Concanavalin A Agglutinability of a Temperature-Sensitive Mutant of Rous Sarcoma Virus

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SUMMARY

Concanavalin A (Con A) concentration dependence of agglutinability of a mutant of Rous sarcoma virus which is temperature-sensitive for transformation was examined. Con A agglutinability of the virion was quantitatively expressed by measuring radioactivities of 60 to 70S RNA extracted from Con A-agglutinated material. The mutant grown at a permissive temperature (35 °C) agglutinated at a significantly lower concentration of Con A, compared with that at a non-permissive temperature (40 °C).

INTRODUCTION

RNA tumour viruses are known to derive their envelopes from pre-existing cellular membranes. Accordingly, the properties of the virus envelope are presumed to reflect, to a certain extent, those of the host cell membrane from which the virus matures. Malignant transformation of cells by Rous sarcoma virus (RSV) is accompanied by several changes of cell membranes such as increased lectin agglutinability (Burger & Martin, 1972; Kapeller & Doljanski, 1972), biochemical changes in cell surface components (Hakomori, Saito & Vogt, 1971; Warren, Critchley & Macpherson, 1972; Russell & Robinson, 1973; Hynes & Wyke, 1975), cell shape changes and so on. In studies to clarify the changes of the cellular membrane accompanied by malignant transformation, the enveloped virus seems to have advantages over the cellular membrane: it contains fewer macromolecular components which are fairly well characterized (Rifkin & Quigley, 1974) and a more homogeneous preparation can be obtained. Con A was used to purify oncornaviruses (Stewart et al. 1973). In the present study, Con A agglutinability of a mutant of RSV (TS-68) which is temperature-sensitive for transformation (Kawai & Hanafusa, 1971) was quantitatively expressed by counts of radioactivity of 60 to 70S RNA extracted from Con A-agglutinated material, and Con A agglutinability of the mutant grown at a permissive temperature was compared with that grown at a non-permissive temperature. The mutant grown at the permissive temperature agglutinated at significantly lower concentration of Con A than that at the non-permissive temperature. The observed differences presumably reflect those of the host cell membrane from which they mature.

METHODS

Virus and cells. The Schmidt–Ruppin strain of RSV belonging to subgroup A (SR-RSV-A), originally derived from the University Laboratories, Highland Park, New Jersey, U.S.A., (lot SR-6) was provided by Dr K. Yamanouchi. A mutant of SR-RSV-A (TS-68) which is
temperature-sensitive for transformation was provided by Dr H. Hanafusa (The Rockefeller University, New York).

RIF-free fertile eggs were obtained from SPAFAS (Norwich, Connecticut). Primary cultures of chick embryo fibroblasts (CEF) were prepared from 10 to 11 day embryos according to the methods of Rubin (1960). Cells for infection were plated at 2 to 10 x 10^6 cells per 10 cm plastic Petri dish in 20 ml of Eagle's minimal medium supplemented with 5% bovine serum and 1.0 μg of fungizone per ml. Three hours after seeding, the medium was removed and the cells were infected with 0.5 to 1.0 ml stock virus. After adsorption of virus for 1 h, the culture was incubated with 20 ml medium in a CO2 chamber.

Radioactive labelling of virus. Seven to 10 days after infection, 200 μCi 3H-uridine (23.3 Ci/mmol, Daiichi Pure Chemicals Co., Ltd, Tokyo, Japan) was added to 5 ml medium of a virus-producing culture in a 10 cm plastic Petri dish. Two hours later, a further 5 ml medium was added. Twelve hours after infection, the viruses were harvested and the culture was incubated with 20 ml fresh medium for another 12 h. The labelled media were pooled and used for the study of Con A agglutinability after one cycle of differential centrifugation.

Con A. Con A was prepared from jack bean meal by specific adsorption on Sephadex G-50 and subsequent elution with glucose as described by Agrawal & Goldstein (1967).

