Induction of a Host-specific Chromatin-associated Glycopolypeptide by
Human Cytomegalovirus

By DAGMAR WEDER AND KLAUS D. RADSACK*
Zentrum für Hygiene und Medizinische Mikrobiologie der Universität Marburg, Pilgrimstein 2,
3550 Marburg, Federal Republic of Germany

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SUMMARY

Analysis of chromatin preparations from \[^{3}H\]glucosamine-labelled human foreskin
fibroblasts revealed that a chromatin-associated glycopolypeptide with the approxi-
mate mol. wt. 130000 (130K) is induced in response to either infection with human
cytomegalovirus (HCMV) or serum treatment. Comparative limited proteolysis
suggested that the \[^{3}H\]glucosamine-labelled 130K polypeptides induced by these
different stimuli were not identical. This observation was in contrast to results obtained
by immunoprecipitation with antisera raised against the 130K glycopolypeptide from
serum-induced cells which favoured a relatedness to the 130K polypeptide from virus-
infected cultures. Two-dimensional separation by isoelectric focusing and SDS-
polyacrylamide gel electrophoresis subsequently showed that the 130K glycopolypep-
tide from serum-induced cells consists of two components, one of which is identical to
the single component observed in samples from HCMV-infected cultures. Experiments
on the effect of glycosylation inhibitors on DNA replication in HCMV-infected as well
as in serum-induced cells support the view that the host-specific chromatin-associated
glycopolypeptide may be involved in DNA replication in infected cells.

INTRODUCTION

There is ample information that inhibition of virus multiplication by sugar analogues or
tunicamycin is primarily due to interference by these drugs with the proper synthesis and
assembly of envelope glycoproteins necessary for virus infectivity (Courtney et al., 1973;
Gallagher et al., 1973; Scholtissek, 1975). On the other hand, glycosylation inhibitors may also
exhibit inhibitory activity during the early phase of virus multiplication, as in the case of adeno-
associated virus (AAV), where evidence has been presented that 2-deoxy-D-glucose (dGlc)
eliminates the function of an 'early' viral glycopolypeptide of AAV and thus prevents initiation
of viral DNA replication (Young & Mayor, 1979). Inhibition of viral DNA synthesis in the
presence of glycosylation inhibitors has recently been reported for a second system, human
fibroblasts infected by cytomegalovirus (HCMV; Radsak & Weder, 1981 a). It is of interest in
this context that two glycopolypeptides have been identified among the 'early' HCMV-induced
proteins (Stinski, 1977). Data on the subcellular distribution, source (i.e. virus-coded or host-
specific) and function of these glycosylated polypeptides were not described in this study. With
respect to subcellular distribution, previous experiments with HCMV-infected cells in this
laboratory had suggested that products whose induction is sensitive to glycosylation inhibitors
may separate with chromatin upon cell fractionation (Radsak & Weder, 1981 a). Here we
present evidence that one 'early' HCMV-induced glycopolypeptide found in association with
chromatin preparations is host-specific and may function in DNA replication in infected cells.
METHODS

Cells and virus. Primary human foreskin fibroblasts (HFF; a generous gift of Dr B. Fleckenstein, Erlangen, F.R.G.) were used between the 12th and 22nd passage for all experiments and for propagation of HCMV (strain Towne; Furukawa et al., 1973). Cells were cultivated in minimal essential medium (MEM, Gibco) with Earle's salt solution supplemented with 50 µg gentamicin/ml and 10% foetal calf serum (FCS). For experimental use cultures were partially arrested by serum starvation (0-2% FCS for 72 h) beginning, on average, 3 days after the cultures reached confluency (DeMarchi & Kaplan, 1977; Radsak & Weder, 1981a). For experimental infection with stocks of HCMV (Radsak & Schmitz, 1980) an m.o.i. of approx. 3 was used. Under these conditions about 90% of the cells contained viral antigen (Fioretti et al., 1973; Radsak & Schmitz, 1980). For heat inactivation adequate dilutions of HCMV stock were incubated for 30 min at 56 °C (St. Jeor et al., 1974). Mock-infection was carried out either with used culture medium or serum-free fresh medium, both procedures yielding identical results. Virus titres were determined by the endpoint dilution method combined with indirect immunofluorescence for virus antigen (Radsak & Schmitz, 1980). For serum stimulation partially arrested cultures were fed with MEM plus 15% FCS. When dGlc was used as an inhibitor or during [3H]glucosamine labelling, glucose in MEM was replaced by 10 mM-sodium pyruvate (Scholtissek, 1975; Radsak & Weder, 1981a).

