POLYMERIZED OROSOMUCOID - AN INHIBITOR OF INFLUENZA VIRUS HEMAGGLUTINATION

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Several mucoproteins with sialic acid residues at the terminal positions in their oligosaccharide side chains are inhibitors of hemagglutination caused by influenza viruses. This inhibitory effect is probably due to the competition between the mucoprotein in solution and the mucoprotein receptors on the surface of red blood cells, both being bound by the virus. In its active state influenza virus acts as a neuraminidase, cleaving the sialic acid bound by alpha-ketosidic linkage to other sugar components of sialomucoproteins (for a review see ref. (1)).

All sialomucoproteins known as strong inhibitors of influenza virus hemagglutination (ovine salivary mucin, uromucoid, M and N antigens from human erythrocytes) have molecular weights of the order 10^6 (3, 7, 8, 13). Orosomucoid and fetuin which are sialomucoproteins with molecular weights 44,100, and 48,100, respectively, are only weak inhibitors (2). On partial proteolytic digestion of M and N antigens (5) and ovine mucin (2), their inhibitory activity is markedly diminished. As a result it was suggested that the size of the sialomucoprotein molecules may be an essential factor for their attachement to influenza virus particles (2).

The experiments described below provide an evidence in support of this hypothesis. On cross-linking orosomucoid a polymer with molecular weight about 10^6 was obtained which proved to be a strong inhibitor of influenza virus hemagglutination.

Materials and Methods

Orosomucoid was obtained from the urine of nephrotic children by a combination of methods described by Weinfeld and Tunis (16), and Hardwicke and de Vaux St.Cyr (4). The details of the procedure will be described elsewhere. The orosomucoid obtained migrated as a single band in paper electrophoresis (veronal buffer, pH 8.6) with the mobility of alpha-1 serum globulins, did not coagulate when boiled in 0,1 N acetic acid, and sedimented in the ultracentrifuge as a single boundary. For its other properties see Table I. In one experiment the orosomucoid obtained by the method of Weimer et al. (15) was used with similar results.

The cross-linking of orosomucoid by acetaldehyde was carried out according to the treatment of tropocollagen with formaldehyde (14) but a high concentration of orosomucoid was employed so as to enhance the possibility of forming the intermolecular cross-links. A five per cent solution of orosomucoid was prepared in 0.2 M phosphate buffer of pH 7 and to this solution acetaldehyde was added to a final concentration of 1.3 %, and the pH was brought to 4.3 with 5 N acetic acid. In some experiments ^{14}C-acetaldehyde (50 μC/g, uniformely labelled) was used. The solution was heated in a sealed ampoule at 50° ± 1° for 20 hours. The resulting gel was dissolved in 5 volumes of water by heating briefly at 60° with stirring. The solution was then passed through a column of Sephadex G 100 in water, and the sialic acid containing material emerging from the column at the front was collected and lyophylized. The yield was about 80 % of the orosomucoid used for polymerization.

In control experiments a 5 % solution of orosomucoid in 0.2 M phosphate brought to pH 4.3 with 5 M acetic acid was heated as described above. The material was then diluted to the desired mucoprotein concentration and assayed for the inhibitory activity, and in the ultracentrifuge.

Sedimentation and viscosity measurements were carried out in 0.2 M phosphate buffer of pH 6.0 at 20°. The results were extrapolated to infinite mucoprotein dilution.

Assays of the inhibitory activity were performed according to Tamm and Horsfall (12). Lee strain of influenza virus inactivated by 30 min. heating at 56° was used. Inactivated virus agglutinates erythrocytes but is devoid of neuraminidase activity.

Sialic acid was determined according to Svennerholm (11), and acetyl groups according to Ludowieg and Dorfman (6).

Results and Discussion

The orosomucoid treated with acetaldehyde under conditions described formed a polymer; its properties are presented in Table I.

Table I

Properties of the orosomucoid and its polymer

	Orosomucoid	Polymerized orosomucoid
Sedimentation constant	2.8 S	18 S*
Limiting viscosity number	0.061 dl/g	0.205 dl/g
Molecular weight	44,100**	870,000
Sialic acid content	10.4 %	9.95 %
Acetyl group content	4.9 %	4.65 %
Acetaldehyde incorporated	---	1.8 %
Inhibitory activity***	0.5 μg/4 a.u.	8×10^{-4} μg/4 a.u.

* Weight average
** From reference (10)
*** Expressed as the smallest amount of the material neutralizing 4 agglutinin units of influenza virus

Ultracentrifugal analysis of the polymer revealed its polydispersity. The mean molecular weight was calculated from sedimentation and viscosity data (9). The β-coefficient was assumed to be 2.16×10^6

as for moderately asymmetric macromolecules. The relatively low value of the limiting viscosity number of the polymer seems to justify this supposition. The partial specific volume of the polymer in solution was assumed to be the same as that of the orosomucoid, i.e.: 0.675 cm^3/g (10).

The polymer did not disaggregate in 0.3 % sodium dodecyl sulfate (at pH 6.0). It seems then that the orosomucoid molecules have been covalently cross-linked by $-CH(CH_3)-$ bridges (comp. ref. 14). In experiments with radioactive acetaldehyde it was found that about 30 aldehyde residues were incorporated per molecule of the orosomucoid.

The content of the most labile components of the orosomucoid: sialic acid and acetyl groups did not change to a marked degree during the polymerization procedure. Therefore we presume that apart from the cross-linking no deep chemical changes took place in the orosomucoid structure.

The inhibitory activity of orosomucoid increased on polymerization about 600-fold. The activity of the polymer was similar to that of the most potent inhibitors of influenza virus hemagglutination.

The orosomucoid heated in concentrated solutions at pH 4.3 without acetaldehyde formed aggregates with sedimentation coefficients 18 - - 19 S, but the inhibitory activity of this material was only 2 - 4 times higher than that of the orosomucoid. This was probably due to the instability of the aggregates. They disaggregated in dilute water solutions within several hours, and immediately in 0.3 % sodium dodecyl sulfate.

Erlier observations did not permit a decision as to whether the weak inhibitory activities of sialomucoproteins with lower molecular weights were due only to the smaller size of the molecules or to deficiences in their receptor structures. The strong enhancement of the inhibitory activity on polymerization indicates that when an adequate number of terminal sialic acid residues bound by alpha-

-ketosidic linkages is present in a macromolecule of appropriate size, the substance acts as a potent inhibitor of virus hemagglutination. The detailed structure of the remainder of the sugar side chains seems to be of minor importance.

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