Deoxyglucose Transport Changes in Murine Sarcoma Virus-infected Cells – a Quantitative Assay for Virus Transforming Activity

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SUMMARY

Dose-response studies were performed in relation to the rate of uptake of [3H]-2-deoxyglucose by murine sarcoma virus-infected murine cells. A linear relationship was observed between the virus dilution used for infection and the rate of dgl uptake by the infected cells. The extrapolated dilution factor (reciprocal of dilution) at which no difference was observed between infected and uninfected cells was directly proportional to the activity estimated by focus assay of MSV. Such end-point dilution factors can be reproducibly and objectively obtained 2 to 4 days after infection.

Cells infected with sarcoma viruses containing RNA undergo characteristic morphological changes and do not show the usual density-dependent inhibition of growth. The rate of transport of a variety of hexoses is increased as compared to that for uninfected cells (Hatanaka, Huebner & Gilden, 1969). Using mutants of Rous sarcoma virus (Kawai & Hanafusa, 1971; Martin et al. 1971) and of murine sarcoma virus (MSV) (Somers, May & Kit, 1973), it has been shown that the increased rate of uptake of 2-deoxyglucose (dgl) was expressed during the culture of the virus-infected cells only at the temperature permissive for the morphological transformation of the cells. In addition to the increased rate of sugar transport, cells transformed by sarcoma viruses also show the ability to divide, developing into foci of multilayered growth when the anchored cells are cultured in either liquid media or under agar. The focus assay has been used to determine the infectivity of sarcomagenic oncornaviruses. Foci induced by the infectious sarcoma viruses become apparent in 3 to 6 days, and although they may be discrete, as with many strains of avian sarcoma viruses, they are often diffuse, as with murine sarcoma viruses and hence difficult to score (Fig. 1). Therefore, the enumeration of foci may be subject to errors depending on the experience of the investigator, and on the conditions of cell culture.

We have investigated the rate of dgl uptake as a function of virus concentration. A linear relationship was found between the rate of uptake of dgl and the concentration of virus in the inoculum. The dilution of the virus sample at which the rates of uptake in the infected and uninfected cells were equal was taken as the titre of the virus preparation. This assay is simple and reproducible, offering an alternative to the focus assay of the sarcoma viruses.

The established murine cell lines Balb 3T3, NIH 3T3, and NRK, were used in this study. The Harvey (Harvey, 1964) and Kirsten (Kirsten & Mayer, 1967) strains of MSV (H-MSV and K-MSV) were grown in Balb and NIH 3T3 cells, respectively. The conditions of cell culture and virus preparation have been described (Bose & Zlotnick, 1973). Infectivities of the MSV-containing samples were determined by counting the H-MSV-induced foci in Balb 3T3 cells according to the procedure described by Aaronson, Jainchill & Todaro (1970). The foci were counted 4 to 6 days after infection, after staining with Giemsa stain. The dgl uptake end-point dilution assay was performed as follows: Day 0 seeding; approx. 2.5 × 10⁶ Balb 3T3 cells or 8 × 10⁴ NRK cells were seeded into 5 cm diam. culture dishes.
Fig. 1. Foci of transformed cells after infection of Balb 3T3 cells with Harvey MSV. Giemsa-stained cultures, 10 days post-infection. Magnification ×66. Note the discrete (left) as well as the diffuse ‘foci’ of transformed cells.

Fig. 2. [H]-dgl uptake assay in 5 cm dishes and in 24-well tissue-culture plates. Balb 3T3 cells were seeded at 3·3 × 10⁵ per 5 cm dish (Nunclon) and 3·6 × 10⁵ per well of a Linbro 24-cup tissue-culture plate (FB-16-24-TC, 4”× 6”). After 24 h the cultures were treated with DEAE-dextran (25 µg/ml) and infected with 0·5 ml of virus dilutions. The dgl uptake assay was performed 4 days after infection, using 1 µCi of [H]-dgl per 5 cm dish (▲—▲) and 0·25 µCi per well (●—●), respectively. The co-ordinate scales are logarithmic. Incubation with the radioactive substrate was for 10 min.
Fig. 3. U.v. inactivation of transforming activity as measured by deoxyglucose uptake. 7·3 × 10^4 NRK cells were plated in 5 cm dishes (Nunclon). K-MSV, obtained as a 24 h harvest of culture fluid from virus-shedding NIH 3T3 cells, was irradiated for 2 min at a distance of 26 cm from a germicidal lamp (Sylvania, G30T8). Cells were infected with various dilutions of the unirradiated and irradiated virus samples. Uptake of [3H]-dgl was measured during a 10 min incubation 2 days after infection. ○, unirradiated virus; ●, u.v.-irradiated. The end-point dilution factors (control, 4 × 10^4 irradiated 1·5 × 10^3) were obtained by extrapolation (dashed lines).