Con A agglutination. To 30 ml labelled infected culture medium was added 170 ml non-labelled infected medium. The combined 200 ml medium was centrifuged at 8000 g for 15 min. The supernatant fluid was then centrifuged at 20000 rev/min (Spinco No. 21 rotor) for 90 min. The pellets were suspended in 3 ml Eagle's minimal medium, and then centrifuged at 8000 g for 15 min. To the supernatant fluid of low speed centrifugation were added different amounts of Con A, and the reaction mixture was kept at 4 °C for 3 h. The resulting agglutinated material (Con A pellet) was separated from its supernatant fluid (post-Con A supernatant) by centrifuging at 3000 g for 30 min in the cold room. RNA was extracted from the Con A pellet by treatment with 0.2 ml of 1% SDS in TEN buffer (0.01 M-tris-HCl, 0.001 M-EDTA, pH 7.3, 0.15 M-NaCl) containing 0.1% β-mercaptoethanol at 37 °C for 2 min. After shaking for 30 s by using an Omni mixer, the RNA was analysed by sucrose density gradient sedimentation (Stewart & Maizel, 1974). The post-Con A supernatant fluid was pelleted by high speed centrifuging after adding 0.7 mg TMV as a carrier and analysed in the same way as the Con A pellet.

Sucrose velocity gradient sedimentation. Sucrose gradients of 15 to 30% (w/w) in TEN buffer were made by using Brakke's technique (Brakke, 1960). Rate zonal centrifugation for 2 h at 45000 rev/min in a SW 50 rotor (Spinco) was performed after 0.2 ml of SDS-treated Con A-agglutinated material was layered on top of each gradient column. After centrifuging, 8 drop fractions were obtained from the bottom of the tube, and 0.2 ml of cold 20% TCA and 200 μg carrier yeast RNA were added to each fraction and kept for 90 min at 4 °C. TCA-insoluble precipitates were collected on Whatman glass fibres (GF/C), dried, dissolved in scintillator and assayed for radioactivity in a Beckman scintillation counter.

RESULTS

Measurement of Con A agglutinability

The RNA extracted from labelled SR-RSV-A agglutinated by treatment with 0.365 mg/ml Con A was analysed by sucrose velocity gradient sedimentation. The sedimentation distribution of the RNA is shown in Fig. 1 a. A major class of RNA appears as 60 to 70 S, since it sedimented ahead of hamster liver ribosome centrifuged in the absence of Mg2+, a
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Fig. 1. Sucrose velocity gradient sedimentation. (a) Labelled RNA extracted from the Con A pellet was centrifuged in a 15 to 30% sucrose gradient in TEN buffer at 45,000 rev/min (Spinco) for 2 h, and the TCA-precipitable radioactivity of each fraction was counted: ••••, SR-RSV-A infected medium; □-----□, uninfected medium. (b) Hamster ribosomes centrifuged in the presence of 0.001 M-MgCl₂ under the same conditions as that of (a) were estimated from their ultraviolet extinction at 260 nm. (c) Hamster ribosomes in the absence of MgCl₂. Sedimentation is from right to left.

A comparison of Con A agglutinability of temperature-sensitive mutants grown at the permissive and non-permissive temperatures

Con A concentration dependence of agglutinability of TS-68 grown at the permissive temperature (35 °C) was compared with that grown at the non-permissive temperature (40 °C). As shown in Fig. 3, at a Con A concentration of 0.365 mg/ml, the mutants grown at both permissive and non-permissive temperatures agglutinated 100 %: almost all radioactivity of 60 to 70 S RNA was recovered from the Con A pellet (a and b) but not from the post-Con A supernatant fluid (c and d). At a Con A concentration of 0.073 mg/ml, in the mutants grown at both temperatures almost all radioactivity of 60 to 70 S RNA was recovered not from the Con A pellet but from the post-Con A supernatant. However, at a Con A concentration of 0.183 mg/ml a pronounced difference was observed. TS-68 grown
Fig. 2. Dissociation of Con A-agglutinated material with α-methylmannoside. RNAs from the Con A pellet followed by treatment with or without α-methylmannoside were analysed by sucrose velocity gradient sedimentation: ••, counts of labelled RNA from the Con A pellet without α-methylmannoside treatment; □□, counts of labelled RNA from the Con A pellet followed by α-methylmannoside treatment.

