VIROLOGY

Presence in Horse Serum of Two Inhibitors of Avid A2 Influenza Virus Strains

RECENTLY it has been shown¹⁻³ that normal sera from some animals contain substances which inhibit specifically the avid⁴ A2 influenza virus strains. Attention has been paid to these substances, as they exhibit not only haemagglutination-inhibiting activity, as the α -inhibitors, but also virus-neutralizing activity.

In our laboratory two inhibitors of avid A2 influenza virus strains, showing different mobilities in the electric field and differing also in other properties, were isolated from normal horse serum.

The faster-moving inhibitor, designated as glycoprotein inhibitor, was isolated from the lyophilized sulphosalicylic acid filtrate of the serum⁵ by extraction with an equal volume of 90 per cent phenol at 65° C. for 30 min.⁶. The water layer was freed of phenol by dialysis and then lyophilized. The substance obtained showed a single boundary on electrophoresis and sedimentation in the analytical centrifuge. It was of a glycoprotein nature, containing 18–20 per cent hexoses, 14.8–16 per cent sialic acid, 11.5–12.5 per cent hexosamines and 38–40 per cent polypeptides. 1 mgm. of this glycoprotein contained 6,400–12,000 haemagglutination-inhibiting units against 4 haemagglutination units of the avid A2 (Singapore) 57 influenza virus strain (eighth egg passage) and neutralized 100–1,000 TCID₅₀ of this virus up to 1:1,024 dilution. The action of a 0.1 per cent solution of crystalline trypsin at pH 8.0 and 37° C. for three days lowered the inhibitory activity of the inhibitor 50–100 times. 400–800 units of receptor destroying enzyme have split 43–50 per cent of the total sialic acid from 2 mgm. of the glycoprotein, without affecting the activity of the inhibitor.

The slowly moving inhibitor, designated as γ -globulin inhibitor, was isolated from horse serum by precipitation with a 0.01 per cent solution of 'Neokal B' (commercial preparation of a butylnaphthalene-sulphonate) in phosphate buffer pH 6.4 and 0.02 ionic strength. The serum was mixed at 0° C. with the 'Neokal' solution in the ratio 1:30, the precipitate separated by centrifugation, redissolved in saline and dialysed against distilled water. During the dialysis the euglobulins, containing the bulk of the biological activity, were precipitated. They were used in this work without further purification. The electrophoretic and sedimentation patterns of this substance showed the presence of two components. 1 mgm. of the γ -globulin inhibitor contained 400–800 haemagglutination-inhibiting and 512–1,024 virus-neutralizing units.

Both inhibitors isolated from horse serum were specific, in that they reacted only with the avid A2 influenza virus strains. Potassium periodate in 0.001–0.005 M concentrations completely inactivated the inhibitors at room temperature in 15 min.

The two inhibitors were separated from their mixture by paper electrophoresis using diethylbarbiturate buffer pH 8.6 as electrolyte and recovered by elution of 1-cm. strips. The maxima of the haemagglutination-inhibiting activity coincided with those of proteins, this being in agreement with the results obtained by other authors⁷ with unfractionated horse serum.

Table 1. HEMAGGLUTINATION-INHIBITING ACTIVITY OF THE GLYCOPROTEIN AND γ -GLOBULIN INHIBITORS AFTER DIFFERENT TREATMENTS

Treatment, fraction, original concentration	Haemagglutination-inhibiting units per ml.	
	Glycoprotein inhibitor	γ -Globulin inhibitor
'Neokal', supernatant, 2 mgm./ml.	32,768	0
'Neokal', precipitate, 35 mgm./ml.	no precipitate	16,384
Sulphosalicylic acid, filtrate, 20 mgm./ml.	131,072	0
Sulphosalicylic acid, precipitate, 35 mgm./ml.	no precipitate	2,048
Heating at 100° C. for 30 min., supernatant, 5 mgm./ml.	131,072	64
Heating at 100° C. for 30 min., precipitate, 5 mgm./ml.	no precipitate	0

Table 2. SOME PROPERTIES OF THE HORSE SERUM INHIBITORS AGAINST AVID A2 INFLUENZA VIRUS STRAINS

Properties	Glycoprotein inhibitor	γ -Globulin inhibitor
Sedimentation constant (S_{20})	3.66 S	14.7 S* and ~5.9 S
Mobility in the electric field at pH 8.6	with α -globulins	between β - and γ -globulins
Solubility in distilled water	soluble	insoluble
Haemagglutination-inhibiting units per mgm.	13,107	409–1,638
Virus-neutralizing units per mgm.	1,024	512–1,024

* Main component.

The differences in some properties of the inhibitors (solubility, sedimentation-rate, mobility in the electric field, haemagglutination-inhibiting and virus-neutralizing activities) as well as the changes in their haemagglutination-inhibiting activity after heating at 100° C. and treatment with 'Neokal', sulphosalicylic acid and phenol are presented in Tables 1 and 2.

On the basis of the results so far obtained we may conclude that normal horse serum contains at least two specific inhibitors of avid A2 influenza virus strains. The inhibitor moving faster in the electric field is an acid serum glycoprotein, and the slow one—so far not characterized in detail—is of a γ -globulin nature.

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SOIL SCIENCE

Soluble Aluminium in Plants and Soils

HIGHLY soluble aluminium concentrations in extracts of certain acid soils were first noted at least as early as 1904¹. It has been commonly explained as being due to aluminium ions held by chemical and physical means within the mineral part of the soil². This mechanism usually requires a close