3a α and human C3 α and β chains.

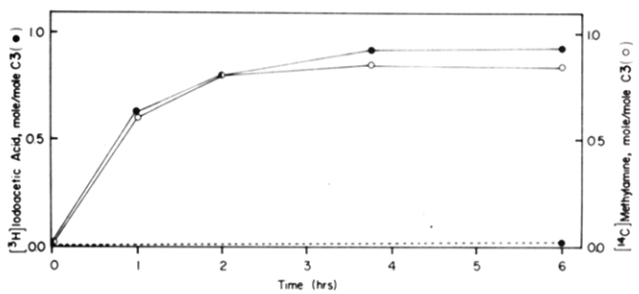

FIGURE 4: [^{14}C]Methylamine and iodo[^{3}H]acetic acid incorporation into guinea pig C3. The dashed line is a control sample of native C3 treated with iodo[^{3}H]acetic acid.

α chain. Corresponding gels, which were stained for protein with Coomassie brilliant blue R250 (data not shown), indicated that comparble amounts of guinea pig and human C3 had been applied. It has been recently reported that mouse C3 β chain appears to be smaller than human C3 β chain (Nonaka et al., 1980). Comparative carbohydrate analysis of mouse and human C3 also indicated a lack of detectable carbohydrate on mouse β chain (Nonaka et al., 1980; Fey et al., 1980).

Amino-Terminal Analysis of Guinea Pig C3 α and β Chains. Guinea pig C3 was reduced and alkylated, and the chains were separated by chromatography on Sepharose CL-4B in 0.2% NaDodSO$_4$. The separated chains were subjected to amino acid analysis and Edman degradation. On comparison with the compositions previously reported for human α and β chains (Tack et al., 1979), no significant differences were observed. These data are available as supplementary material (Table II). In Figure 3 amino-terminal structures of guinea pig α and β chains are compared with the corresponding regions of rat C3a (Jacobs et al., 1978) and the α and β chains of human C3 (Tack et al., 1979). The guinea pig β-chain sequence was previously published elsewhere (Goldberger et al., 1981). The N-terminal sequence of the guinea pig α chain and rat C3a were identical for the first 12 residues, while four replacements were observed on comparing human and guinea pig α chains. This is analogous to other results, indicating that there is approximately 70% homology among the primary structures of human, rat, and porcine C3a (Hugli, 1975; Jacobs et al., 1978; Corbin & Hugli, 1976). Sequence analysis of the amino terminus of the β chain indicated a high degree of conservation between guinea pig and human C3; only the N-terminal residue differed (Figure 3). Pro-C3, the biosynthetic precursor to C3, consists of a single chain which is subsequently processed into the two-chain structure found in plasma. These sequence data were used to identify the chain order in the guinea pig pro-C3 molecule as β–α (Goldberger et al., 1981).

Incorporation of Methylamine into Guinea Pig C3. Guinea pig C3 was treated with 50 mM [^{14}C]methylamine and

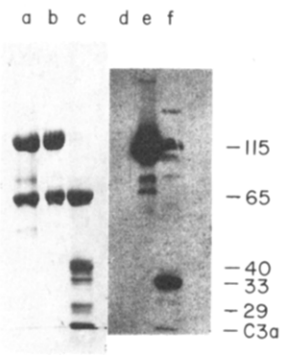

FIGURE 5: Incorporation of [^{14}C]methylamine into the α chain and C3d subdomain of guinea pig C3 as determined by NaDodSO$_4$–polyacrylamide gel electrophoresis and autofluorography. C3 (4.6 mg/mL) was reacted with [^{14}C]methylamine and digested with 2% (w/w) elastase for 2 h at 37 °C. (Tracks a–c) Coomassie brilliant blue R250 stain; (tracks a and d) native C3; (tracks b and e) [^{14}C]methylamine-inactivated C3; (tracks c and f) [^{14}C]methylamine-inactivated C3 digested with elastase.

iodo[^{3}H]acetic acid for varying times (Figure 4). A stoichiometric incorporation of methylamine into C3 was observed, accompanied by the simultaneous appearance of an SH group. Control samples were treated with alkylating reagent in the absence of methylamine incorporated less than 0.1 mol of iodo[^{3}H]acetic acid/mol of C3. The reactive thiol residue appeared to be susceptible to oxidation, so that it was essential that the alkylating reagent be presented along with the nucleophile in order to fully titrate this group. The thiol, however, could be partially protected provided the incubation was done under nitrogen.