Fig. 4. Linear relationship between logarithm of focus forming units (f.f.u./ml) and logarithm of dgl uptake end-point dilution.

(Nunclon) with 5 ml of growth medium, or alternatively, 3 × 10^4 Balb 3T3 cells were seeded into each of the 24 wells of a Linbro plate (FB 16-24-TC, 4” x 6”) with 2 ml of medium. The dishes were incubated at 37 °C in a humidified CO₂ incubator. Day 1, infection; the cultures were treated with 25 μg/ml of DEAE-dextran (Sigma International) for 1 h to enhance their susceptibility to infection (Somers & Kirsten, 1968). The monolayers were then washed twice with media. The fluid containing virus was filtered through a membrane filter (0·22 μm) and diluted in growth medium. The dilution factors (reciprocal of dilutions) were 1 × 10^1, 2 × 10^1, 5 × 10^1, 1 × 10^2, 2 × 10^2, 5 × 10^2, and 1 × 10^3 and 2 × 10^3. A 0·5 ml aliquot of each of the dilutions was used to infect the cells. After an adsorption period of 1 h at 37 °C, 5 ml of growth medium was added to each 5 cm dish, or 2 ml to each well of the Linbro plate, and incubation continued at 37 °C. Day 4; the rate of uptake of [3H]-dgl was measured (Bose & Zlotnick, 1973). In the experiments represented in Fig. 2, the rates of dgl uptake exhibited by Balb 3T3 cells infected with various concentrations of H-MSV were plotted against the dilution factors on logarithmic co-ordinates. A linear relationship was found between the logarithm of the rate of dgl uptake and the logarithm of the reciprocal of the virus dilution (dilution factor). Such a relationship should reflect the proportion of virus-transformed cells in the population. These results were obtained by using identical samples of H-MSV to infect Balb 3T3 cells grown either in 5 cm culture dishes or in the wells of a Linbro plate. At high concentrations of the virus a plateau value was seen with the rate of uptake of [3H]-dgl, representing the maximum number of cells which could be transformed. When lower concentrations of H-MSV were used, the rate of dgl uptake decreased. At 1:5 × 10^9 dilution in 5 cm culture dishes and at 1:4 × 10^8 in the 24-well plate, the rate of uptake by the infected cells was identical to that by mock-infected Balb 3T3 cells.
Short communications

In order to ascertain that the dose-response results described above were indeed reflecting the activity of the sarcoma virus, a sample containing K-MSV was exposed to u.v. radiation for 2 min. The irradiated and unirradiated samples were appropriately diluted and the diluted virus samples were used to infect NRK cells. The results summarized in Fig. 3 indicated that the end-point dilution factor was \(4 \times 10^8\) in the control and decreased to \(1.5 \times 10^5\) after 2 min irradiation of the virus preparation. The ability of MSV to produce an increased rate of dgl uptake after infection of susceptible cells was therefore a u.v.-sensitive function of virus.

The results summarized in Fig. 4 show that the agreement is good between focus forming units and the end-points as determined by dgl uptake rates. In addition to the piled-up growth resulting in foci, one can determine the transforming capacity of at least two strains of MSV by infecting cells with different concentrations of the virus, and measuring the rates of uptake of \([3H]\)-dgl at 3 to 4 days post-infection. Such determinations are extremely useful when high virus inputs are used.

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Department of Microbiology
St Louis University School of Medicine
St Louis, Missouri 63104 U.S.A.

Barbara J. Zlotnick
S. K. Bose*
R. C. Roa

REFERENCES


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* To whom all correspondence should be directed.