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STUDIES ON THE POLYPEPTIDES OF VARICELLA-ZOSTER (V-Z) VIRUS
II. SYNTHESES OF VIRAL POLYPEPTIDES IN INFECTED CELLS

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SUMMARY Synthesis of varicella-zoster virus (VZV)-induced polypeptide was examined at intervals after infection by immunoprecipitation, SDS-polyacrylamide gel electrophoresis (PAGE) and fluorography. Human embryonic lung (HEL) cells were inoculated with VZV-infected cells at a ratio of about 1 infected cell to 5 uninfected cells and pulse-labelled with ^{35}S-methionine or ^{14}C-glucosamine. Under this experimental condition, virus-induced protein and glycoprotein began to appear 4 to 6 and 18 to 20 h after infection, respectively, and a capsid protein (MW 145,000) was prominent 18 h after infection. The synthesis of these proteins reached maxima at 46 to 48 h after infection. In all, 34 polypeptides and 10 to 13 glycoproteins with molecular weight of 145,000 to 22,000 and 115,000 to 38,000, respectively, were detected. A few polypeptides (MW 130,000, 95,000, 72,000) were detected in infected cells treated with phosphonoacetic acid (PAA) at an early stage of infection.

Proteins and glycoproteins of virion of VZV were analyzed by immunoprecipitation with antisera against partially purified virion of VZV. At least 12 polypeptides with MW of 145,000 to 35,000 and 7 glycoproteins (MW 105,000, 90,000, 84,000, 72,000, 70,000, 60,000 and 56,000) were identified. A polypeptide with a molecular weight of 145,000 was considered to be the major capsid protein.

INTRODUCTION

Infection of cells with VZV at high multiplicities is at present impossible because it is not possible to obtain high doses of infectious VZ cell-free virus, and little is known about synthesis of VZV protein and glycoprotein in infected cells. In a previous study, we detected 33 polypeptides and 13 glycoproteins in infected cells at a late stage of infection by immunoprecipitation with hyperimmune monkey or guinea pig serum followed by SDS-PAGE and fluorography (Asano and Takahashi, 1979). For more detailed study of VZV proteins and glycoproteins, we pulse-labelled the infected cells with radioactive pre-

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cursors at various times after infection and analyzed them by the same methods. In the present study also, we inoculated HEL cells with VZV at a ratio of about 1 infected cell to 5 uninfected cells, which is reported to give the maximal yield of cell-free infectious virus at a later stage of infection (Schmidt and Lennette, 1976).

We attempted also to identify the proteins and glycoproteins of virion of VZV. We prepared antiserum against partially purified virions, then treated lysates of infected cells highly labelled with ^{35}S-methionine or ^{14}C-glucosamine with this antiserum and analyzed the resulting immunoprecipitates by SDS-PAGE and fluorography.

MATERIALS AND METHODS

1. *Cell culture*

Monolayer cultures of human embryonic lung (HEL) cells at the 3rd to 10th passage level were grown in a mixture of equal volumes of Eagle's MEM and medium 199 supplemented with 10% fetal calf serum (FCS) in Falcon 100 mm dishes. Cultures were maintained in a similar medium but with 3% FCS.

2. *Virus*

The Oka strain of varicella virus was used in this study (Takahashi et al., 1975).

3. *Partially purified virion*

The method used was essentially that of Martin and Palmer (1973). Monolayers of HEL cell were infected with VZV. When extensive CPE had appeared, the infected cells were collected with trypsinization, washed three times with phosphate buffered saline (PBS), sonicated for 3 min at 20 kc/sec and then centrifuged at 3,000 rpm for 15 min. The supernatant was harvested, layered onto a cushion of 30% sucrose and centrifuged at 30,000 rpm for 2 h. The resulting pellet was resuspended in PBS, layered onto linear 30 to 50% glycerol-potassium tartrate viscosity-density gradients and centrifuged at 39,000 rpm for 18 h. Two bands were visible in the gradients. Each band was collected and examined by electron microscopy. The upper hazy band appeared to consist of homogeneous membranelike material with no viral particles. The lower, stringy band was composed of enveloped particles and a few nucleocapsid. The latter material was dialyzed against PBS and used as partially purified virions.

