Studies on the Structural Localization of Rabbit H Chain Allotypic Determinants Controlled by the a Locus

PURIFICATION AND IMMUNOLOGICAL PROPERTIES OF AN IMMUNOPEPTIDE BEARING α3 ALLOTYPIC DETERMINANT(S)

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An immunopeptide bearing α3 allotypic determinant(s) was isolated from the γ chain of an α3 homozygous rabbit (G222-2) immunized with type III pneumococcal vaccine. Immunological properties of peptides were studied using a radioimmunoassay that involved inhibition by these peptides of a reaction between 125I-labeled anti-α3 antibody and Sepharose-bound α3 immunoglobulin G (IgG). The γ chain was isolated from IgG of restricted heterogeneity and then citraconylated and digested with trypsin. The tryptic digest (TD1) was passed through an anti-α3 immunoabsorbent column either directly or after an intermediate step of Sephadex G-75 chromatography. The bound peptides (T1) were eluted with 0.1 M acetic acid and further digested with trypsin. The digest (TD2) was again run on the anti-α3 immunoabsorbent column to purify the bound immunopeptide T2. In the radioimmunoassay this immunopeptide was found to have major α3 determinant(s). Its molecular weight was found to be approximately 6,000, which decreased to about 3,000 after reduction and alkylation. These data, together with NH2- and COOH-terminal analyses and cysteine peptide mapping, demonstrated that T2 is composed of two polypeptide chains linked by a disulfide bond, one from the cysteine 22 region having lysine at the COOH terminus and the other from the cysteine 92 region having arginine at the COOH terminus. The lysine peptide was separated from the arginine peptide and its NH2-terminal sequence was found to be Gly-Asx-Glx-Ser-Thr-Cys. Since the cysteine is at position 22, the lysine peptide starts at position 17. It has approximately 22 residues. The framework sequence from 17 to 33 of the lysine peptide and 67 to 79 and 84 to 85 which may be present in the arginine peptide are fully exposed on the surface and are far removed from the antibody combining site.

The antigenic determinants of rabbit IgG include isotypic specificities common to all rabbits and allotypic specificities by which rabbits can be subdivided into several groups (1). The heavy chain allotypes α1, α2, and α3, detectable by precipitating alloantisera, behave in breeding studies as if controlled by three allelic genes at an autosomal locus designated as the α locus (reviewed in Refs. 2 and 3). Several studies support the view that the genes of the α locus are controlling or structural genes for the variable region, but because of the considerable sequence heterogeneity of this region it has been difficult to establish the exact chemical nature of the sequences which define the allotypes (4, 5). Chemical analyses have established that there are reproducible total compositional differences in the Fd fragments and in the heavy chains from α1, α2, and α3 normal and antibody IgG pools (6–11). Most of the compositional differences are accounted for as differences in the sequence of the first 94 residues from the NH2 terminus of the heavy chain (4, 10). Approximately 16 positions in the variable regions of heavy chains from pooled IgG have been correlated with a locus specificities (4). Work with rabbit antibodies to
bacterial polysaccharides with limited heterogeneity has confirmed this correlation for some, but not all of these positions (12-17).

Structural definition of the antigenic determinants controlled by the a locus is of decisive importance for understanding the genetics of heavy chain variable regions and interpreting breeding studies such as those which suggest that recombination occurs between closely linked genes coding for the variable and constant portions of heavy chains (18, 19). Correlation of structure with specificities can be misleading because the genes for heavy chains are closely linked. Direct proof for the localization of an antigenic determinant could come from isolation of peptides carrying a allotypic specificities. Florent and co-workers (20) succeeded in isolating such peptides from normal heavy chains but because of their heterogeneity, could not determine their sequence or localization. In the present work, we have confirmed and extended their observations. We have found that γ chains from antibodies of restricted heterogeneity elicited by immunization with pneumococcal vaccines facilitate the study of allotype-related peptides by eliminating some of the problems inherent in the use of heterogeneous pools.

