Proteins of Herpesvirus Saimiri: Identification of Two Virus Polypeptides Released into the Culture Medium of Productively Infected Cells

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SUMMARY

Two virus-induced polypeptides are preferentially released into the culture medium of owl monkey kidney cells productively infected with herpesvirus saimiri (HVS). These polypeptides have apparent mol. wt. of $160 \times 10^3$ and $28 \times 10^3$ and are the major virus-induced polypeptides in a membrane fraction from infected cells. Both these excreted polypeptides co-migrate with virus structural proteins and are selectively released from enveloped virus particles by treatment with Nonidet-P40.

Herpesvirus saimiri (HVS) is a lymphotropic virus which naturally infects squirrel monkeys (Saimiri sciureus) and can be isolated from 50 to 80% of non-selected animals (Falk et al. 1972; Deinhardt et al. 1973) in the absence of overt disease. In contrast, experimental infection with HVS causes a rapidly fatal T-cell lymphoma in at least five other species of New World monkeys (for review, see Fleckenstein, 1979). Partial protection of these susceptible species has been achieved by immunization with a crude suspension of inactivated fluid from infected cultures (Laufs, 1974; Laufs & Steinke, 1975) and by prior immunization with an attenuated derivative of HVS strain 11 (Schaffer et al. 1975; Falk et al. 1976). However, there is no information on which virus proteins are effective in eliciting a protective immune response. Cells infected with at least some other herpesviruses excrete a subset of virus-specified polypeptides in a soluble form into the tissue culture medium (Ben-Porat & Kaplan, 1970; Kaplan et al. 1975; Norrild & Vestergaard, 1979; Randall et al. 1980). These polypeptides are antigenically related to the envelope glycoproteins of the herpesvirus particle. In the case of herpes simplex virus type 1, these excreted polypeptides are capable of absorbing out all virus-neutralizing antibody raised against glycoproteins of the virus particle and of the infected cell (Randall et al. 1980). In this paper we identify those HVS proteins that are excreted from productively infected cells in tissue culture.

For these experiments confluent monolayers of a continuous line of owl monkey kidney cells (OMK-210, kindly provided by L. V. Melendez, New England Regional Primate Centre, Southborough, Boston, Mass., U.S.A.) grown in 80 oz Winchester bottles, were infected with 0.05 to 0.2 p.f.u./cell of the attenuated derivative of HVS strain 11 (Schaffer et al. 1975). When approx. 20% of cells showed a virus-specific c.p.e., the growth medium was replaced with 50 ml Eagle’s medium lacking calf serum and containing one-tenth of the normal concentration of unlabelled methionine with 2 μCi/ml $^{35}$S-methionine (500 Ci/mmol; The Radiochemical Centre, Amersham, U.K.). Cultures were re-incubated until 90 to 100% of the cells showed c.p.e. (i.e. a further 24 to 36 h at 37 ºC). The culture medium was then harvested and centrifuged (3000 rev/min for 10 min) to sediment cells and large cellular debris. The supernatant was removed and centrifuged at 14,000 rev/min for 2 h in order to sediment virus and other particulate material into a pellet designated in this communication as a medium pellet. Soluble proteins in this supernatant were then precipitated by adding an equal volume of neutral, saturated ammonium sulphate. After incubation
overnight at 4 °C, the precipitate was sedimented (3000 rev/min for 10 min) and the pellet resuspended in 2 ml 0.05 M-tris-HCl pH 7, and dialysed against the same buffer. Fig. 1 illustrates a comparison of the 35S-methionine-labelled polypeptides present in these fractions from infected and uninfected cells. The labelled polypeptides present in total infected cell lysate (slot 1) were characteristic of those made late in the growth cycle of the virus and were clearly distinct from the population of proteins made by uninfected cells (slot 2). The majority of these infected cell polypeptides were structural components of virus particles which comprised most of the radioactive proteins in the medium pellets from infected cells as shown by a comparison of slots 1 and 3 (P. O'Hare, R. W. Honess & R. E. Randall, in preparation). In contrast to the relatively complex mixtures of labelled polypeptides in the total infected cell and medium pellet, two polypeptides predominated in the mixture of labelled proteins excreted by infected cells (slot 5). These polypeptides, with apparent mol. wt. of 160 x 10^6 (160K) and 28 x 10^6 (28K), were also specific to infected cultures (compare slots 5 and 6). A number of other labelled polypeptides were present in lower amounts and showed less specific enrichment in the soluble fraction (e.g. 130K; see also Fig. 2, slot 6).

It should also be emphasized that the soluble proteins excreted from infected cultures contained other minor labelled proteins, as well as unlabelled polypeptides characteristic of uninfected cells and serum components from the tissue culture media. The radiochemical purity of the virus-specific components of this fraction is due to the inhibition of incorporation of labelled amino acids into host proteins at late times in the virus growth cycle (P. O'Hare et al., in preparation).