4. *Antisera*

Partially purified virions were injected into guinea-pigs with an equal volume of Freund's adjuvant twice with an interval of 1 month. Blood was collected 10 days after second injection. Hyperimmune monkey or guinea pig antiserum against VZV was prepared as described previously (Asano and Takahashi, 1979).

5. *Radioactive labelling of infected or mock-infected cells*

Monolayer HEL cells were inoculated with cells infected with the Oka strain of varicella virus at a ratio of about 1 infected cell to 5 uninfected cells. After adsorption for 2 h at 37°C, the cells were washed 3 times with PBS and incubated at 37°C in the presence or absence of phosphonoacetic acid (PAA; ICN Pharmaceuticals, Inc., Plainview, NY) at a concentration of 200 μg/ml, and labelled with 10 μCi/ml of ^{35}S-methionine (1220 mCi/m mole, Amersham) or 6 μCi/ml of ^{14}C-glucosamine (318 mCi/m mole, Amersham) at the indicated times.

Labelled cells were solubilized in TD buffer (1% Triton X-100, 0.5% sodium deoxycholate, 0.15 *M* NaCl, 1 m*M* phenylmethyl-sulfonylfluoride, 10 m*M* sodium phosphate buffer, pH 7.2) and the lysate was centrifuged at 30,000 rpm for 2 h at 4°C. The resulting supernatant fluid (TD-lysate) was used for immunoprecipitation.

6. *Immunoprecipitation*

The method used was described previously (Asano and Takahashi, 1979). The TD-lysate was mixed with antiserum and reincubated with a suspension of *Staphylococcus aureus* (Cowan I strain). The mixture was centrifuged at 5,000 rpm for 6 min and the immunoprecipitate was washed three times with TD buffer and once with 10 m*M* sodium phosphate buffer, pH 7.2. The immune complex was separated from the bacterial adsorbent by heating the resulting pellet in "sample buffer" for SDS-PAGE (1% SDS, 1% 2-mercaptoethanol, 10% glycerol, 0.001% phenol red, 50 m*M* Tris-HCl buffer, pH 8.2) for 3 min at 100°C, and the mixture

was centrifuged at 10,000 rpm for 5 min. The supernatant was subjected to SDS-PAGE.

7. *SDS-PAGE (SDS-polyacrylamide gel electrophoresis)*

Samples were analyzed by SDS-PAGE in 9% slab gels as described previously (Asano and Takahashi, 1979), by the method of Maizel (1971). The ratio of acrylamide to bisacrylamide was 30 to 0.8. Electrophoresis was carried out at a constant voltage of 100 V and continued until the phenol red marker reached the anodal end of the gel. After electrophoresis, the gel was fixed with trichloroacetic acid (TCA)-isopropanol and stained with Comassie brilliant blue R250, and then destained as described by Stern and Dales (1976). Then the gel was treated with 2, 5-Diphenyloxazol (PPO) in dimethylsulphoxide by the method of Bonner and Laskey (1974). Autoradiograms were prepared by exposing the dried gels to Kodak "X-Omat" R film at −70°C or at room temperature.

8. *Chemicals*

Phosphonoacetic acid (PAA; ICN Pharmaceuticals, Inc., Plainview, NY) was dissolved in distilled water at a concentration of 20 mg/ml, adjusted to pH 7.4 with 1 *N* NaOH, and sterilized by filtration.