**EXPERIMENTAL PROCEDURE**

**Materials**—Ribonuclease and insulin B chain were obtained from Sigma Chemical Co. (St. Louis, Mo.). TPCK-trypsin, carboxypeptidase A, and carboxypeptidase B were purchased from Worthington (Freehold, N. J.). Guanidine hydrochloride and urea were ultrapure grade from Schwarz/Mann (Orangeburg, N. Y.). Aminopropyl glass and p-phenylene disothiocyanate were obtained from Pierce (Rockford, Ill.). Polyamide sheets were obtained from Cheng Ching Trading Co., Ltd., No. 75 Sec. 1, Hankow St. Taipei, Taiwan.

**Rabbit Antiserum**—Rabbits of known allotype were bred and maintained in our own colonies at the National Institutes of Health, Bethesda, Md. They were immunized with type III (285RE-3 al, a3b4b4) and antibody are arranged to produce a symmetrical pattern. This correlation of structure with specificities can be misleading. The Sepharose was activated with CNBr for 1 h; the column was washed with 0.1 M sodium acetate buffer, pH 5.5, and finally against 1% acetic acid, after oxidative sulfitolysis of interchain disulfide bonds as described previously (27). For carboxylation (28), the heavy chains were suspended in water, the pH adjusted to 8.0 with NaOH, and a 50-fold molar excess of citraconic anhydride over the lysine content was added in 50-μl portions with stirring at room temperature. The pH was maintained between 7.5 and 8.0 by addition of 5 N NaOH with a pH-stat. When the base uptake ceased, the clear solution was dialyzed against several changes of 0.1 M NH₄HCO₃, pH 8.0 at 3°. Tryptic digestion was carried out with TPCK-treated trypsin (Worthington) in 0.1 M NH₄HCO₃ at 37° with an enzyme to substrate ratio of 1:100 (w/w). The reaction was stopped after 19 h by diazotization. To remove citraconyl groups (29), the trypic digest was suspended in 0.04 M pyridine/acetic buffer, pH 3.5, and left at room temperature for 24 h with stirring. The suspension was then lyophilized.

**Affinity Chromatography**—For isolation of active peptides from tryptic digests by affinity chromatography, we first coupled purified normal a3 IgG to Sepharose by the cyanogen bromide method as described by Goldman and Mage (30). The antigen/Sepharose column was then used to prepare pure anti-a locus allotype antibodies from pooled antisera. The purified antibodies were in turn coupled to Sepharose 2B by a similar procedure with the following modifications. The Sepharose was activated with CNBr for 1½ h; the ratio of IgG to Sepharose was about 9 mg of IgG/ml of activated Sepharose; after coupling for 2 h at room temperature and overnight in the cold, the antibody/Sepharose was washed with borate buffer and allowed to react with 1 ml of lactoperoxidase for 2 to 3 h at room temperature with stirring, to block any activated sites still available on the Sepharose. The immunoabsorbent was then washed extensively with 0.1 M NH₄HCO₃ and packed in a chromatographic column. Before each experiment, the column was washed with 0.1 M acetic acid and then reequilibrated with 0.1 M NH₄HCO₃, Usually, 40 to 50 mg of tryptic digest of heavy chain was dissolved in 5 ml of 0.1 M NH₄HCO₃ and applied to an antibody/Sepharose column containing 250 mg of bound antibody. The nonbound peptides were washed through with 0.1 M NH₄HCO₃, pH 8, at a flow rate of approximatly 30 ml/h. The bound, active fraction was eluted with 0.1 M acetic acid, pH 2.9 and an increased flow rate of 100 ml/hr. The eluted fractions (immunopeptide T1) were lyophilized and examined for antigenic activity. The antibody/Sepharose columns showed considerable loss of capacity to absorb active peptides if used more than five times.

The immunopeptide (T1) was further digested with TPCK-trypsin (TD2) and the active peptide (T2) purified on the immunoabsorbent column as described above.