Isotopic labelling of cells. For pulse labelling of induced cultures [3H]thymidine (sp. act. 79 Ci/mmol), a combination of [3H]leucine and [3H]lysine (sp. act. 59 and 74 Ci/mmol respectively) or [3H]glucosamine hydrochloride (sp. act. 32 Ci/mmol) were included in the culture medium at the concentrations given in Results. All radiochemicals were purchased from Amersham Buchler.

Cell fractionation and incubation of chromatin. Most of the cell fractionations, in particular those for preparation of biologically active chromatin, were carried out according to the method of Yamada et al. (1978) as published previously (Radsak & Weder, 1981a). Chromatin preparations contained on average 50 µg DNA/10^6 cells; the protein : DNA ratio was in the range of 5:1. The standard assay for cell-free precursor incorporation by chromatin (Radsak et al., 1980; Radsak & Weder, 1981a) contained 50 mM-Tris–HCl pH 8, 5 mM-MgCl_2, 2.5 mM-dithiothreitol, 0.25 mM-ATP, 0.1 mM each of dATP, dGTP, dCTP, 0.001 mM-[3H]TTP at a specific activity of 1000 ct/min/pmol and chromatin equivalent to about 8 to 10 µg DNA in a total volume of 0.1 ml. Incubation was for 30 min at 37 °C prior to addition of 1 ml cold trichloroacetic acid (TCA) plus 50 mM-sodium pyrophosphate and determination of acid-insoluble radioactivity. Incorporation was calculated from the mean of acid-insoluble radioactivity of duplicate assays. For analysis of chromatin-associated polypeptides in some experiments, chromatin was isolated using the method of Rizzo & Bustin (1977) which yields chromatin not active in in vitro incorporation.

Polyacrylamide gel electrophoresis (PAGE) and fluorography. Analytical PAGE was done in 8%, 10% or 7 to 20% polyacrylamide slab gels in the presence of SDS according to the method of Laemmli (1970) as described by Gallwitz et al. (1978). If quantification of protein in the gel was needed slabs were scanned in a Quick scan densitometer (DESAGA, Heidelberg, F.R.G.) after staining with Coomassie Brilliant Blue, using tracks with known amounts of bovine serum albumin as a standard. Fluorography was performed following the procedure of Bonner & Laskey (1974) using Rotifluoroszint (Roth, Karlsruhe, F.R.G.) for impregnation.

Two-dimensional separation (2D-PAGE) of chromatin extracts by isoelectric focusing and SDS–PAGE. Chromatin extracts were prepared for 2D-PAGE by incubation of chromatin from 2 × 10^7 cells with 0.5 ml solubilization buffer (see below under 'Immunoprecipitation') for 20 min at 4 °C and subsequent sedimentation of insoluble material. Separation in the first dimension consisted of isoelectric focusing with ampholines in the range of pH 3 to 10, and in the second dimension of SDS–PAGE (10% polyacrylamide) exactly as described by O'Farrell (1975).

Electrophoretic elution of individual polypeptides from stained gels. For isolation of individual chromatin-associated polypeptides gel slabs were briefly stained after SDS–PAGE of a chromatin fraction, destained, the band of interest cut from the gel slab and loaded onto short cylindrical gels of 4-10% polyacrylamide in glass tubes. This was followed by electrophoretic elution at 3 to 5 mA per tube overnight into a dialysis bag attached to the tubes (Cleveland et al., 1977). The eluate was either used for immunization of rabbits, or concentrated by addition of TCA to a final concentration of 20% (Cleveland et al., 1977) if the polypeptide was to be subjected to analysis by partial proteolysis.

Antiserum. For production of antiserum against the 130000 mol. wt. (130K) polypeptide from serum-induced cultures, three rabbits were immunized by a schedule of 10 subcutaneous injections of approx. 50 µg per animal of SDS–PAGE-purified polypeptide combined with an equal volume of Freund’s complete adjuvant. Preimmune sera were obtained from the same rabbits prior to immunization. Antisera against ‘early’ HCMV-induced antigens were from individuals shown to be positive by indirect immunofluorescence (Fioretti et al., 1973) on HCMV-infected HFF treated with phosphonoacetic acid (PAA; 100 µg/ml for 24 h).