RESULTS

1. *Relationship of the appearance of cytopathic change, viral replication and incorporation of ^{35}S-methionine into infected whole cells*

Monolayer cells were inoculated with infect-

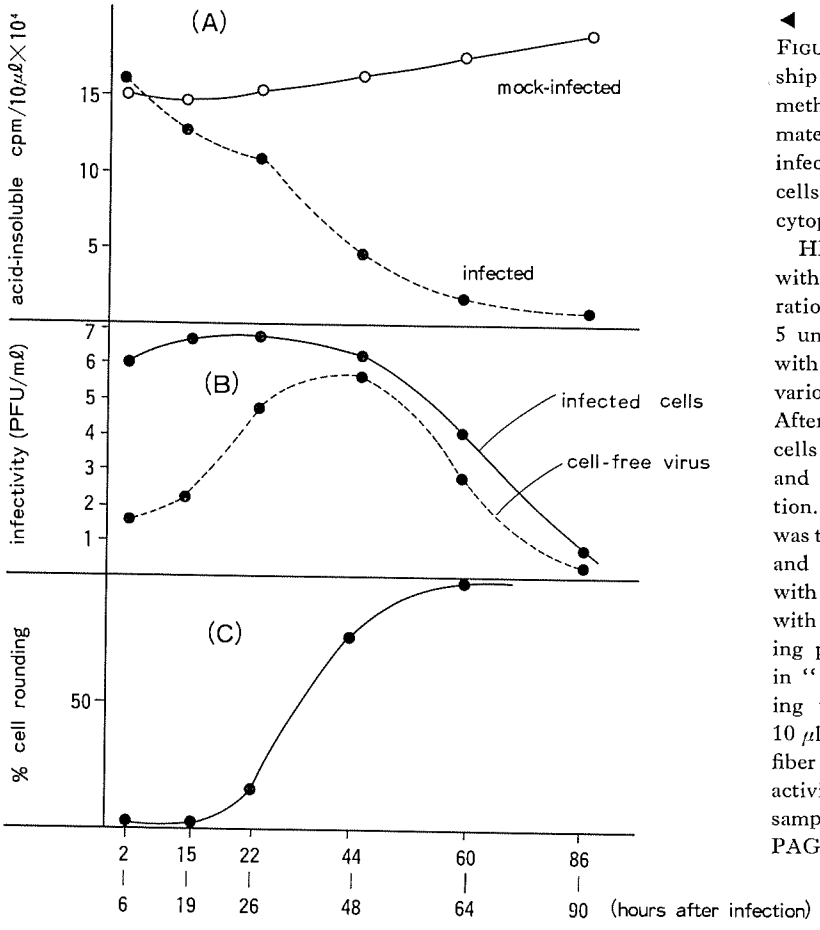

FIGURE 1. Temporal relationship of incorporation of ^{35}S-methionine into acid-insoluble materials (A), production of infectious virus and infected cells (B) and appearance of cytopathic effect (C).

HEL cells were inoculated with VZV-infected cells at a ratio of about 1 infected cell to 5 uninfected cells, and labelled with ^{35}S-methionine for 4 h at various times after infection. After each pulse-labelled period, cells were washed with PBS, and precipitated by centrifugation. One fifth of the pellet was treated with cold 10% TCA and then washed three times with cold 5% TCA and once with cold acetone. The resulting precipitate was solubilized in "sample buffer" in a boiling water bath. Samples of 10 μl were collected on glass fiber disc, counted for radioactivities (A). The remaining samples were examined by SDS-PAGE and radioautography.

ed cells at a ratio of about 1 infected cell to 5 uninfected cells, and labelled with ^{35}S-methionine for 4 h at various times after infection. After each radioactive pulse period, monolayers of HEL were washed three times with cold PBS, and precipitated by centrifugation. One fifth of the pellet was treated with cold 10% TCA and then washed three times with cold 5% TCA and once with cold acetone. The resulting precipitate was solubilized in " sample buffer " in a boiling water bath. Samples of 10 μl were collected on glass fiber discs (24 mm GF/C, Whatman), dried, and counted in a scintillation counter. The remaining sample was analyzed by SDS-PAGE and autoradiography. Under this experimental conditions, protein synthesis did not show a peak, but gradually decreased as shown in Fig. 1. Autoradiograms of this sample were shown in Fig. 2. A gradual decrease of protein synthesis is also seen from the figure. In these autoradiograms, it was not easy to determine the virus-induced polypeptides precisely, because of the high background radioactivity of the host cells. However, 9 polypeptide bands, which could not be found in mock-infected cells, were observed in infected cells labelled 15 to 64 h after infection. These polypeptides incorporated large amounts of ^{35}S-methionine during 22 to 48 h after infection. The highest yields of cell-free virus were obtained by harvesting cells 44 to 48 h after infection, when viral cytopathic effect was still not maximal, as shown in Fig. 1.

