Isatin-β-Thiosemicarbazone Causes Premature Cessation of Vaccinia Virus-Induced Late Post-Replicative Polypeptide Synthesis

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SUMMARY

The effects of isatin-β-thiosemicarbazone on vaccinia virus-induced polypeptide synthesis has been examined by polyacrylamide gel electrophoresis and autoradiography. The synthesis of pre-replicative and early post-replicative polypeptides proceeded normally in the presence of the drug; the onset of late post-replicative polypeptide synthesis occurred with normal timing under these conditions but the rate of synthesis of all virus polypeptides then declined rapidly.

Isatin-β-thiosemicarbazone (IBT) and related compounds inhibit the growth of poxviruses. Studies on the mode of action of these compounds have shown that virus DNA and virus-induced pre-replicative polypeptides are made in their presence (Easterbrook, 1962; Woodson & Joklik, 1965; Katz, Margalith & Winer, 1973a). Late in infection, however, protein synthesis in infected cells declines rapidly, the stability of virus mRNA being reduced (Woodson & Joklik, 1965). Virus induced post-replicative polypeptide synthesis has been demonstrated in drug-treated cells (Katz et al. 1973a), although immunological studies have shown that IBT prevents the synthesis of those virus antigens made late in infection (Appleyard, Hume & Westwood, 1965). The experiments reported here were designed to investigate in more detail the effects of IBT on vaccinia virus-induced polypeptide synthesis late in infection.

Vaccinia virus (Evans vaccine strain) was grown in BHK 21 cells, and purified by the method of Joklik (1962). Monolayers of cultures of BSC 1 cells were grown in Eagle’s medium (Glasgow modification) containing 10% foetal bovine serum and were infected with purified virus at an input multiplicity of 50 p.f.u./cell in Eagle’s medium containing 2% foetal bovine serum and 0.02 M-MgCl₂. Absorption was allowed to continue for 30 min at 38 °C. The monolayers were then washed and maintained in Eagle’s medium containing 5% foetal bovine serum. Proteins were labelled by the addition of 14C-protein hydrolysate (5 μCi/ml, 57 mCi/mAtom; obtained from the Radiochemical Centre, Amersham) contained in amino acid-free Eagle’s medium; before labelling, cell monolayers were washed twice with warm amino acid-free Eagle’s medium. In some experiments labelling was done using 35S-methionine (40 μCi/ml, 100 000 mCi/mmol; Amersham) in phosphate-buffered saline. After labelling for 15 min at 38 °C the medium was removed and the cells from each plate were scraped into 0.4 ml of 0.001 M-tris-hydrochloride, pH 9.0. Samples were reduced and dissociated by boiling in 1% mercaptoethanol and 2% SDS and were electrophoresed in acrylamide gels using a discontinuous buffer system. Electrophoresis and subsequent procedures leading to autoradiography were done using methods previously described (Pennington, 1974). Isatin-β-thiosemicarbazone (a gift from Dr D. J. Bauer) was dissolved in a small volume of acetone; the solution was then added slowly to the appropriate volume of Eagle’s medium.

Analysis of the time course of virus-induced polypeptide synthesis showed that pre-replicative polypeptides were synthesized normally in the presence of IBT (10 μg/ml).
Fig. 1. Effect of IBT on the synthesis of representative virus-induced polypeptides. Infected cells were maintained in the presence or absence of IBT (10 μg/ml) and labelled at the times indicated with \(^{14}C\)-protein hydrolysate (5 μCi/ml) for 15 min. Densitometer tracings were prepared from autoradiograms of dried gels and the peaks were cut out and weighed. The amounts of each polypeptide are plotted in arbitrary units. (a) Polypeptide 25K (pre-replicative): • •, no IBT; ○ — ○, IBT. (b) Polypeptide 28K (early post-replicative): • — •, no IBT; ○ — ○, IBT. Polypeptide P4a (late post-replicative): △ — △, no IBT; △ — △, IBT.

