Binding of Concanavalin A to the Envelope of Two Murine RNA Tumour Viruses

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Concanavalin A (Con. A), a phytagglutinin derived from Canavalia ensiformis, binds specifically to several branched carbohydrates (Goldstein, Hollerman & Merrick, 1965), containing terminal non-reducing α-D-glucopyranosyl, α-D-mannopyranosyl or β-D-fructofuranosyl residues (Goldstein, Hollerman & Smith, 1965; Goldstein & So, 1965).

Recently Bernhard & Avrameas (1971) developed a method to visualize the reaction of a cell surface with Con. A with the electron microscope. This gave us the opportunity to use Con. A to study the structure of the envelope of two RNA tumour viruses that mature at the cell membrane. B particles of the mouse mammary tumour virus (MTV-s) and C particles of a murine leukaemia virus (Rauscher) were used for this purpose.

The similarity of the Con. A-polysaccharide interaction to antibody-antigen precipitation is striking (So & Goldstein, 1967). We therefore tried to observe directly the interaction between the virus particles and Con. A after negative staining with phosphotungstic acid (PTA), as has been done with the virus-antibody complexes (Lafferty & Oertelis, 1963). Con. A has a molecular weight of 55,000 (Kalb & Lustig, 1968), so it is a smaller molecule than an antibody. The dimensions of antibody molecules are such that they fall within the limit of resolution of the electron microscope. On the other hand, the structure of the surface of the B particles is favourable for these observations, because the outer membrane is covered by spines of about 85 Å. If Con. A is attached to the virus particles, the spines on the virus surface will be almost completely obscured.

The B particles were purified from mammary tumours of BALB/c DeA fC3H/HeA or C3H/HeA mice as previously described by Calafat & Hageman (1968), with some modifications. A 0.01 M-tris buffer pH 7.0+0.25 M sucrose was used. The continuous urografin gradients were replaced by continuous gradients of 5 to 20 % sucrose in 0.01 M tris buffer pH 7.0. The C particles were purified according to the same method from spleens of BALB/cDeA mice infected with Rauscher leukaemia virus.

After fixation of the virus particles (250 μg. virus protein/ml.) for 1 hr in glutaraldehyde 2.5 % in 0.1 M phosphate buffer pH 7.2 they were incubated with different concentrations of Con. A (125–5000 μg./ml.) for 10, 30 or 60 min. at room temperature. The treatment with peroxidase and the diaminobenzidine (DAB) reaction was done following the method described by Bernhard & Avrameas (1971). The control preparations were treated in the same way but 0.1 M α-methyl-D-glucoside (Sigma) was added to the solutions of Con. A and peroxidase. The material was post-fixed in osmium tetroxide for 1 hr and embedded. Unstained or slightly stained thin sections were examined.

The virus was incubated with different concentrations of Con. A in phosphate buffer for 30 or 60 min. at room temperature and overnight at 4 °C. The virus-Con. A complex was sedimented at low speed and the pellet was washed 2 times in phosphate buffer to eliminate the excess of Con. A. Finally the pellet was suspended in a small quantity of phosphate buffer and negatively stained with 1 % PTA.

The B particles were incubated with a specific antiserum against B particles, kindly provided by Dr J. H. Daams, or with an antiserum against serum of C57Bldr mice, for 1 hr at
37°, overnight at 4°, the precipitate was spun down, washed 2 times and negatively stained with 1 % PTA. The preparations were examined with a Philips EM-200 microscope, operating at 80 kv. The double condenser system was used routinely with an average spot size of 15 μm.; in order to prevent specimen contamination a cooling device was used.

The B and C particles were agglutinated by Con. A. Thin sections (Fig. 1 a) showed that the virus particles were agglutinated in groups, and there was a thick layer of electron-dense, rather homogeneous precipitate along the virus membrane (Fig. 1 a, b). If the competitive

inhibiting sugar was added practically no agglutination and no precipitate was observed (Fig. 1 c). Virus treated with peroxidase alone followed by the DAB reaction showed no attached precipitate. After negative staining Con. A can be seen attached to the virus particles especially at the periphery (Fig. 2 c), forming a fine meshwork between the particles (Fig. 2 b). As Con. A is a small molecule sometimes the spines at the surface of the virus particles are still visible. The agglutination could be best observed if rather high Con. A concentrations, from 1000 μg./ml. and higher, were used. The different incubation times gave nearly the same results.

Fig. 2(d) shows B particles after incubation with the anti-B particle serum; the virus en-
velope is covered by antibody and aggregates are formed. The distance between the particles is slightly larger than in Fig. 2(b) owing to the larger size of the antibody molecules. On B particles incubated in phosphate buffer (Fig. 2(a) or anti-C57Bl serum, the spines are clearly visible and no aggregation occurred.

Virus particles of the two RNA tumour viruses have many binding sites for Con. A on their envelope, so they must contain exposed carbohydrates with terminal non-reducing sugars. These viruses are formed by budding from the cell membrane, which also contains binding sites for the agglutinin, because in preliminary experiments we found agglutination of cells of mammary tumours of C3H mice with Con. A.

Inbar & Sachs (1969a, b) reported that the surface of cells transformed by DNA tumour viruses and of leukaemic mouse cells contained many exposed binding sites to Con. A or to an agglutinin from wheat germ (Burger, 1969). Untransformed cells also could bind the agglutinin, but part of the binding sites would be in a cryptic form and could only be exposed after trypsin treatment.
Short communications

It is possible that the RNA tumour viruses contribute to the surface changes in tumour cells. However, Moore & Temin (1970) found that transformation of chicken cells by several RNA tumour viruses did not increase their agglutinability by Con. A, though rat cells transformed by B 77 virus showed markedly increased agglutination, comparable with the effect of trypsin treatment. Experiments on the binding capacity for agglutinins of surfaces of cells infected by mammary tumour virus or Rauscher leukaemia virus are in progress.

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REFERENCES


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