**Gel Diffusion**—The mixture of tryptic peptides as well as subsequently isolated fractions were initially screened for antigenic activity by an Ouchterlony-type immunodiffusion test in which control antigen and antibody are arranged to produce a symmetrical pattern. This pattern is distorted by asymmetric introduction of an inhibiting or precipitating antigen. (30) Where possible, as specified under "Results," several antisera produced in different homozous and heterozygous recipients were utilized for activity tests, and controls to test for nonspecific inhibition of inappropriate allotype/anti-allotype precipitation were included.

**Radioimmunoassay**—Radiolabeling of purified anti-a3 antibody was done by the lactoperoxidase method (31) at pH 5.5 (0.05 M sodium acetate buffer). The protein (2 x 10⁻⁶ mol) was mixed with 2 x 10⁻¹ mol of lactoperoxidase, 2 x 10⁻¹ mol of sodium iodide (carrier), and 0.2 μCi of ¹²⁵I in the form of NaI in a total volume of 490 μl. Laddation was started by the addition of 20 μl of 10⁻¹ M H₂O₂. After 1 h at room temperature, the solution was dialyzed in the cold against several changes of 0.05 M sodium acetate buffer, pH 0.5, and finally against 1% NH₄HCO₃. After dialysis, 10% bovine serum albumin in 1% NH₄HCO₃ was added to the solution and the counts were diluted by the addition of unlabeled antibody to a specific activity of 30,000 cpm/μg. About 40% of the added iodide was determined to have bound to the protein, and 99% of the counts in the protein sample were precipitable by 10% trichloroacetic acid. The direct binding curve (see below) among a3 IgG immunoaosorbent and ¹²⁵I-labeled purified anti-a3 antibody showed that only 25% of the antibody bound to the immunosorbsent, probably because of exposure to low pH values during antibody purification and exposure to oxidizing agent and high radioactivity during its labeling. The labeled antibody was repurified by mixing with a3 IgG immunosorbent for 1 h followed by centrifugation and washing two times with 1% NH₄HCO₃. The antibody was then eluted sequentially with glycine/HC1 buffer (ionic strength 0.1), pH 2.8, followed by pH 2.4. Only 0.5% of the bound counts were eluted at pH 2.8, whereas 8.3% eluted at pH 2.4. The latter fraction was used in the radioimmunoassays. Its maximum binding to a3 IgG immunosorbent was found to be 80%.

For direct binding curves, 50 μl of the labeled antibody solution in 10% albumin (pH adjusted to 8) containing about 15 ng of antibody (4,500 cpm) were mixed in microfuge tubes with different volumes of 50- to 100-times diluted a3 IgG-immunosorbent (approximately 1 mg of IgG/ml of gel), and the volume made up to 200 μl with 1% NH₄HCO₃. The reaction was carried out at room temperature with mixing on a rotary wheel for 3 to 4 h. The tubes were then centrifuged at room temperature and 100 μl of supernatant was transferred to a
clean tube. Radioactivity was determined in each supernatant and in the portion remaining in each tube and the percentage of counts calculated. A blank was also run in which no immunoadsorbent was added. Labeled antibody solutions were centrifuged immediately before use in the assay in order to obtain low blank values (±2%). Solutions used without prior centrifugation gave high blank values (>10%).

The concentrations of all the inhibitors used in the inhibition assay were determined by amino acid analysis. For inhibition assays, varying amounts of inhibitor and a fixed amount of labeled antibody (50 μl, 15 ng, 4,500 cpm) were mixed in microfuge tubes in a total volume of 185 μl. The reactants were incubated with mixing overnight at room temperature in order to allow sufficient time for the inhibitors to react with the antibody. Fifteen microliters of the immunoadsorbent (an amount giving approximately 60% of the maximum binding as determined from the direct binding curve) were added and stirring continued for 1 h. The tubes were centrifuged and 100 μl of supernatant was withdrawn and the per cent of counts per min bound was calculated. Each reaction mixture contained 2.5% albumin. For each inhibitor concentration, four tubes were set up; two of them received 15 μl of immunoadsorbent while the other two (blanks) did not receive any immunoadsorbent, and thus served as controls to check if there was any nonspecific adsorption of the inhibitor-containing complex. The various inhibitors tested, IgG, CH, TD1, and TD2 gave less than 5% blank values in the concentration range studied. The remaining two inhibitors, H and TD2, also gave low blank values (<5%) except that H gave 10 to 12% precipitation of labeled antibody in the concentration range 40 to 100 μl, and TD2 was volatile and gave to 11% precipitation. The precipitates were removed by centrifugation and the percentage of counts per min found in the supernatants (P) was subtracted from the respective test sample values. A control was also set up in which no inhibitor was added. The percentage of inhibition was calculated as follows. % Inhibition = (A - P) x 100/A where A and P are the per cent of counts per min bound in absence and in presence of inhibitor, respectively.