Quantitative analysis of the kinetics of synthesis of a prominent member of this class, polypeptide 25K, showed that the drug did not affect the timing of onset and cessation of synthesis; the relative rates of synthesis at various times after infection were also unaffected by the drug (Fig. 1).

Post-replicative polypeptides can be divided into two main classes, early and late, the division depending on the time of their appearance during infection (Pennington, 1974). IBT had no effect on the kinetics of synthesis of early post-replicative polypeptides; quantitative results obtained with a prominent member of this class, polypeptide 28K, are shown in Fig. 1. The onset of synthesis of late post-replicative polypeptides occurred with normal timing in the presence of IBT (Fig. 1 and 2). However, the rate of synthesis of these polypeptides declined rapidly in drug-treated cells (Fig. 1); late in infection total protein synthesis in these cells was reduced to very low levels (Fig. 1 and 2). No decline in protein synthesis occurred in uninfected cells exposed to IBT for similar periods (results not shown).

The possibility that the inhibitory effect of IBT required prolonged drug treatment of
infected cells was investigated by adding IBT (10 μg/ml) to cells at various times after infection. The drug was added at hourly intervals between 2 h and 6 h post-infection, and cells were labelled at 9 h post-infection. A profound inhibition of virus-induced polypeptide synthesis was observed in all drug-treated samples (results not shown). These results show that prolonged exposure of infected cells to IBT is not required for manifestation of its inhibitory effect on virus polypeptide synthesis and indicate that the action of the drug is not mediated by an effect on early events in the virus growth cycle.

Pre-replicative polypeptide synthesis persists for long periods in infected cells treated with inhibitors of DNA synthesis (Pennington, 1974). The effect of IBT on polypeptide synthesis in such cells was investigated. Cells were infected and maintained in cytosine
arabinoside (25 μg/ml) for 9 h in the presence or absence of IBT (10 μg/ml) and were then labelled. IBT had no effect on virus polypeptide synthesis under these conditions; identical amounts of pre-replicative polypeptides were synthesized in IBT-treated and control cells (results not shown). These results show that post-replicative polypeptide synthesis is required for manifestation of the inhibitory effect of the drug, and again demonstrate that prolonged exposure of infected cells to the drug is not in itself sufficient to produce this effect.

These results confirm and extend those of Appleyard et al. (1965), Woodson & Joklik (1965), Joklik et al. (1967) and Katz et al. (1973a), and demonstrate clearly that IBT inhibits virus growth by interfering with the production of virus-induced late post-replicative polypeptides. This class of virus polypeptide contains many structural proteins and their precursors, polypeptides P4a and P4b, for example, being precursors of the two major virion structural proteins (Moss & Rosenblum, 1973). The premature cessation of synthesis of these proteins readily explains the thiosemicarbazone-induced defects in virion assembly described by Easterbrook (1962) and by Woodson & Joklik (1965).

The molecular basis of the effect described here is unknown. The isolation of thiosemicarbazone resistant and dependent mutants (Appleyard & Way, 1966; Katz et al. 1973b) supports the hypothesis that the effect is mediated through a virus coded macromolecule, probably a polypeptide. As the effect is only manifested after the onset of late post-replicative polypeptide synthesis, it is not unreasonable to speculate that the drug interacts with one of these polypeptides to produce a general inhibition of virus protein synthesis. Joklik et al. (1967) have suggested that IBT may switch off virus protein synthesis by interacting with a protein which, under normal conditions, selectively abolishes the function of early mRNA. These workers postulated that this interaction alters the protein in such a way that it now prevents all mRNAs from functioning. The data presented here suggests that a possible candidate for such a protein is one which is responsible for the switch-off of early post-replicative polypeptide synthesis, since this event occurs not long after the onset of late post-replicative polypeptide synthesis at about the time when the effect of IBT becomes manifest.

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REFERENCES


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