Fig. 3. Sucrose velocity gradient analysis of RNAs from materials agglutinated with different concentrations of Con A. TS-68 virus stocks grown at the permissive and non-permissive temperatures were treated with different concentrations of Con A, 0.073, 0.183 and 0.365 mg/ml, and from the resulting Con A pellet RNAs were extracted and analysed by sucrose velocity gradient sedimentation. The post-Con A supernatant fluid pelleted by centrifuging at 25,000 rev/min (SW50, Spinco) for 90 min after adding 0.7 mg TMV as a carrier and analysed in the same way as the Con A pellet. (a) The Con A pellet of TS-68 grown at the permissive temperature: •—•, Con A concentration, 0.365 mg/ml; □—□, 0.183 mg/ml; ▲—▲, 0.073 mg/ml. (b) The Con A pellet of TS-68 at the non-permissive temperature: •—•, 0.365 mg/ml; □—□, 0.183 mg/ml; ▲—▲, 0.073 mg/ml. (c) The post-Con A supernatant fluid of TS-68 grown at the permissive temperature: •—•, 0.365 mg/ml; □—□, 0.183 mg/ml; ▲—▲, 0.073 mg/ml. (d) The post-Con A supernatant fluid of TS-68 grown at the non-permissive temperature: •—•, 0.365 mg/ml; □—□, 0.183 mg/ml; ▲—▲, 0.073 mg/ml.

at the permissive temperature agglutinated about 80% of that at a Con A concentration of 0.365 mg/ml (Fig. 3a), while the mutant grown at the non-permissive temperature agglutinated only 27% (Fig. 3b). Percent agglutinability was also calculated from amounts of labelled RNA remaining at the 60 to 70S region without agglutination (Fig. 3c and d).
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Fig. 4. The effect of Con A concentration on agglutination. Percent agglutination, calculated by taking a total radioactivity at Con A concentration of 0.365 mg/ml as 100, is plotted as a function of Con A concentration: O—O, TS-68 grown at 40 °C; •—•, TS-68 at 35 °C; □—□, SR-RSV-A at 40 °C; ■—■, SR-RSV-A at 35 °C.

Twenty-one and 70% radioactivities (a total radioactivity at a Con A concentration of 0.073 mg/ml was taken as 100) remain in the post-Con A supernatant fluid, giving the values for percent agglutinability of 79 and 30% for the mutant grown at the permissive temperature and that at the non-permissive temperature, respectively. These values agree fairly well with those obtained from the Con A pellet.

In summary, both mutants grown at the permissive and non-permissive temperatures agglutinated 100% at a Con A concentration of 0.365 mg/ml, but remained in the supernatant fluid without agglutination at 0.073 mg/ml. However, at a Con A concentration of 0.183 mg/ml, a pronounced difference was observed. The mutant grown at the permissive temperature agglutinated about 80%, but that grown at the non-permissive temperature agglutinated only about 30% (Fig. 4).

A possibility that the difference thus observed in Con A agglutinability was due not to transformation of the cell but to the difference in temperature at which the virus was produced was examined by using wild type RSV, SR-RSV-A. As shown in Fig. 4, a significant difference was not observed in Con A agglutinability whether wild type RSV multiplied at 35 °C or 40 °C, indicating that the difference observed in TS-68 is not due to growing the virus at the different temperatures.

DISCUSSION

Cells transformed by a variety of oncornaviruses are in most cases more readily agglutinated with Con A than non-transformed cells (Rapin & Burger, 1974). Oncornaviruses mature at the cell surface and are presumed to be coated with host cell membrane. Therefore, it is most likely that their envelopes share a common property with the host cell membrane from which they mature. TS-68 produced at the permissive temperature is expected
to be coated with transformed cell membrane and to be more agglutinable with Con A than TS-68 produced at the non-permissive temperature. The result obtained in the present study was in accordance with this expectation. Burger & Martin (1972) found that hyaluronidase treatment significantly enhances the agglutinability of cells transformed by RSV, while having little effect on that of uninfected cells. However, such differentiating effect by treatment with hyaluronidase was not observed on virions produced at the permissive and non-permissive temperatures (T. Miki, unpublished data). Increased Con A agglutinability of the mutant produced at the permissive temperature is presumed to reflect increased fluidity of virus membrane or increased affinity to Con A, or both, by analogy to the mechanism of Con A-transformed cell interaction. However, its molecular basis and real mechanism remain for further study.

In the present study, we used a crude virus preparation after one cycle of differential centrifugation. Therefore, a possibility exists that Con A-induced virus agglutination is mediated by some constant contaminant in the virus preparation. However, this possibility remains to be proved experimentally.

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REFERENCES


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