Immunoprecipitation. Immunoprecipitation was performed essentially as described by Blanton & Tevethia (1981). Cell fractions to be used were mixed with an equal volume of solubilization buffer [40 mM-Tris–HCl pH 9, 0.6 M-NaCl, 20% (v/v) glycerol 2 mM-CaCl_2, 1 mM-MgCl_2, 4 mM-EDTA, 1% (v/v) Nonidet P40 (NP40)] and incubated at 4 °C for 20 min. For pre-clearing, rabbit preimmune serum or, if human sera were to be used...
subsequently, human serum negative for 'early' HCMV-induced antigens were added to the mixture at a final concn. of 5% and incubated for 90 min at 4°C prior to addition of 100 μl/ml Immunoprecipitin (Bethesda Research Laboratories). Agitation of the suspension for 15 min at room temperature was followed by sedimentation in a Beckman model B microfuge, and incubation of the precleared extracts overnight at 4°C with 20 to 50 μl/ml specific antisemur. Immune complexes were analysed by SDS–PAGE and fluorography after adsorption on Immunoprecipitin as described for the preclearing step and four subsequent washings with 50 mM-Tris–HCl pH 7.4, 0.5 M-LiCl, 0.1 M-NaCl and 1% (v/v) NP40.

Partial proteolysis. TCA-precipitated polypeptides obtained after electrophoretic elution from gel slices were partially digested with various amounts of V8 protease from Staphylococcus aureus (Miles Laboratories) during SDS–PAGE exactly as described by Cleveland et al. (1977). After SDS–PAGE, gel slabs were processed for fluorography.

Determination of radioactivity, protein and DNA content. Acid-insoluble radioactivity was determined by collecting TCA precipitates on filters as described previously (Radsak & Weder, 1981a). DNA was extracted by phenol–chloroform (Hirai & Watanabe, 1976) before TCA precipitation. Protein content was estimated by the method of Lowry et al. (1951), and DNA content by the procedure of Giles & Myers (1965).

RESULTS

Identification of glycopolypeptides in chromatin preparations from HCMV- or serum-induced HFF

In order to show the presence of glycopolypeptides in chromatin preparations of HFF, confluent cultures (2 × 10^7 cells) of serum-starved (DeMarchi & Kaplan, 1977; Radsak & Weder, 1981a) cells were exposed to HCMV infection in the presence of PAA and 0.2% serum. In addition to mock-infected cultures, serum-starved cells stimulated by 15% serum were used in a parallel experiment as controls. Untreated HCMV-infected cultures were not examined in this context as late viral functions which include numerous glycopolypeptides would have complicated this initial analysis and are unlikely to participate in early regulatory events of DNA replication in infected cells. For glycopolypeptide labelling 5 μCi/ml [3H]glucosamine was added to the culture medium from 36 to 48 h after onset of the experiment prior to harvest and cell fractionation (Yamada et al., 1978). The proteins were solubilized from the chromatin fraction (see legend to Fig. 1 and Methods) and analysed by SDS–PAGE (Laemmli, 1970) and subsequent fluorography (Bonner & Laskey, 1974). Fig. 1 shows that glycopolypeptides were present under our experimental conditions in all chromatin preparations examined. The main band of radioactivity in each track appeared in the range of mol. wt. 130K. In addition, bands containing minor amounts of radioactivity with higher as well as lower electrophoretic mobilities were also observed. In chromatin extracts from serum-induced cells (Fig. 1c) those of higher molecular weight were more prominent, whereas extracts of chromatin from PAA-treated HCMV-infected cultures (Fig. 1b) consistently exhibited relatively strong diffuse radioactivity in the 90000 to 95000 mol. wt. range which could not be detected in the other samples.