DEAE-cellulose Chromatography—The immunopeptide T2 was reduced and 14C-citrooxyethylated and chromatographed on a DEAE-cellulose column (1 × 30 cm) in the presence of 0.2 M NaHCO3. The starting buffer was 0.01 M Tris/HCl, pH 8.35. A gradient from 0.01 to 0.05 M Tris/HCl buffer was generated with the help of an Ultragard (LKB) using a 4-h setting at a flow rate of 25 ml/h. The fractions were analyzed by measuring radioactivity.

Determination of Molecular Weight—The molecular weight was calculated as follows:

\[
\text{Molecular Weight} = V_r / V_t \times 10^4
\]

where \(V_r\) is the elution volume and \(V_t\) is the total volume of the column.

Tryptophan Content—The tryptophan content of the immunopeptide T2 was determined by hydrazine with 4,5-dimethoxyaniline (35) and chromatographed on a Durrum D-500 analyzer. The tryptophan content of the peptide was calculated as follows:

\[
\text{Tryptophan Content} = \frac{\text{counts per min found in the supernatants} (P)}{\text{counts per min found in the supernatants of the control} (P_c)} \times 100
\]

RESULTS

Antipneumococcal antisera from rabbits homologous at the α locus were selected because they appeared partially or highly restricted in heterogeneity by microelutte acetate electrophoresis (22). IgG fractions were prepared from these sera and examined by isoelectric focusing (Fig. 1). Heavy chains were prepared from fractions containing major components of limited heterogeneity, citraconylated, and digested with trypsin. Separation of Active Peptides by Sephadex G-75 Gel Filtration—Initial attempts to separate tryptic peptides from α3 heavy chain of rabbit G222-2 (Sample 1, α3 in Fig. 1) were made utilizing Sephadex G-75 in 0.1 M NaHCO3. Figure 2 shows a typical separation. The mixture of tryptic peptides and each of the major ultraviolet absorbing fractions eluted from the Sephadex column were assayed for antigenic activity. Results similar to those shown in Fig. 3 were obtained using six different active α3 antisera made in a2b4, a2b5, a1b4 and a1a2b5 recipients. The controls shown in the figure were a2b4 anti-a2 and a3b4b5 anti-a1 antisera reacting with a2 and a1 control antigens (IgG), respectively. It can be seen in Fig. 3 that specific inhibitory activity was clearly present in the whole tryptic digest and in Sephadex G-75, peak IV. Some activity was also present in peaks II and III. The inhibitory activity was specific for the α3 allotype since no inhibition was observed of precipitation of α1 with anti-a1 or a2 with anti-a2 (Fig. 3).
peptides were bound and eluted from specific immunoabsorbsents. In the present paper, further studies done on the peptides from G-222-2 heavy chain (a3) are reported. Peak IV (Fig. 3) which displayed strongest inhibitory activity was rechromatographed on the same Sephadex column and its purity checked by isoelectric focusing. Although a single major band was observed, a scan of cysteine-containing pep- tides after tryptic and chymotryptic digestions gave clear evidence for contamination by peptides from the constant region of the heavy chain. Moreover, automated sequence analysis of the peptide also revealed a sequence starting with alanine 124 in the heavy chain constant region.

