The Amino Acid Composition of Normal Human Urinary Mucoprotein

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The fibrous urinary mucoprotein of Tamm and Horsfall, which is a temporary inhibitor of the hemagglutination reaction of the myxoviruses, exists in normal human urine in two molecular forms, one a tetramer of the other (1). The smaller form, of molecular weight \(7 \times 10^4\), length \(6 \times 10^4\) A, and width \(40\) A, can be dissociated further by transverse cleaving into half molecules (2) and into quarter molecules (3), each of which is stable and obtainable as a purified component in solution. Urinary mucoprotein may be dissociated systematically into much smaller fragments, such as eighths and sixteenths. It is therefore polymeric, and the question of the nature and size of the monomeric unit is raised. Physicochemical studies indicate that the monomeric unit is sufficiently small to make an amino acid analysis meaningful and of value in determining a minimal molecular weight.

Earlier studies (4) have demonstrated a difference in behavior between urinary mucoprotein obtained from patients with cystic mucoproteins, in the search for which the amino acid analysis of the No. 30 rotor of the Spinco model L ultracentrifuge immediately before hydrolysis. Mucoprotein concentrations were determined in a Zeiss interferometer, which was calibrated against the dry weight of mucoprotein (105 A for 48 hours).

Lyophilized samples (6 mg) of protein were hydrolyzed with 15 ml of redistilled constant boiling HCl in Pyrex tubes sealed in a vacuum and placed in boiling toluene for 12, 24, 48, and 72 hours. The HCl was removed by lyophilization. The residue was dissolved in pH 2.2 citrate buffer and analyzed according to the procedures of Spackman, Stein, and Moore (5) and Kassel and Laskowski (6) with a Phoenix K-5000 A amino acid analyzer. Cysteine was determined by a modification of the performic acid oxidation method of Schram, Moore, and Bigwood (7). Tryptophan and tyrosine were determined by the spectrophotometric method of Benee and Schmid (8). Nitrogen was determined by the micro-Kjeldahl method. Glucosamine and galactosamine also were determined with the automatic amino acid analyzer. These two substances emerge before the lysine peak after the tyrosine-phenylalanine peaks on the 50 cm column (pH 5.28, 0.35 n buffer, 50°C) and separate cleanly.

RESULTS

Table I shows the amino acid composition of urinary mucoprotein. Except for hydroxyproline, all of the common naturally occurring amino acids are present. The absence of hydroxyproline, as well as the presence of tryptophan, renders remote the relationship of collagen to this mucoprotein.

The excess of acidic amino acids correlates with the acidic nature of the mucoprotein. The acidic isoelectric point of T & HE is usually related to its sialic acid content. The greater proportion of aspartic acid to glutamic acid is an unusual property of urinary mucoprotein shared with influenza virus (type B, Lee strain), ovomucoid, tobacco mosaic virus, and a few other substances that have been analyzed. We did not find in the literature analyses of any other protein whose amino acid composition corresponds to that of T & HE.

A minimal molecular weight of 28,100 ± 400 was calculated from the half-cystine, histidine, isoleucine, lysine, methionine, and phenylalanine values. The number of residues of each amino acid was calculated from this molecular weight. From each residue value rounded out to the nearest integer a complete series of molecular weights was calculated to confirm the analysis (Table I). A minimal mucoprotein molecular weight of 28,100 is consistent with the measured amounts of all of the amino acids. This unhydrated minimal molecular weight should be compared with the weight of the smallest fragment of T & HE detectable by physicochemical means, namely, \(27 \times 10^4\), and is further evidence that urinary mucoprotein is comprised of a multitude of identical units.

The summation of residues indicates that T & HE is comprised of 70.4% protein and 6.5% hexosamine. We could account for all of the nitrogen in the “Total N” column of Table I. Because the sialic acid is decomposed, some of its nitrogen may appear as amide nitrogen.

It is interesting to compare the minimal molecular weight from amino acid analysis with the literature carbohydrate analyses (Table II). Only those data are presented that were obtained on samples of urinary mucoprotein that are probably identical with T & HE. Table II also includes elemental analyses, selected values of percentage composition, and the number of...
In a study of this type involving the comparison of our own data with data from the literature, we must be certain that the urinary mucoprotein examined in each case was identical with the others. We feel that our starting material is identical with that of Tamm et al. (9, 14, 15), Klenk and Lauenstein (11), Curtail (16), Pye (17), Gottschalk (13), and Odin (10).

The relationship of T & HE to the uromucoid of Boyce and Swanson (12) is uncertain at this time. The yield of T & HE from normal human urine obtained by us averages 17 mg per liter of urine and corresponds to the yield reported by Tamm and Horsfall (9) and by Ada and Gottschalk (18). The yield reported by Boyce and Swanson (12) was 44 mg per day. The method of preparation of the two mucoproteins is different. Boyce and Swanson have also reported (12) a yield of uromucoid of 12 and 18 mg per 24 hours from ureterostomy urine. The carbohydrate and amino acid composition of T & HE is not identical with that of uromucoid. The amino acid analysis of T & HE presented here should be compared with that obtained by King, Fielden, and Boyce (19) on uromucoid.

