HUMAN MN GLYCOPROTEINS: DEPENDENCE OF BLOOD-GROUP AND ANTI-INFLUENZA VIRUS ACTIVITIES ON THEIR MOLECULAR SIZE*

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The MN erythrocyte antigens constitute the second human blood-group system; in addition they are potent influenza virus receptors and have been characterized chemically as glycoproteins (1-5). An influenza virus receptor preparation of related nature possessing only traces of blood-group MN activity has also been isolated from human red cells (6).

We have recently found that the gentlest isolation procedures resulted in a preponderance of very large molecules of the MN substances. These blood-group substances were obtained in physicochemically homogeneous form from a population of molecules of varying size by a series of fractionations (7-11). Harsher treatment such as raising the temperature above 45°C or working outside a range of pH 5.5-7.2 at any step during isolation and purification gave a predominance of smaller molecules. It appears that the MN glycoproteins possess molecular weights which are multiples of 30,000 and that the large molecules tend to disaggregate upon manipulation (7-12). Major chemical differences were not demonstrable as the result of slightly more rigorous conditions; minor chemical alterations do occur at elevated temperatures, however (7,8).

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TABLE 1

IN VITRO ACTIVITY OF HUMAN MM AND NN BLOOD-GROUP ANTIGENS: DEPENDENCE ON MOLECULAR SIZE.*

Antigen	Molecular weight	Smallest amount (μg/ml) completely inhibiting agglutination of human blood-group O erythrocytes by 4 agglutinating doses**		
		—Human Sera— Anti-M	Anti-N	Influenza virus PR8
MM				
Ca 979	12×10^6	1		0.05
Ca 980	6×10^6	3		0.2
Ca 1014	1.8×10^6	5		0.8
NN				
Ca 825	5.9×10^5		10	1.5
Ca 745	1.5×10^5		50	
M and N***	3.1×10^4	500	3,500	10

* For procedures see (8).
** Homologous, homozygous erythrocytes used for blood-group determination.
*** Highest blood-group activity out of 6 samples kindly furnished by Dr. R.H. Kathan.

We have now found that the in vitro blood-group activity as measured with human sera and the capacity to inhibit hemagglutination by some myxoviruses shows a marked dependence on the molecular size of the isolated glycoprotein, i.e., the molecular size appears to be one of the important factors which determine activity. Blood-group as well as antiviral activities were highest for the largest molecules. The findings on substances isolated in this laboratory under the gentle conditions described above are depicted in Table 1. It can be seen that virus inhibitory capacity increases

with molecular size and that the glycoproteins of large molecular weight appear to be the most powerful myxovirus receptors yet isolated from human cells. Likewise a substantial increase in molecular size is accompanied by a similar increase in blood-group activity of these substances as measured with human sera. For comparison the human red cell virus receptor material which had been extracted at 68°C and pH 8 with aqueous phenol (6) was included. Its antiviral activity as measured with the PR8 strain influenza virus was 0.5% of that of the most active sample listed and it had <0.5% of the blood-group activities of the largest molecules listed in the Table and <1.5% of the smallest. This latter activity is lower than that of haptens with a molecular weight of <5000 which were obtained by pronase digestion from highly active M and N active macromolecules (8,10) and indicates some damage of the substance listed last in Table I. The difference in activity of the substances listed first and last respectively in the Table becomes even more striking if the activities are expressed on a molar basis: it is 10^4 to 10^6 fold.

Dependence of antiviral and blood-group activity on molecular size was less marked when virus inhibitory activity was measured with influenza virus B strain Maryland and blood-group activity determined with rabbit sera.

It is noteworthy in relation to the data presented here that the importance of molecular size for influenza virus inhibitor activity has been deduced from the decrease of both size and activity of ovine submaxillary mucin following treatment with trypsin (13). Also, orosomucoid (Mw 41,000) which does not significantly inhibit hemagglutination by influenza viruses became a potent inhibitor of hemagglutination by a number of influenza virus strains when it was polymerized (14,15).

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