Immunopeptide T1—Further purification of the active peptide was achieved with an immunoabsorbent column prepared by coupling anti-a3 antibody to Sepharose 2B. The active fraction (peak IV) from the Sephadex G-75 column, or the whole tryptic digest of heavy chain was applied on the immunoabsorbent column. Unbound peptides were washed through with 0.1 M NH₄HCO₃, pH 8, and the bound active fraction was eluted with 0.1 M acetic acid, pH 2.9. The efficiency of the immunoabsorbent decreased with reuse. The yields of active peptides (immunopeptide T1) from immuno- absorbent columns decreased from 0.8 to 0.1 mol/mol of heavy chain on repeated chromatographies of whole tryptic digests of heavy chains on the same immunoabsorbent. The yield was somewhat lower when the intermediate step of G-75 purification was employed.

Immunopeptide T2—Exposure of the T1 peptide to low pH values during its purification through the immunoabsorbent column also hydrolyzed off citraconyl blocking groups and generated free amino groups of lysine. Thus it was now subjected to a second tryptic digestion to cleave newly exposed lysine peptide bonds. The resulting digest (TD2) was passed through the anti-a3-immunoabsorbent column to purify the bound immunopeptide T2. This immunopeptide also had inhibitory activity detected by the immunodiffusion method. Its activity was also assayed by a radioimmunoassay in which inhibition by the immunopeptide of the binding of ¹²⁵I-labeled anti-a3 antibody to Sepharose-bound a3 IgG was measured quantitatively. The inhibition curves for the different inhibitors tested are shown in Fig. 5, which also includes points for heterologous inhibition by a2 IgG, H chain, and H chain tryptic digest. In each case, the heterologous inhibition was less than 10%. Duplicate or triplicate measurements of counts per min bound generally agreed within 2%. The straight lines in Fig. 5 were drawn by the method of least squares.

The molecular weight of T2 was found to be approximately 6,000 (Fig. 4). The amino acid composition of T2 is given in Table 1 (first column). After full reduction and alkylation, a single peak of approximately 3,000 molecular weight was obtained (Fig. 4). NH₂-terminal analysis of T2 by the dansylation method as well as by automated Edman degradation gave two amino acids, glycine and threonine, in equal amounts, and a third amino acid, serine, in traces. COOH-terminal analysis of T2 by carboxypeptidase B treatment for 4 h gave two amino acids, arginine and lysine, in about 90% yield. These data suggest that the immunopeptide T2 is composed of two chains of about 3,000 daltons each, held together by a disulfide bond.
Convincing evidence as to the localization of the immunopeptide in the heavy chain sequence was obtained by radioautographic analysis of labeled half-cystine-containing peptides. After electrophoresis at pH 3.5 and radioautography, \(^{14}C\)-containing peptides were identified on the basis of their mobilities (35). In the radioautograph shown in Fig. 6, no half-cystine-peptides with mobilities characteristic of those from the constant regions were found. The only half-cystine-peptides found had mobilities compatible with peptides containing cysteines 22 and 92 of the variable region of heavy chain. The two bands in the cysteine 22 region indicate some sequence heterogeneity as has been observed previously with normal immunoglobulins (34). Further evidence that cysteine 92 is indeed present in the T2 fragment was obtained from the kinetic analysis of carboxypeptidase A plus B digestion of reduced and carboxymethylated T2 and a peptide isolated from the reduced and carboxymethylated T2 by DEAE-cellulose chromatography (see below) that had lysine at the COOH terminus. When the latter peptide was digested with carboxypeptidase A plus B, the following amino acids were liberated in decreasing order of yield: lysine, valine, serine, threonine, tyrosine, and phenylalanine. (Tryptophan was also liberated but its amount could not be quantitatively estimated.) Digestion of the total reduced and alkylated T2 (mixture of both peptides) liberated, in addition to the above amino acids, arginine, alanine, and carboxyamidomethyl cysteine. When the values for the amino acids liberated from the lysine peptide were subtracted from the values of the amino acids liberated from equimolar amount of T2, the following COOH-terminal sequence for the arginine peptide became evident: Tyr-Phe-Cys-Ala-Arg. These amino acids correspond to the sequence of the rabbit heavy chain from position 90-94.

