The Phosphorylation of Ribosomal Protein S6 in Hamster Fibroblasts Infected with Pseudorabies Virus Inactivated by Ultraviolet Radiation

By IAIN M. KENNEDY, DAVID P. LEADER AND WILLIAM S. STEVELY*

Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, U.K.

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SUMMARY

Infection of baby hamster fibroblasts with pseudorabies virus at high multiplicities resulted in a substantial increase in the phosphorylation of ribosomal protein S6. However, the phosphorylation was still observed with virus that had been completely inactivated by u.v. irradiation. We therefore conclude that expression of the viral genome is not required for the virus to elicit this effect.

In previous studies we have shown that infection of baby hamster fibroblasts (BHK cells) by pseudorabies virus or by herpes simplex virus results in a substantial increase in the phosphorylation of ribosomal protein S6 (Kennedy et al., 1981). The delay of at least 3 h before this phosphorylation could be observed, and the fact that another ribosomal protein (S16 or S18) was phosphorylated in cells infected with pseudorabies virus, but not in those infected with herpes simplex virus, allowed the possibility that the phosphorylation of ribosomal protein S6 might be catalysed by a virus-coded protein kinase. We now report results that appear to eliminate such an explanation for the phosphorylation.

Descriptions or references to most of the methods used in this work are given in a previous paper (Kennedy et al., 1981). Briefly, BHK-21/C13 cells maintained in monolayer culture in modified Eagle’s medium containing 10% calf serum were infected with pseudorabies virus at a multiplicity of 20 p.f.u./cell (unless otherwise indicated), using stock virus with a particle:plaque-forming unit ratio of 20:1, and harvested 5 h after the start of infection. Inactivation by u.v. light was as follows. Stock virus was diluted tenfold with phosphate-buffered saline containing 1% (w/v) glucose. Aliquots (2.5 ml) were irradiated in 50 mm Petri dishes under a UVSL-58 Mineralight (Ultra-Violet Products Inc.). Dosage was calculated from the distance between source and sample and the time of irradiation.

The extent of phosphorylation of ribosomal protein S6 was determined by its position of migration during two-dimensional gel electrophoresis. Such changes in migration have previously been shown to correlate with the change in the labelling of the protein by radioactive orthophosphate, but are generally accepted as giving a more reliable and informative indication of the phosphorylation state (see Leader, 1980, for a discussion of this point).

The effect of infection with pseudorabies virus on the phosphorylation of ribosomal protein S6 is well illustrated in Fig. 1(a, b), where the change in position of migration from that of the poorly phosphorylated state to that of the multiply phosphorylated state of the protein is almost complete. [In Fig. 2(a, b) the effect is also apparent, but is less.] It can be seen from Fig. 1 that irradiation of the virus with intensities of up to 50 kiloergs of u.v. radiation did not prevent this effect, even though such preparations of virus had no detectable plaque-forming ability (less than 0.002% of that of the unirradiated virus). Fig. 2 suggests that it is most unlikely that within these limits any residual active virus could be responsible for the phosphorylation, because decreasing the multiplicity of infection as little as from 20 to 1 p.f.u./cell prevented the phosphorylation. It should be mentioned that induction of viral DNA polymerase activity was still obtained after infection with pseudorabies virus at 1 p.f.u./cell.

The results presented here recall those of Kaerlein & Horak (1978), who studied the
Fig. 1. Effect of u.v. irradiation of pseudorabies virus on the phosphorylation of ribosomal protein S6 in infected BHK cells. The frames show the two-dimensional gel electrophoretic separation of total ribosomal proteins extracted from (a) uninfected cells, or cells infected with virus irradiated with doses of (b) 0, (c) 1000, (d) 5000, (e) 10000 or (f) 50000 ergs. The positions of the maximally and minimally phosphorylated species of ribosomal protein S6 in each frame are indicated by the left-hand (anodic) and right-hand (cathodic) arrows, respectively, a broken arrow being used to indicate the less abundant form. The titre of virus after u.v. irradiation at 1000, 10000 and 50000 ergs was 2%, 0.4% and 0.002% respectively that of the untreated virus.
Fig. 2. Effect of multiplicity of infection on the phosphorylation of ribosomal protein S6. Infection was with (a) 0, (b) 20, (c) 1, (d) 0·1 or (e) 0·01 p.f.u. pseudorabies virus per BHK cell. Other details are as for Fig. 1.
phosphorylation of ribosomal protein S6 in HeLa cells infected with vaccinia virus inactivated by u.v. irradiation. These authors suggested that protein kinase from the inoculated virus particles might be responsible for the phosphorylation of ribosomal protein S6. In the case of pseudorabies virus this is unlikely as we have examined the protein kinase activities present in the virion, finding that these seem to reflect the spectrum of cellular protein kinases in infected cells, with no enrichment for any of the kinases that are able to phosphorylate S6 (W. S. Stevely, M. Katan & D. P. Leader, unpublished results). Both pseudorabies virus (Chowdhury, 1980) and herpes simplex virus (Fenwick & Walker, 1978) inactivated with u.v. radiation still elicit the early phase of inhibition of host cell protein synthesis, and it is possible that the phosphorylation might be a host cell response to such inhibition. Inhibition of protein biosynthesis by drugs such as puromycin and cycloheximide (Gressner & Wool, 1974) and (in the case of liver) by a variety of pathological conditions (e.g. Gressner, 1980; Leader, 1981) has been shown to cause increased phosphorylation of ribosomal protein S6. However, it cannot be excluded that the phosphorylation is a cellular stress response to the penetration by the virus of the cell membrane, independently of any effect this may have on protein synthesis. The higher multiplicity of infection required for the phosphorylation relative to that required for infection is perhaps more consistent with such an alternative.

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REFERENCES


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