Induction of glycosylation of chromatin-associated polypeptides by 'early' functions of HCMV or serum

Following a comparable experimental schedule to that described, pulse labelling with 5 μCi/ml [3H]glucosamine for 12 h intervals (see legend to Fig. 2) was used to study the temporal relationship of precursor incorporation to onset of HCMV infection or serum treatment. The fluorogram in Fig. 2 shows a representative result: mock-infected controls (Fig. 2, tracks a' to c') exhibited an unchanged pattern of radioactive bands with decreasing intensity during the second and third pulses. HCMV infection and serum treatment (Fig. 2, tracks d' to f' and g' to j', respectively), on the other hand, resulted after initial depression during the first pulse in induction of glycosylation of the prominent 130K polypeptide during the second pulse interval (Fig. 2, tracks e' and h'). Enhanced labelling of this polypeptide which evidently persisted after serum stimulation during the consecutive pulses was, however, found to decline beyond 48 h in HCMV-infected preparations (Fig. 2, tracks f', i' and j'). A further difference between the two stimuli is obvious with regard to the kinetics of labelling of the glycopolypeptides in the 150000
Fig. 1. Fluorogram of SDS–polyacrylamide gel (8% polyacrylamide) of chromatin extracts from (a) mock-infected, (b) PAA-treated HCMV-infected, and (c) serum-induced HFF labelled with 5 μCi/ml [3H]glucosamine from 36 to 48 h post-infection following serum addition prior to harvest at 48 h. Extracts were prepared by mixing chromatin with an equal volume of solubilization buffer, subsequent incubation and pelleting of insoluble material (see Methods). Approx. 5 μg of protein was analysed in each track.

Effect of dGlc on synthesis of chromatin-associated polypeptides

The following experiments served to (i) clarify whether induction of [3H]glucosamine incorporation was paralleled by induction of polypeptide synthesis and (ii) trace the influence of dGlc on synthesis of the glycopolypeptides. The identical experimental sequence as above was thus repeated using [3H]leucine and [3H]lysine (2.5 μCi of each/ml) for pulse labelling at various times post-infection or after serum addition respectively (Fig. 3). One set each of HCMV-infected and serum-treated cultures were kept in the presence of dGlc. Identification of the 130K polypeptide during electrophoretic analysis was attempted by co-electrophoresis of [3H]glucosamine (Fig. 3, tracks b) and 3H-amino acid-labelled (Fig. 3, tracks a) samples.

It is evident from Fig. 3(A, tracks a) that under our conditions synthesis in PAA-treated infected cells of a 130K polypeptide concomitant to its glycosylation is only observed during the first pulse. Subsequently these two events are dissociated, in particular during the second pulse when glycosylation is increased (Fig. 3A, track P2, b) and the 3H-amino acid label is almost completely absent in the identical position of Fig. 3 (A, track P2, a). Serum treatment (Fig. 3 B),

to 200000 mol. wt. range. HCMV infection apparently rapidly inhibits glycosylation of these high molecular weight polypeptides whereas serum treatment results only in an eventual decrease. With respect to the low molecular weight polypeptides kinetic variations were inconsistent and are not considered here.
Fig. 2. (a to j) Stained polyacrylamide slab gel (gradient of 7 to 20% polyacrylamide) and (a' to f) corresponding fluorogram of chromatin preparations from (a to c) mock-infected, (d to f) PAA-treated HCMV-infected and (g to j) serum-induced HFF pulse-labelled with 5 μCi/ml [3H]glucosamine from 12 to 24 h post-infection (a, d, g, and a', d', g'), 36 to 48 h post-infection (b, e, h and b', e', h'), 60 to 72 h post-infection or following serum addition (c, f, i and c', f', j') and 84 to 96 h following serum addition (j and f). As evidenced by the stained slab gel (a to j) care was taken to analyse equal amounts of protein (50 μg ± 10%) from the various chromatin preparations in order to obtain an estimate of the specific radioactivity of individual polypeptides. M, Mol. wt. markers: phosphorylase b, 94000; bovine serum albumin, 68000; ovalbumin, 43000; carbonic anhydrase, 30000; trypsin inhibitor, 20100; α-lactalbumin, 14400.
Fig. 3. Fluorograms of SDS-polyacrylamide gels of chromatin preparations from (A) mock-infected (co), and PAA-treated HCMV-infected (10% polyacrylamide), and (B) serum-induced (gradient of 7 to 20% polyacrylamide) HFF, pulse-labelled with 2.5 µCi/ml each of [3H]leucine and [3H]lysine (a) or with 5 µCi/ml [3H]glucosamine (b) from 12 to 24 h (P1), 36 to 48 h (P2, co) and 60 to 72 h (P3) post-infection or following serum addition. Again care was taken to analyse equal amounts of protein in each track (see legend to Fig. 2).

on the other hand, results in both induction of glycosylation as well as synthesis of the 130K polypeptide. The increase of 3H-amino acid label during the second and third pulse (Fig. 3B, tracks a of P2, P3) is, however, much less pronounced than that of [3H]glucosamine label (Fig. 3B, tracks b of P2, P3).

