Localization of a Single Binding Site for Immunoglobulin Light Chains on Human Tamm-Horsfall Glycoprotein

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Abstract

Cast nephropathy is a severe complication of multiple myeloma. Binding of filtered monoclonal light chains (LC) with Tamm-Horsfall glycoprotein (THP) triggers heterotypic aggregation of these two proteins to form casts in the distal nephron of the kidney. To localize the LC binding site on THP, human THP was deglycosylated and underwent limited trypsin digestion in the presence or absence of a nephrotoxic LC known to bind THP. A 29.6-kDa band was protected from trypsin digestion by the addition of LC. NH2-terminal amino acid sequence and amino acid analyses revealed this band was located between the 6th and 287th amino acid residues of THP. Six peptides located within this 29.6-kDa fragment were synthesized and used as potential inhibitors of binding or aggregation of five different nephrotoxic LCs with THP. Peptide AHWSGHCC1L (from amino acid 225 to 233) completely inhibited binding and aggregation of these proteins. Optimal inhibition required a cystine residue in this peptide. Truncation experiments demonstrated the entire sequence was necessary for ideal inhibition and the histidine residue explained the effects of pH on binding. These studies provided a basis for further study of LC–THP interaction and a potential approach toward the prevention of cast nephropathy. (J. Clin. Invest. 1997. 99: 732–736.) Key words: Bence Jones protein • cast nephropathy • acute renal failure • multiple myeloma • myeloma kidney

Introduction

Tamm and Horsfall described in 1950 a unique urinary protein that inhibited viral hemagglutination in vitro (1). This Tamm-Horsfall glycoprotein (THP),1 which has since been characterized as a 616–amino acid glycoprotein (2), is synthesized specifically by cells of the thick ascending limb of the loop of Henle (3, 4). About 30% of the molecular weight of THP is carbohydrate (5). Although the physiologic significance of THP remains undefined, this protein plays an integral role in the pathologic condition known as cast nephropathy. Cast nephropathy, or “myeloma kidney,” is a common complication of multiple myeloma. Binding and subsequent coaggregation of filtered immunoglobulin light chains (LCs) with THP obstruct flow through the renal tubule and result in renal failure (6, 7). Both κ and λ LCs share a common binding site on THP, although different nephrotoxic LCs possess different affinities for THP (6). Identifying and characterizing the protein–protein interaction between LCs and THP allow development of new therapeutic strategies to decrease incidence of this complication. The current experiments were designed to determine the light chain binding site on human THP.

Methods

Protein and peptide preparations. THP was purified from urine of a normal male in standard fashion (8) using original method of Tamm and Horsfall (1). THP was precipitated by using 0.64 M NaCl, dialyzed against water adequately and lyophilized. Five LCs, κ (κ isotype), and don, bol, wil, and hor (all λ isotypes), were used in this study. These LCs were purified from urine of patients who had multiple myeloma and renal failure by precipitation using 70% ammonium sulfate, followed by ion exchange chromatography (9). Purified LCs were dialyzed against water adequately and lyophilized. The isoelectric point was 7.7 for mic, 5.7 for don, and 5.1 for bol. Peptides used in these studies were synthesized by the protein synthesis core facility of the Comprehensive Cancer Center of the University of Alabama at Birmingham and by Research Genetics Inc. (Huntsville, AL). Peptides were kept lyophilized at −20°C until use. To determine the role of cystine in the binding interaction, some peptides containing cysteine residues were diluted to 10 mg/ml in 0.1% trifluoroacetic acid (Sigma Chemical Co., St Louis, MO), and then further purified using reverse-phase high performance liquid chromatography with a C18 preparative column (Novapack; Waters Processing Supplies, Inc., Marlborough, MA). The flow rate of mobile phase was 1 ml/min. An acetonitrile gradient from 0 to 100%, which was controlled using a two-pump delivery system (501 pump solvent delivery system; Waters, Marlborough, MA). Ellman’s reagent (Caymen Chemical Co., Inc., Ann Arbor, MI) was used to detect free sulfhydryl groups of these peptides.

One peptide, the light chain binding peptide (LCBP), was used as a template to design a complementary peptide according to Weigent et al. (10). The complementary peptide (PBCL) was constructed based upon the RNA sequence that was complementary to the RNA strand encoding the amino acid sequence of LCBP. The hydrophyty profile of each amino acid of the complementary and template peptides was determined using computer software (MacVector; Eastman Kodak Co., New Haven, CT).