Incorporation of Methylamine into Guinea Pig C3d. Guinea pig C3 was reacted with [^{14}C]methylamine and digested with elastase prior to analysis by NaDodSO$_4$ gel electrophoresis (Figure 5). The ^{14}C label was incorporated preferentially into the α chain of undigested C3. Following elastase digestion, the label was confined to the C3d fragment. The β chain was resistant to digestion with elastase. The α chain derived C3d fragment was seen as a doublet. This could be due to multiple elastase cleavage sites, heterogeneity in the amount of carbohydrate, or contaminating proteases. Tracks d–f of the corresponding autofluorogram (Figure 5) indicated labeled bands other than C3d after prolonged exposure. In track e, these are most likely breakdown products of the α chain, while in track f, they are uncleaved α and α' chains.

Isolation of Guinea Pig C3d. Guinea pig C3d was isolated by coupling [^{14}C]methylamine-treated C3 to activated thiol–Sepharose via the free SH group and digesting with elastase. The bound fragment was then eluted with L-cysteine and radioalkylated with iodo[^{3}H]acetic acid. Alternatively, C3d was isolated by digesting C3 with elastase and then separating C3d from C3c by gel chromatography. When activated thiol–Sepharose was employed, the yields were approximately 35%; when digestion of native C3 followed by gel chromatography was used, the yields were 70%. C3d purified by either method appeared to be identical. Analysis by NaDodSO$_4$ gel electrophoresis under reducing conditions indicated a double band for C3d (Figure 6). Edman degradation, however, revealed a single amino-terminal sequence (Figure 7). If the doublet is indicative of a partial cleavage by elastase, this must occur toward the carboxy-terminal end of the fragment. The ^{3}H and ^{14}C cpm values observed on Edman degradation of C3d (isolated on activated thiol–Sepharose) are shown in Figure 8. The release of *S*-([^{3}H]carboxymethyl)cysteine at step 23 corresponds to the SH group that appears on nucleophilic inactivation of C3. The ^{14}C cpm values

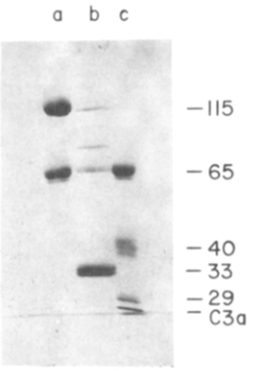

FIGURE 6: NaDodSO$_4$–polyacrylamide gel electrophoresis of guinea pig C3d purified for sequence analysis. (Track a) Starting material (native C3); (track c) eluate from the thiol–sepharose after digestion; (track b) the L-cysteine eluate from thiol–Sepharose following digestion. The band at the running front is assumed to be C3a.

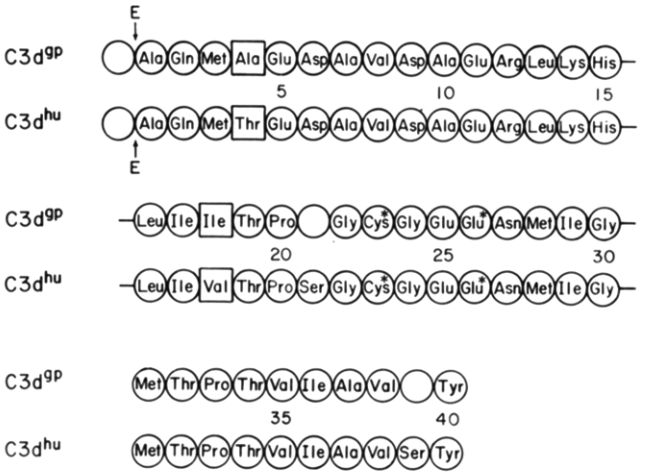

FIGURE 7: Amino-terminal structure of guinea pig C3d compared to the human C3d structure. (E) denotes the elastase cleavage site. The cysteinyl and glutamyl residues labeled with iodo[^{3}H]acetic acid and [^{14}C]methylamine, respectively, are indicated by an asterick.