Evaluation of the fluorograms of preparations from cultures treated with dGlc (3 to 5 mM) which inhibits cellular glycosylation nearly completely as measured by [3H]glucosamine incorporation (not shown) yielded the following observations. The most obvious effect of dGlc on both HCMV-infected as well as serum-stimulated cells is the prominent induction of synthesis of a polypeptide with approx. mol. wt. 80000 (80K) which is labelled with about equal intensity during all three pulses (Fig. 4A and B, tracks P1 to P3). Furthermore, it is apparent in Fig. 4(B, tracks P1 to P3), representing serum-stimulated cells, that radioactivity in the 130K position is selectively reduced after dGlc treatment. In the case of chromatin from dGlc-treated HCMV-infected cells a comparable loss of label in this molecular weight range is not observed (Fig. 4A, tracks P1 to P3).

By comparing the pattern of chromatin-associated polypeptides from drug-treated infected cells labelled at late times post-infection (Fig. 4A, dGlc P3) with that from PAA-treated and untreated infected cells (Fig. 4A, E and L respectively) it becomes evident that dGlc is apparently effective in preventing synthesis of late viral proteins. This observation is in agreement with the inhibitory effect of dGlc on HCMV-induced DNA replication (Radsak &
Weder, 1981 a). The sugar derivative does, however, allow expression of most of the ‘early’ virus-induced polypeptides that are distinguishable in chromatin preparations of PAA-treated cells (Fig. 4A, tracks dGlc P3 and E respectively).

**Relatedness of the 130K glycopolypeptides from HCMV-infected and serum-stimulated cultures**

The induction of glycosylation of the 130K chromatin-associated polypeptides by HCMV as well as serum prompted experiments to examine their relatedness, i.e. to determine whether the 130K glycopolypeptide from HCMV-infected cultures represents an ‘early’ virus-specific or virus-induced host-specific product. Three methods, comparative limited proteolysis (Cleveland et al., 1977), immunoprecipitation (Blanton & Tevethia, 1981) with antisera of various specificities and two-dimensional separation by isoelectric focusing and SDS–PAGE (2D-PAGE; O’Farrell, 1975) were employed in an attempt to solve this question.

For limited proteolysis the 130K glycopolypeptides were isolated by electrophoretic elution from SDS–slab gels of chromatin extracts from [3H]glucosamine-labelled induced cells (pulse 2 in most of the experiments) and subjected to treatment with V8 protease. The fluorogram in Fig. 5 demonstrates that partial digestion yielded patterns for the glycopolypeptide from serum-stimulated cells that were significantly different from those for the polypeptide from infected cultures, inferring that the latter may be virus-specific.

Contrasting results were obtained during immunoprecipitations using sera of various specificities. In addition to human sera, known to be positive for ‘early’ viral antigens, an
Fig. 5. Fluorogram of SDS-polyacrylamide gel (15% polyacrylamide) of partial proteolysis by 0.5 μg (a) and 0.125 μg (b) V8 protease of isolated 130K glycopolypeptides (w/o) from PAA-treated HCMV-infected and serum-induced HFF pulse-labelled with 5 μCi/ml [3H]glucosamine from 36 to 48 h post-infection or following serum addition respectively. M, Mol. wt. markers: globulin, 150000; phosphorylase b, 92000; bovine serum albumin, 68000; carbonic anhydrase, 30000; cytochrome c, 12300.

antibody raised in rabbits against the 130K glycoprotein isolated from chromatin preparations of serum-stimulated cultures by preparative SDS-PAGE and electrophoretic elution from gel slices (see Methods) was used. This latter antiserum specifically immunoprecipitated the [3H]glucosamine-labelled 130K polypeptide from chromatin extracts of serum-induced as well as that from HCMV-infected cultures (Fig. 6) as revealed by subsequent SDS-PAGE of the precipitates and fluorography. The assays resulted consistently in precipitation also of polypeptides with molecular weights higher than 130K (Fig. 6), an observation whose significance is unclear at present.