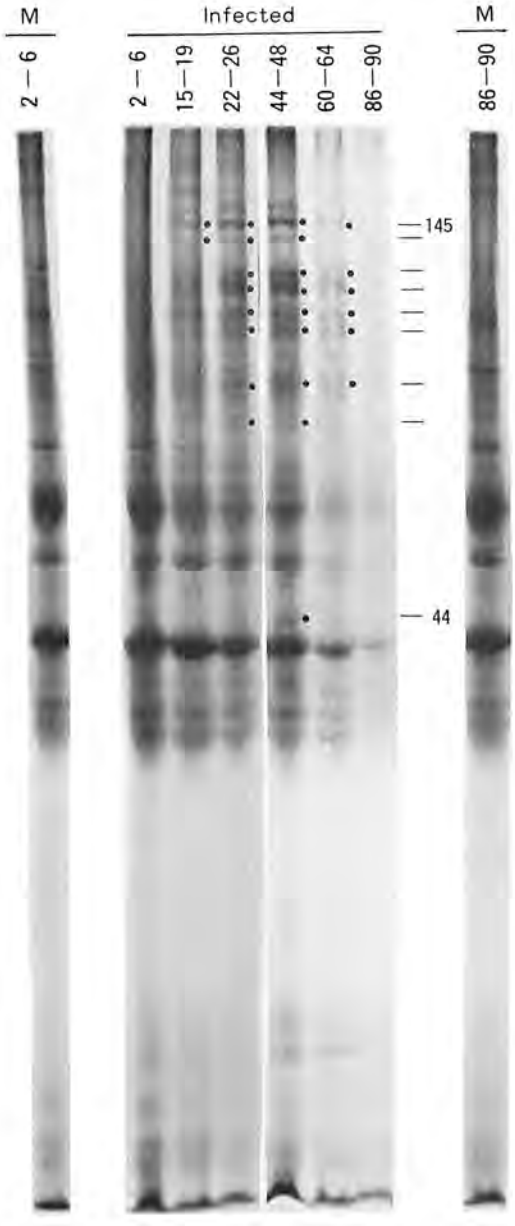

FIGURE 2. Autoradiogram of polyacrylamide gel after electrophoresis of whole cells (mock-infected and infected). HEL cells were inoculated with infected cells of the Oka strain of VZV at a ratio of about 1 infected cell to 5 uninfected cells and then labelled with 2 μCi/ml of ^{35}S-methionine for 4 h at various times after infection. Whole cells were concentrated by TCA precipitation, solubilized in "sample buffer" and applied to slab gels.

2. *Analysis of virus-induced polypeptides in infected cells by SDS-PAGE and radioautography*

For more detailed information on viral protein synthesis in infected cells up to 48 h post infection, infected or mock-infected cells were labelled with large amounts of ^{35}S-methionine for 2 h at various times after infection in the