were released at step 26 and corresponded to γ-glutamyl-[^{14}C]methylamide. Significant radioactivity was also released, with the same ^{3}H and ^{14}C spacing, early in the sequence analysis, indicating minor internal cleavages in this C3d preparation; this does not account for the doublet seen in Figure 6, since a second sequence was not detected by HPLC analysis. The guinea pig C3d sequence (Figure 7) was very similar to that determined for human (Thomas et al., 1982). Two changes, both conservative, were found: an Ala-Thr replacement at step 4 and an Ile-Val replacement at step 18. Two residues which were unidentified in the guinea pig sequence corresponded to Ser resiudes in the human sequence. As with human C3, the data indicate that in the native molecule, the Cys and second glutamyl residues in the sequence Cys-Gly-Glu-Glu- bridge to form an internal thiol ester bond. The importance of this site is indicated by the extensive homology observed on comparing these two species.

Guinea pig C3 therefore shows homology with human C3 in several respects. It behaves similarly during purification, has a two-chain structure with similar amino-terminal sequences, and contains an intrachain thiol ester bond located near the amino-terminal end of the C3d fragment. In contrast, guinea pig β chain appears to be considerably lighter than human β chain on electrophoretic analysis and does not stain for carbohydrate. In this respect, guinea pig C3 is similar to mouse C3 (Nonaka et al., 1980). These observations may relate to incompatibilities observed between human and guinea

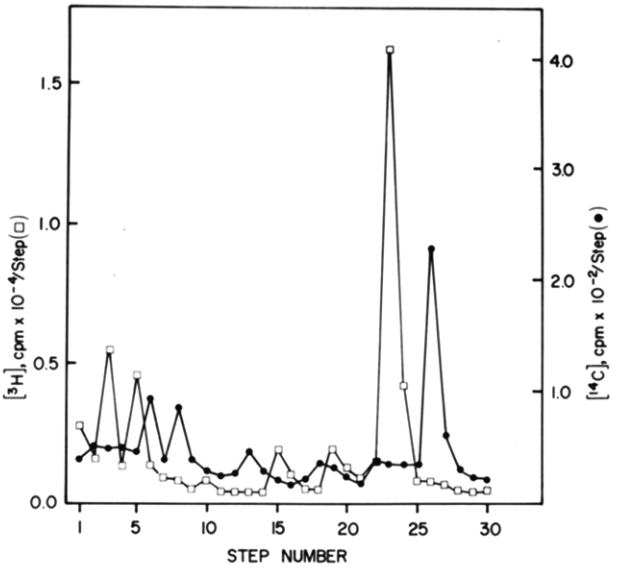

FIGURE 8: ^{3}H and ^{14}C cpm values obtained for each step of automated Edman degradation of guinea pig C3d.

pig C3/C5 convertases (von Zabern et al., 1979).

The amino-terminal region of human and guinea pig C3d are highly conserved. Thirty-five out of 37 residues identified in the amino-terminal region of guinea pig C3d were identical with those of human C3d, representing a 95% homology between the respective primary structures. Initial experiments indicated that the SH group released upon methylamine inactivation was more labile in guinea pig than in human. Since the primary structure in this region is conserved, the explanation for this increased lability is still obscure.

The full extent of conservation between human and guinea pig C3d is of future interest. Law et al. (1979b) have shown that C3d remains bound to cell surfaces following cleavage reactions which release the C3c fragment. Ross & Polley (1975) have demonstrated that bound C3d is subsequently reactive with specific receptors present on a subset of B lymphocytes. Through further comparative analysis, it may be possible to map other sites of functional importance.

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Supplementary Material Available

Quantitation of PTH-amino acids on sequence analysis of guinea pig C3 (Table I) and amino acid composition of guinea pig C3 (Table II) (2 pages). Ordering information is given on any current masthead page.

Registry No. C3, 80295-41-6; C3d, 80295-45-0.

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