In contrast, all attempts with antisera specific for 'early' viral antigens remained negative when chromatin extracts of [3H]glucosamine-labelled infected cells were examined. These antisera did, however, recognize virus-specific polypeptides in cytoplasmic extracts from 3H-amino acid-labelled infected cells (not shown). Analysis by 2D-PAGE subsequently solved the discordant results observed with comparative limited proteolysis and immunoprecipitation. As shown in Fig. 7 the 130K glycoprotein in chromatin extracts from [3H]glucosamine-labelled serum-induced cultures evidently consists of two main components in the appropriate molecular weight range, whereas only one main component in the corresponding position is detected in extracts from infected cells. The more acidic polypeptides in samples from seruminfected cultures appears to be identical with the single virus-induced glycoprotein.
**HCMV-induced chromatin-associated glycoprotein**

Fig. 6. Fluorogram of SDS–polyacrylamide gel (8% polyacrylamide) of chromatin extracts (extr) (see legend to Fig. 1) and the corresponding immunoprecipitates with anti-130K polypeptide (ipp) from mock-infected (a and a'), PAA-treated HCMV-infected (b, b') and serum-induced (c, c') HFF pulse-labelled with 5 µCi/ml [3H]glucosamine from 36 to 48 h post-infection or following serum addition.

**Effect of dGlc on induction of DNA replication by HCMV or serum**

We have recently shown that glycosylation inhibitors like dGlc and tunicamycin prevent HCMV-induced DNA replication in infected cells (Radsak & Weder, 1981a). Toxicity of the drug and effects via altered nucleotide pools (Scholtissek, 1975) could essentially be excluded (Radsak & Weder, 1981a). Furthermore, chromatin preparations of HCMV-infected cells which have been shown previously to continue DNA replication under cell-free conditions (Radsak et al., 1980) exhibited significantly reduced endogenous replication activity when isolated from dGlc-treated infected cells (Radsak & Weder, 1981a), an observation which favours the view of an association of 'dGlc-sensitive factors' with chromatin. Here we present evidence that a chromatin-associated glycopolyptide is indeed induced by 'early' functions of HCMV concomitant to induction of DNA replication in infected cells (Radsak & Weder, 1981a,b). To substantiate the function of this glycosylated polypeptide in DNA replication, the action of dGlc on DNA synthesis, by serum-starved cells stimulated by serum, was examined. Table 1 shows drug-mediated prevention of induction of DNA replication in this non-viral model. Delayed addition of dGlc is effective until 12 h after onset of stimulation (Table 1, line 9).
suggesting a sensitivity somewhat different from that of HCMV-infected cultures (Radsak & Weder, 1981a). When dGlc is added at later times enhancement of precursor incorporation is observed (Table 1, line 11) a phenomenon which has to remain unexplained at present. Chromatin preparations isolated from cultures treated with serum in the presence of the drug again are essentially inactive, which is in line with the results for chromatin from HCMV-infected cells (Table 2; Radsak & Weder, 1981a). Secondly, in a more direct approach to the function of the 130K glycopolypeptide, chromatin assays were performed in the presence of antisera raised against the 130K polypeptide from serum-induced cells. Both chromatin preparations from HCMV-infected and those from serum-stimulated cultures were consistently partially inhibited in comparison to the appropriate controls with preimmune serum (Table 2).
Table 2. Precursor incorporation by chromatin preparations*

<table>
<thead>
<tr>
<th>Induction of DNA synthesis by</th>
<th>Inhibitor treatment of induced cultures</th>
<th>In vitro addition of antisera†</th>
<th>[3H]TTP incorporated (pmol) by chromatin preparations‡</th>
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<tr>
<td>Expt. 1, HCMV, infectious</td>
<td>–</td>
<td>–</td>
<td>6-50</td>
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<tr>
<td>HCMV, heat-inactivated</td>
<td>–</td>
<td>–</td>
<td>0-19</td>
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<tr>
<td>HCMV, infectious</td>
<td>100 μg PAA/ml</td>
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<td>3-77</td>
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<td>–</td>
<td>0-66</td>
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<tr>
<td>Expt. 2, HCMV, infectious</td>
<td>–</td>
<td>–</td>
<td>14-06</td>
</tr>
<tr>
<td>Mock-infection</td>
<td>5 mM-dGlc</td>
<td>–</td>
<td>0-17</td>
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<tr>
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<td>2-26</td>
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<tr>
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<td>2-50</td>
</tr>
<tr>
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<td>2-40</td>
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<tr>
<td>Expt. 4, 0.2% FCS</td>
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<td>–</td>
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<td>–</td>
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<td>6-80</td>
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<tr>
<td>15% FCS</td>
<td>5 mM-dGlc</td>
<td>–</td>
<td>0-92</td>
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<td>15% FCS</td>
<td>–</td>
<td>Anti-130K-2, 20 μl</td>
<td>1-70</td>
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</table>