In a further series of experiments the 160K and 28K polypeptides from the medium of infected cells were compared with those of a membrane fraction from infected cells and intact or detergent-disrupted virus particles. Enveloped virus particles were partially purified from the medium pellet of infected cultures labelled with 35S-methionine (e.g. Fig. 1, slot 3). The pellet was resuspended in 1 ml 0.05 M-tris-HCl pH 7, placed over a 10 to 45 % sucrose gradient and centrifuged at 23000 rev/min for 30 min in an MSE (Crawley, U.K.) 6 x 14 ml swingout rotor. A band, visible half-way down the gradient, was harvested and shown to contain enveloped virus particles by electron microscopy. In addition, a band of membrane fragments at the top of the gradient was also collected. The labelled polypeptides of the partially purified virus particles were separated by SDS–polyacrylamide gel electrophoresis, together with those of the total infected cell, the soluble excreted proteins and the membrane fraction from infected cells (Fig. 2). The major labelled polypeptides of the membrane fraction of infected cells co-migrated with the 160 K and 28 K polypeptide excreted into the medium of infected cultures (slots D and E respectively). These polypeptides were not found in an analogous membrane fraction from uninfected cells (not shown). Both the 160 K and 28 K polypeptides also co-migrated with polypeptides present in preparations of partially purified enveloped virus particles (slot A). Moreover, these polypeptides were solubilized by detergent treatment of enveloped virus particles. In this experiment a sample of partially purified virus was made up to 1 % Nonidet-P40 (NP40), 0.1 M-NaCl, 2 mM-EDTA and 2 mM-mercaptoethanol and incubated at 37 °C for 30 min. A control sample of virus, to which buffer without detergent was added, was treated in the same manner. These mixtures (0.25 ml) were placed over 10 % sucrose cushions (0.6 ml) and centrifuged at 30000 rev/min for 30 min in 1 ml adaptors of an MSE 6 x 5.5 ml swingout rotor. In the control sample more than 95 % of the applied radioactivity sedimented to a pellet, with less than 2 % being recovered in the top 0.4 ml of the gradient. In contrast, 60 % of the applied radioactivity was sedimented from the detergent-treated sample and 35 % was recovered in a soluble form in the top 0.4 ml of the gradient. Fig. 2 shows an analysis of the polypeptides present in samples of the soluble (slot C) and sedimented (slot B) material from the detergent-treated virions. A similar analysis of the control samples failed to reveal any virus polypeptides in
Fig. 1. Autoradiograms of $^{35}$S-methionine-labelled polypeptides separated by electrophoresis on (a) 12% and (b) 8.5% polyacrylamide slab gels from: unfractionated infected (slots 1 and 7) and uninfected (slot 2) cells; the medium pellets from infected (slot 3) and uninfected (slot 4) cells; and the soluble proteins excreted into the medium of infected (slots 5 and 8) and uninfected (slot 6) cells. Methods for sample preparation and electrophoresis on SDS–polyacrylamide gels were as described previously (Heine et al. 1974). The basis for determinations of apparent mol. wt. will be given elsewhere (P. O’Hare et al., in preparation).
Fig. 2. Autoradiograms and derived densitometer tracings of 35S-methionine-labelled polypeptides separated by electrophoresis on a 10% polyacrylamide slab gel from: partially purified virus (slot A); the pellet (slot B) and soluble fractions (slot C) obtained after sedimentation of detergent-treated virions (see text); a membrane fraction from the medium pellet of infected cells (slot D; see also Fig. 1) and the soluble excreted proteins from these cells (slot E). The absorbance tracings of slots A, B and C were made with a Joyce–Loebl scanning microdensitometer. The samples of polypeptides from the pellet (B) and soluble fractions (C) of detergent-treated virus were from approx. one-quarter of the amount of partially purified virus as that shown in slot A (note absorbance scales). The mol. wt. of the major polypeptides and the position of actin, a contaminating polypeptide of these virus preparations, are shown.
the soluble fraction and the ratios of labelled polypeptides in the sedimented material were indistinguishable from a sample of the untreated virus (not shown). The detergent treatment of virions efficiently removed a polypeptide of 160K and a minor polypeptide of 28K which co-migrated with the 160K and 28K polypeptides excreted from infected cells (slot C and E). A polypeptide of 130K was also released from detergent-treated virions and co-migrated with a polypeptide present in relatively low concentrations in the population of proteins excreted from infected cells.

By analogy with the effects of detergent on other enveloped viruses, including herpes simplex virus (Gibson & Roizman, 1972; Sarmiento & Spear, 1979), it seems likely that the 160K and 28K polypeptides which are removed efficiently by detergent treatment of the virus, are components of the envelope. Thus, the virus-induced polypeptides released in a soluble form into the medium of infected cells co-migrate with polypeptides present in the envelope of the virus particle and in a membrane fraction from infected cells. Therefore, as with other herpesviruses (Randall et al. 1980), polypeptides excreted from HVS-infected cells should provide ideal starting materials for purification and antigenic analysis of protein components of the virus envelope.

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REFERENCES


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