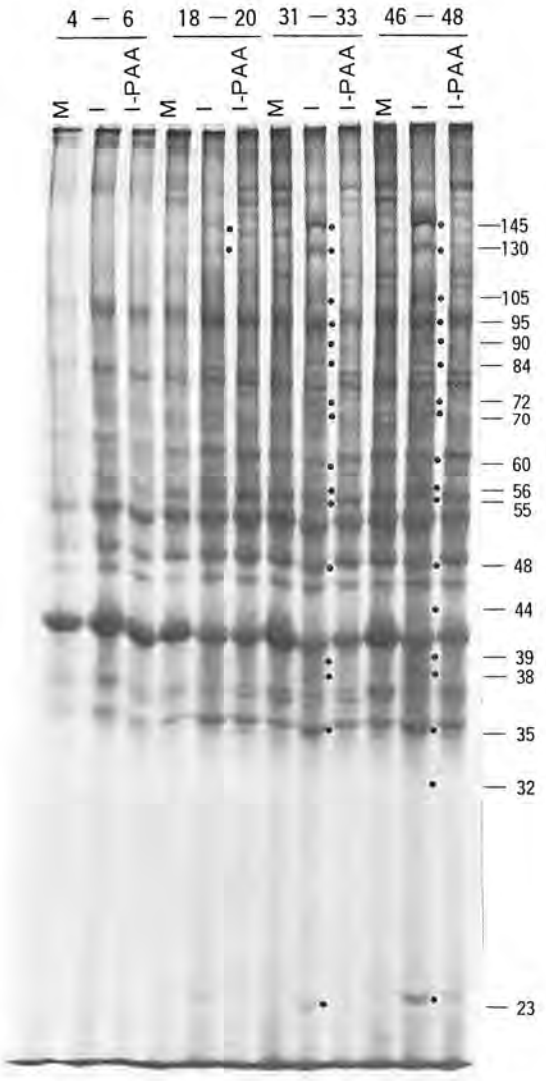

FIGURE 3. Polypeptide synthesis in VZV-infected whole cells up to 48 h after infection. HEL cells were infected as described in the legend to Fig. 2 and labelled with 10 μCi/ml of ^{35}S-methionine for 2 h at various times after infection. TCA precipitated samples were solubilized in "sample buffer" and analyzed by slab SDS-PAGE and autoradiography as described in the text. M: mock-infected, I; infected, I-PAA; infected in the presence of PAA (200 μg/ml).

presence or absence of PAA, and polypeptides were analyzed by SDS-PAGE and autoradiography. Figure 3 shows the profile of polypeptides synthesized in infected and mock-infected cells. During the early phase of protein synthesis, ^{35}S-methionine mainly labelled host cell polypeptides, and virus-induced proteins were not easily demonstrable. But, two polypeptides (145,000 and 130,000) were detected at 18 to 20 h after infection and the syntheses continued to increase at relatively constant rates. Later the number of virus-induced polypeptides and the rate of these syntheses increased, and at least 18 polypeptides were identified 46 to 48 h after infection. The molecular weights of these polypeptides ranged from about 145,000 to 23,000.

3. *Analysis of virus-induced polypeptides and glycoproteins in infected cells by immunoprecipitation followed by SDS-PAGE and fluorography*

In the above experiment, precise identification of virus-induced polypeptide and glycoprotein was difficult because of the high background of those of the host cells. Therefore, lysates of infected or mock infected cells were immunoprecipitated with VZV-hyperimmune monkey or guinea pig serum and analyzed by SDS-PAGE and fluorography. First, to exclude the possibility of nonspecific interaction of immune serum with possible FC receptors produced in infected cells, we mixed lysate of infected cells labelled with ^{35}S-methionine during the late phase of protein synthesis with immune or non-immune guinea pig serum and analyzed the resulting precipitates by SDS-PAGE and fluorography. As shown in Fig. 4, no band was observed with the precipitate of a lysate of infected cells mixed with non-immune guinea-pig serum, thus excluding the possibility of a nonspecific reaction.

In previous work, we identified 30 polypeptides and 10 glycoproteins in VZV infected cells at a late phase of protein synthesis by immunoprecipitation with green monkey antiserum. In the present study, we examined the