Trypsin protection experiment. Purified THP, 10 mg/ml, was suspended in 1% SDS and boiled for 5 min. Denatured THP was then mixed in sodium phosphate buffer, pH 8.6, containing 160 mM so-
Table I. Effect of Synthetic Peptides in Preventing Binding between LCs and THP. The IC₅₀ Was Calculated using Data from the Binding Analysis Studies

<table>
<thead>
<tr>
<th>Position on THP</th>
<th>Peptide sequence</th>
<th>mic</th>
<th>don</th>
<th>holl</th>
</tr>
</thead>
<tbody>
<tr>
<td>72–81</td>
<td>EGFRILSPGLG</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>118–120</td>
<td>RGD</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
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<tr>
<td>127–130</td>
<td>SPGS</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>157–169</td>
<td>DEYWRSTEGEGY</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>176–186</td>
<td>RGWYRFVGGQGG</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>225–233</td>
<td>AHWSGHHCCL</td>
<td>1.5±0.7</td>
<td>0.6±0.1</td>
<td>0.5±0.1</td>
</tr>
</tbody>
</table>

Modifications of LCBP (peptide 225–223)

- AHWSGHHCCL (D-amino acids)
  - 0.7±0.3
  - 0.6±0.2
  - 0.4±0.2

- AHWSGSCCL
  - 0.6±0.04
  - 0.4±0.05
  - 0.5±0.05

- AHWSGHSSL
  - 8.2±2.6
  - 9.8±1.2
  - 7.4±0.7

- AHWSGHCC*L
  - NE
  - NE
  - NE

- GHCCL
  - 6.5±1.2
  - 5.1±0.7
  - 4.0±1.5

- GSCCL
  - 4.2±0.2
  - 3.5±0.5
  - 4.0±0.3

- AHWSG
  - NE
  - NE
  - NE

- AHWSGHG
  - NE
  - NE
  - NE

Complementary peptides

- QAAVAPVR
  - 0.5±0.04
  - 0.2±0.03
  - 0.2±0.03

- QAAVA
  - 2.6±1.4
  - 2.3±0.3
  - 0.6±0.3

C*, cysteines that have covalent modifications on the sulphydryl group; NE, no effect; *extrapolated values.
Huang and Sanders calculate the IC₅₀ of the inhibitory peptides. Differences between data sets were determined using unpaired t test, with significance set at 5%.

Results

One band located at 29.6 kD was found to be protected by mic from digestion by trypsin (Fig. 1). NH₂-terminal amino acid sequence analysis and amino acid analysis showed this band was located between the 6th and 287th amino acid residues of THP. This region of THP was examined using Kyte-Doolittle hydrophobicity data to determine those segments that had a high probability to be on the surface of the molecule, allowing potential interaction with light chains. Six peptides predicted to be on the surface of the region between the 6th and 287th amino acid residues were synthesized (Table I). Only one of these six synthesized peptides, which was identical to the amino acid sequence from 225 to 233 of human THP and designated LCBP, blocked the binding of LCs with THP in a concentration-dependent manner. The ability to inhibit binding differed among the three LCs (Table I) and was consistent with the affinities of these LCs for THP (6). Mic possessed the highest affinity for THP (6), while bol had the lowest affinity. This LCBP also inhibited binding of wil and hor to THP in a concentration-dependent fashion. In turbidity studies, this same peptide (LCBP) inhibited heterotypic aggregation of LCs with THP (Fig. 2).

Seven peptide fragments were synthesized, representing truncation or replacement of amino acid residues of the LCBP (Table I). Peptides AHWSG and AHWSGHG did not inhibit binding between LCs and THP. Replacement of either or both cysteines with serine decreased or eliminated this inhibitory effect. Furthermore, peptide AHWSGHC*CSS, which was synthesized using acetylimidomethyl-cysteines (represented by C*), had no effect on the binding of LCs to THP. Removal of AHWS residues from the NH₂-terminal of the LCBP reduced the inhibitory capability of the peptide; inhibition remained incomplete even at high doses of peptide. Inhibitory activity was partially restored by changing H to S to produce GSCCL. AHWSGSCCL produced greater (P < 0.025) inhibition of the interaction between mic and THP than AHWSGHCCCL (Table I). Holo-LCBP synthesized with D amino acids (D-LCBP) inhibited binding (Table I) and heterotypic aggregation of LCs with THP in turbidity studies (Fig. 2).

GHCCCL, holo-LCBP, and D-LCBP were further subjected to HPLC analysis. Similar patterns with multiple peaks were found with all three peptides. The major peaks represented single molecules by mass spectrometry. Only these peaks, which did not possess free sulfhydryl groups demonstrated using Ellman’s reagent, inhibited the binding interaction of LCs with THP.

Complementary peptide PBCL (QAAVAAPVR) also inhibited binding of LCs to THP in a dose-dependent manner (Fig. 3). This peptide also inhibited binding of wil and hor to THP.

Figure 2. Aggregation rates of LCs, 2.7 mM, to THP, 5 μM, in the presence of LCBP, LCBP synthesized with D amino acids (D-LCBP), and PBCL. LCBP and D-LCBP, 100 μM, and PBCL, 15 μM, were added simultaneously with THP into PBS containing LCs. All three peptides completely inhibited heterotypic aggregation of LCs to THP.