Separation of the two polypeptide chains from fully reduced and carboxymethylated immunopeptide T2 was attempted on a DEAE-cellulose column. The resulting elution profile is shown in Fig. 7. The peaks were pooled separately and analyzed for amino acid composition and COOH- and NH\(_2\)-terminal residues. COOH-terminal analysis with carboxypeptidase B gave 1 mol of lysine/mol of each peptide peak. Dansylation showed dansyl glycine as a major spot for each
peak. Thus it appeared that several variants of the peptide containing COOH-terminal lysine had been separated. The arginine-containing peptide was not recovered in this experiment. The total yield based on ¹⁴C counts in peaks 1 to 4 was only 52%. Table I gives the amino acid compositions of peaks 1 through 4 from the DEAE-cellulose column. The composition of a putative arginine-containing peptide is also given in Table I, as calculated by difference. Both the arginine peptide and the lysine peptide have 0.5 mol of methionine/mol of chain. The reported sequence of the variable region of an α3 heavy chain shows variable methionine residues at positions 34 and 79. The amino acid composition of the CNBr fragment containing residues 1 through 34 of the G222-2 H chain isolated according to Friedenson et al. (40) is also given in Table I. It is noteworthy that this fragment has a histidine residue, as does the lysine peptide. The latter peptide, as is seen below, starts at position 17 and does not have any histidine up to position 22. This would mean that the histidine residue is present between 23 and 34.

The major peak (pk2) from the DEAE-cellulose column was coupled to derivatized glass beads. Edman degradation carried out on the Sequamag gave evidence for Asx-Glx-Ser-Thr-Cys, in positions 2 through 6. Since the peptide was reduced and ¹⁴C-carboxymethylated, cysteine could be identified in the liberated thiazolinones simply by determining radioactivity in each step. Fig. 8 shows the counts for each step. The sequence obtained clearly establishes the position of cysteine in our purified lysine peptide.
### TABLE I

**Amino acid composition of a3 peptides**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>T2</th>
<th>Peptides</th>
<th>CNBr peptide residues 1 to 34&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Average of peaks 1 to 4&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Arginine peptide&lt;sup&gt;c&lt;/sup&gt;</th>
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</thead>
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<tr>
<td>Aspartic acid</td>
<td>4.3</td>
<td>1&lt;sup&gt;*&lt;/sup&gt; 2&lt;sup&gt;o&lt;/sup&gt; 3&lt;sup&gt;o&lt;/sup&gt; 4&lt;sup&gt;o&lt;/sup&gt;</td>
<td>2.3</td>
<td>1.9&lt;sup&gt;*&lt;/sup&gt;</td>
<td>2.4</td>
</tr>
<tr>
<td>Threonine</td>
<td>6.0</td>
<td>2&lt;sup&gt;o&lt;/sup&gt; 2.2 2.2 1.5 1.1 3.0</td>
<td>2.6</td>
<td>1.9</td>
<td>5.6</td>
</tr>
<tr>
<td>Serine</td>
<td>7.5</td>
<td>2&lt;sup&gt;o&lt;/sup&gt; 2.0 1.6 1.6</td>
<td>2.0</td>
<td>2.1 &lt;sup&gt;b&lt;/sup&gt; 2.4</td>
<td></td>
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<tr>
<td>Glutamic acid</td>
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<td>1&lt;sup&gt;o&lt;/sup&gt; 1.8 2.0 2.3</td>
<td>2.5</td>
<td>2.1</td>
<td>2.4</td>
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<td>1.3</td>
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<tr>
<td>Glycine</td>
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<tr>
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<td>Methionine</td>
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<td>0.9</td>
<td>0.3&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
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<td>0.7</td>
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<tr>
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<td>1&lt;sup&gt;o&lt;/sup&gt; 1.3 1.3 1.2</td>
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<tr>
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<td>1.0</td>
<td>0.3&lt;sup&gt;g&lt;/sup&gt;</td>
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<tr>
<td>Tryptophan</td>
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<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

* Peaks from the DEAE-cellulose column (Fig. 7).
* Isolated from C, fragment of G222-2 H chain after reduction and alkyiation followed by Sephadex G-50 chromatography (40).
* The average is corrected for the percent contribution of each peak based on their yields (Fig. 7).
* Calculated from the difference between the compositions of T2 and average of peaks 1 to 4.
* Obtained as homoserine.
* Determined as carboxymethyl cysteine.
* N.D. Not determined.