It is of interest to tally the results to account for as much of the molecule as possible. Addition of all of the known residues (on the assumption of fully acetylated amino sugars) and adding the one sulfate group and the 8 unaccountable sulfur atoms yields a minimal molecular weight of 27,322, which is in good agreement with our value of 28,100. Still to be accounted for are the three small chromatography peaks.

Our picture of the urinary mucoprotein molecule is like a string of beads, much as proposed by Porter and Tamm (15). The diameter of a spherical molecule with a molecular weight of 28,100 and partial specific volume of 0.70 is 40 Å. This is consistent with the diameter of the $i \times 10^8$ molecular weight form of T & HE (1), although it should be pointed out that no evidence exists that the monomeric form is spherical.

The results of our amino acid analysis do not correlate well with the titration data of Curtail (16), which show a total of 18 titratable groups per minimal molecular weight of 18,100 in urea-degraded urinary mucoprotein. This corresponds to our analytically determined minimum of 41 that would be expected to appear in the titration curve. The discrepancy is equally apparent between the analytical data and the titration curve for normal urinary mucoprotein (16). This discrepancy may be attributable to the intramolecular blocking of groups. More likely the cause lies in the physical state of the mucoprotein during the titration. Access of the hydroxyl ions to the titratable groups may be hindered unless the mucoprotein is in true solution.

**SUMMARY**

Normal human urinary mucoprotein, designated T & HE, was quantitatively analyzed for amino acids. From these results the minimal molecular weight of the mucoprotein is 28,100. The mucoprotein contains 6.45% hexosamine and 70.36% amino acids. More aspartic acid than glutamic acid is present. The known amino acid and carbohydrate residues account for almost all of the molecular weight and all of the nitrogen. Still unaccountable are three small chromatography peaks and 8 atoms of sulfur.

*This minimal figure includes the 15 glutamic acid, 21 aspartic acid, 5 histidine, 5 lysine, 5 tyrosine, the minimum of 1 sulfhydral residue, less the maximum of 11 amide groups. The arginine and half-cystine residues are not included.
### Table II

**Analytical data on T & HE from the literature**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Amount reported in Grams per 100 ml</th>
<th>Grams of protein</th>
<th>Value to be used in calculations</th>
<th>No. of residues per 28,100 molecular weight</th>
<th>Minimal molecular weight calculated from integral number of residue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>12.24 (9)</td>
<td>11.83 (9)</td>
<td>12.16</td>
<td>X 10^3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.9 (10)</td>
<td>10.23 (11)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.33 (11)</td>
<td>12.16 (our value)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>S</strong></td>
<td>3.00 (9)</td>
<td>1.82 (9)</td>
<td>1.80</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.80 (11)</td>
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<td></td>
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<tr>
<td>Sulfate S</td>
<td>0.4 (10)</td>
<td>0.37 (12)</td>
<td>0.38</td>
<td>1</td>
<td>25.2</td>
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<tr>
<td>F</td>
<td>0 (9)</td>
<td>0 (9)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.09 (10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>N-Acetylglucosamine</strong></td>
<td>6.1 (9)</td>
<td>7.5 (our value)</td>
<td>7.5</td>
<td>10</td>
<td>29.3</td>
</tr>
<tr>
<td><strong>N-Acetylgalactosamine</strong></td>
<td>1.5 (hexosamine from (13) minus glucosamine from (9))</td>
<td>1.3 (our value)</td>
<td>1.3</td>
<td>2</td>
<td>33.9</td>
</tr>
<tr>
<td>Galactose</td>
<td>5.4 (13)</td>
<td></td>
<td>5.4</td>
<td>9</td>
<td>26.5</td>
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<tr>
<td>Mannose</td>
<td>2.7 (13)</td>
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<td>2.7</td>
<td>5</td>
<td>27.0</td>
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<tr>
<td>Fucose</td>
<td>1.0 (13)</td>
<td>1.1 (13)</td>
<td>1.1</td>
<td>2</td>
<td>29.2</td>
</tr>
<tr>
<td></td>
<td>1.1 (11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sialic acid</td>
<td>9.2 (10)</td>
<td>9.1 (10)</td>
<td>1.3-9.2</td>
<td>1-9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.7 (11)</td>
<td>5.0 (11)</td>
<td>1.3 (our value)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parentheses refer to sources in the literature.

b The discrepancy between the percentage composition of glucosamine and galactosamine here and in Table I is because the hexosamines here are assumed to be acetylated.

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**REFERENCES**

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