* Isolation of chromatin was performed at 48 h post-infection or serum stimulation respectively. Inhibitor treatment was initiated immediately post-infection or onset of serum stimulation. When dGlc was used as an inhibitor glucose was replaced by sodium pyruvate in the culture medium.
† Chromatin preparations were incubated with antisera for 30 min at 4 °C prior to use in the assay.
‡ Conditions of incubation are described in Methods.

DISCUSSION

It has been shown recently that expression of HCMV antigens like that of herpes simplex virus is regulated by the sequential function of virus-specific ‘immediate early’ (IE), ‘early’ (E) and ‘late’ products (DeMarchi et al., 1980; Wathen et al., 1981). With regard to the antigens expressed at IE and E times post-infection a minimum of nine and ten polypeptides respectively, have been identified by in vitro translation of viral mRNA (Wathen et al., 1981). Analysis of infected cells had previously yielded the additional observation that two of the ‘early’ virus-induced polypeptides with mol. wt. 145K and 100K are modified post-translation by glycosylation (Stinski, 1977). Interestingly, no candidate correlate has been found, at least for the large polypeptide, in the in vitro translation studies reported (Wathen et al., 1981). The chromatin-associated glycopoly peptide discussed in this study exhibits a molecular weight somewhat different from that of the large virus-induced glycopoly peptide described by Stinski (1977). Considering, however, the difference in conditions used, e.g. cell cultures, virus strain and gel systems, as well as the fact that analysis of cell fractions other than chromatin has not yielded evidence for an additional virus-induced ‘early’ glycopoly peptide in that molecular weight range (D. Weder & K. D. Radsak, unpublished observation) we tend to believe that the 130K glycopoly peptide corresponds to the 145K product previously described (Stinski, 1977). With regard to our results from immunoprecipitations with antisera against the 130K polypeptide and against ‘early’ antigens of HCMV as well as to those obtained by comparative two-dimensional analysis it is suggested that the larger glycopoly peptide induced by HCMV under our conditions is a host-specific chromatin-associated product. The glycopoly peptide of 100K described by Stinski (1977) does not clearly separate with the chromatin fraction in our hands and will not be considered here.

Further work is needed to clarify why only the induction of glycosylation could be demonstrated but not the incorporation of amino acid into the 130K polypeptide of HCMV-infected cells. Furthermore, it is unclear at this point why serum stimulation results in induction of an additional glycosylated 130K component. It may be speculated that the more alkaline component represents a differently glycosylated form of the same polypeptide.
As to the function of the 130K glycopolypeptide, several arguments are thought to favour the view that this product is involved in DNA replication: the 130K glycopolypeptide is chromatin-associated and is induced in temporal relation to cellular DNA replication (Hirai & Watanabe, 1976; Radsak & Weder, 1981). Induced cultures treated with low concentrations of glycosylation inhibitors do not proceed to DNA replication and yield chromatin with significantly reduced in vitro replication activity (Radsak & Weder, 1981a). It is pertinent to point out in this context that dGlc does not apparently interfere with expression of 'early' virus-induced chromatin-associated polypeptides other than glycopolypeptides. The prominent induction of an 80K polypeptide in the presence of dGlc has to remain unexplained at this point.

Further, more direct support for involvement of the 130K glycopolypeptide in cellular DNA replication comes from the use of the anti-130K sera in chromatin assays which produced only partial but consistent reduction in precursor uptake.

In conclusion, our results suggest that IE or E functions of HCMV are effective in inducing glycosylation of a host-specific 130K polypeptide which is essential for DNA replication. Preliminary experiments with our anti-130K serum do not support the assumption that the 130K polypeptide is a host cell DNA polymerase. Work is in progress to define the precise action of this cellular glycopolypeptide which possibly mediates stimulation of host cell DNA replication as a prerequisite for HCMV multiplication.

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REFERENCES


HCMV-induced chromatin-associated glycoprotein


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