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**DISCUSSION**

Numerous attempts have been made to explain the genetic and structural basis for a-locus allotypes in the variable region of rabbit heavy chains. In addition to prototype sequences of a1 and a3 H chains (4), partial or complete sequences of the V<sub>H</sub> regions of several rabbit antibodies are available (5, 12-17). Comparisons of these sequences have suggested that three major areas in variable regions of the H chains have amino acid substitutions that seem to correlate with allotypes of the a group. The first region comprises residues 1 to 31, the second region comprises residues 63 to 73 and the third, residues 80 to 85 (4, 5, 41). Our results clearly establish that an immunopeptide (T2) of approximately 6,000 molecular weight can be isolated and purified from a heavy chain carrying a3 allotypic determinants. This immunopeptide is composed of two chains held together by a disulfide bond. The cysteine-peptide map and partial sequences we obtained suggest that peptide T2 contains cysteine 22 and cysteine 92 of the variable region. One peptide starts at position 17 and contains COOH-terminal lysine, and a total of 22 or 23 residues. The second peptide is about 30 amino acids in length and ends with arginine 94. The fact that our lysine peptide starts at position 17 does not rule out a role for residues 1 to 16 in some allotypic determinants. Kindt et al. (42) observed differences in the ability of homogeneous antibodies to absorb antisera against the group a allotypes. These results suggested that each group a allotype is expressed as an array of subspecificities of which only a limited number are expressed on individual antibody molecules. In our studies we have used a heavy chain preparation obtained from IgG of limited heterogeneity. Thus all of the a3 determinants might not have been present in the IgG or in immunopeptide T2. All inhibitors tested could inhibit more than 50% of the binding of the anti-a3 antibody to a3 IgG. G222-2 a3 IgG, although partially restricted in heterogeneity, was an excellent inhibitor (98%) and appears to express allotypic determinants recognized by essentially all of the anti-a3 antibodies in our purified fraction. However, it is not certain that 100% inhibition could be reached with the other inhibitors. Some of the
Experiment I, Edman degradation of the lysine peptide (Fig. 7, peak 2) obtained at the individual steps of automated Edman degradations. Using the solid phase Sequencer sequamat 12K; Experiment 2, Edman degradation of the reduced and ^[14]C-carboxymethylated T2 using Beckman Sequencer 890 B.

anti-a3 antibodies in the purified anti-a fraction studied may have been directed toward determinants which were destroyed or removed at the various steps. However, since more than 50% inhibition (61%) was achieved with peptide T2, this portion of the V H region appears to contain a major antigenic determinant or determinants associated with the a3 allotype.

We have shown that the cyanoem bromide Fragment 1-34 of this a3 H chain has a histidine residue. The lysine peptide from T2 also has a histidine, but not in the region 17-22. This indicates that the histidine is present in the region 23-34, most probably in the first hypervariable region. The presence of histidine in the first hypervariable region of rabbit H chain has never been reported previously. However, histidine has been found in the third hypervariable region of two other rabbit H chains from antipneumococcal antibodies (16, 17).

The framework sequence 17-20 of T2 is also very different from the ones reported so far. In addition, most heavy chains so far sequenced have arginine in position 38. However, a human variable region (V H III) has recently been reported (43) to have proline at this position. It is possible that our peptide ends with arginine peptide which are included in immunopeptide T2.

TheInv determinants of human K chains also involve two regions of the polypeptide chain. Substitutions are found at positions 153 and 191 (50). A three-dimensional model of the light chain shows that these residues are adjacent.

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a3 Immunopeptide

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A A Ansari, M Carta-Sorcini, R G Mage and E Appella


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