Figure 3. Inhibition of the binding of THP to wells containing five different LCs. PBCL effectively prevented binding in a dose-dependent manner.
THP, with IC$_{50}$ of 0.4±0.03 and 0.6±0.06 mM, respectively. Hydropathy plots showed PBCL possessed a pattern opposite that of LCBP (Fig. 4). To test further the interaction between the complementary peptide and THP, a solid-phase binding assay was performed. As expected, THP bound to peptide PBCL in a dose-dependent manner (Fig. 5). PBCL also completely inhibited aggregation of LCs to THP (Fig. 2). Finally, truncation of PBCL to produce QAAVA resulted in a peptide that was complementary to GHCCL. This peptide also inhibited binding of LCs to THP, although the IC$_{50}$ was higher and did not completely prevent binding of mic to THP.

**Discussion**

THP and LCs are the two major components of casts that obstruct the nephron and produce cast nephropathy or “myeloma kidney.” Our previous studies showed that LCs of both isotypes bound to a common site on the peptide backbone of THP, although with different affinities. This noncovalent binding interaction triggered subsequent heterotypic aggregation of LCs with THP (6). These biochemical interactions between LCs and THP form the basic mechanism of cast formation in vivo (6, 7). In the current study, limited trypsin digestion of THP with the simultaneous addition of a light chain known to bind THP localized the binding site of LC on THP to a 29.6-kD peptide fragment between amino acids 6 and 287. One synthesized peptide corresponding to amino acids 225–233 (AHWS-GHCCL), designated LCBP, inhibited binding and aggregation of THP with five different light chains. The cystine residue was important for optimum inhibition. The NH$_2$-terminal portion of this peptide, AHWSG, did not inhibit the binding between LCs and THP. However, removal of AHWS from the LCBP caused an obvious decline in inhibiting capability (Table I). Thus, the entire 9–amino acid residues appeared to form the light chain binding domain. Finally, a complementary peptide, PBCL, which had a hydrophobic profile opposite that of LCBP, bound THP and completely inhibited THP–LC interactions. These combined studies demonstrated the single binding site on THP for LC.

The current studies also complemented our previous experiments that examined the binding interaction between LCs and THP. Pretreatment of THP with a variety of reducing agents inhibited LC–THP interactions (6). This finding was consistent with the observation that the intramolecular disulfide bond between cysteine 231 and 232 provided an ideal conformation to produce optimal inhibition. Without the cystine residue, the binding interaction was much less effective or disappeared. The inhibitory effect of binding between LCs and THP by synthetic LCBP containing a cystine residue indicates the effect of reducing agents was due to cleaving the disulfide bond between cysteine 231 and 232.

We also determined that varying pH between 5 and 8.5, which alters the charge of the imidazole ring on histidine, modified LC–THP interactions (11). In the current study, replacement of the negatively charged histidine with serine enhanced the capability of the peptide to inhibit binding of mic, but not don and bol, to THP at neutral pH (Table I). Thus, the histidine residue may explain the effect of pH in altering the binding interaction between THP and some of these proteins (11). Ambient pH may also affect the charges of side chains of certain amino acid residues on LCs, causing changes in binding affinity.

To characterize further the LC–THP interaction, these studies also employed a recently described method that designs novel peptides based on the genetic code of the binding site for ligands on peptide hormone receptors (10). This molecular recognition theory proposed by Weigent et al. (10) suggests that a peptide synthesized according to the DNA sequence complementary to the DNA sequence encoding another peptide will interact with that peptide. In our experiments, PBCL was designed according to the complementary sequence of DNA encoding LCBP (Fig. 3) and was shown to bind THP (Fig. 4). This peptide completely inhibited LC–THP interactions and provided further evidence that the LCBP defined the
LC binding site of THP. Because the hydropathy plot of PBCL was opposite that of the LC binding peptide (Fig. 3), we have proposed that cast-forming LCs contain peptide sequences that possessed this same inverted hydropathic profile to allow interaction with the LC binding site. Thus, a defined linear hydropathic profile and not a specific amino acid sequence per se may be the most important determinant in the interaction with THP. The results using PBCL are extremely useful for further study of the THP binding site on LCs, and may facilitate the definition of those sequences on LCs that interact with THP.

In summary, the light chain binding site on THP was shown to be localized to a linear sequence of nine amino acids: AH-WSGHCCCL. Immunohistochemistry studies of kidney biopsy specimens from patients with cast nephropathy have shown that THP is an integral part of these casts (12). Modification of THP by colchicine inhibited aggregation with LCs in human subjects (11), and prevented cast formation in a rat model of cast nephropathy (7), thus further showing an important role for THP in this process. Identification of the light chain binding site on THP will help to produce strategies that inhibit interaction of LCs with THP and potentially prevent a severe renal complication of multiple myeloma.

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References