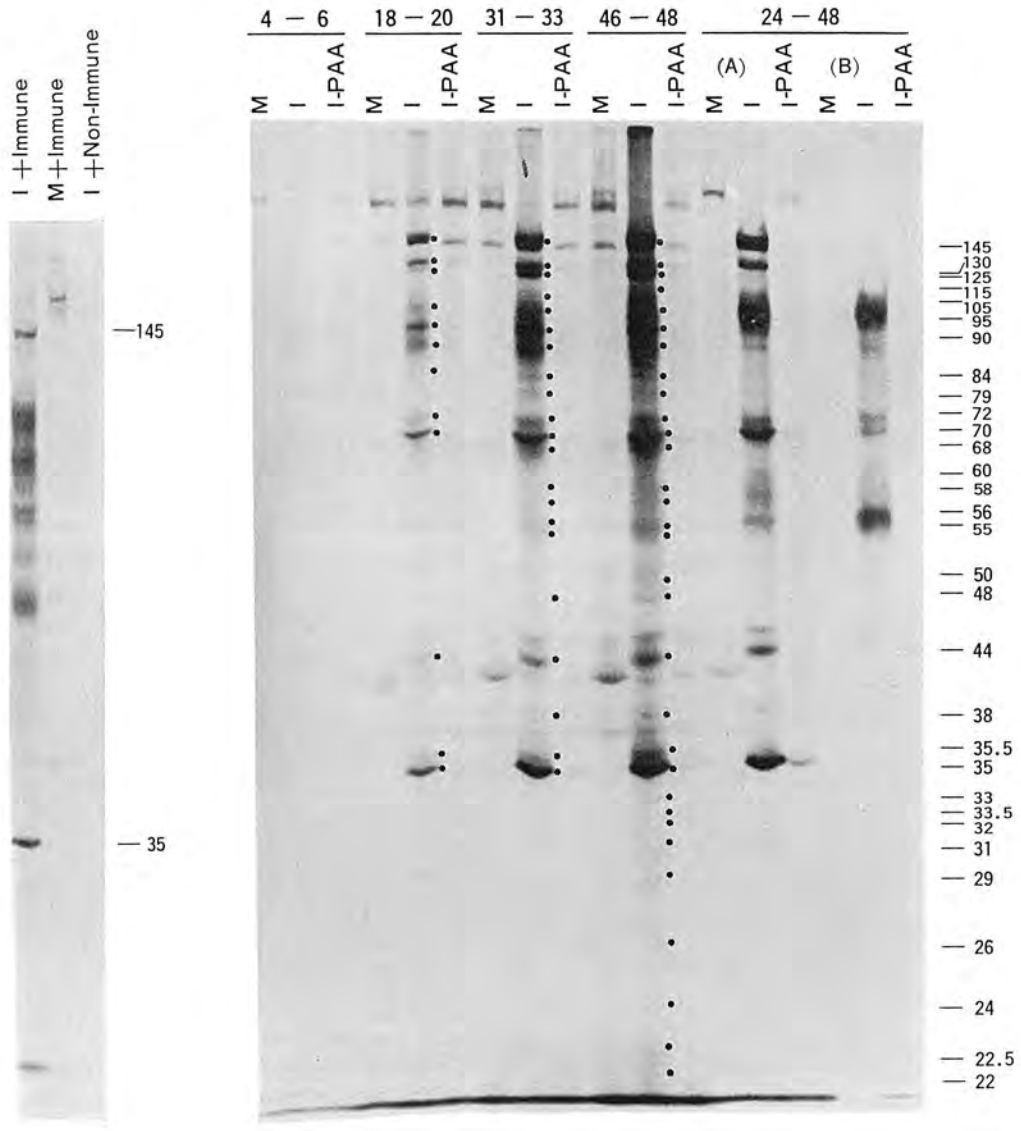

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FIGURE 4. Examination of nonspecific interaction of immunoglobulin G with Fc Receptor protein, possibly induced in VZV-infected cells. HEL cells were infected as described in the legend to Fig. 2, labelled with 2 μCi/ml of ^{35}S-methionine 24 to 48 h of infection and fractionated as described in the text. Then, TD-lysates prepared from infected or mock-infected cells were precipitated with anti-VZV or nonimmune guinea pig serum, and the resulting immunoprecipitates were analyzed by SDS-PAGE and fluorography.

syntheses of these proteins at intervals after infection by the same method, and the results are shown in Fig. 5. About 12 polypeptide bands were identified at 18 to 20 h after infection, and a polypeptide with a molecular weight of 125,000 that appeared 18 to 33 h after infection was seen, which was not seen in the previous study. The number and densities of the bands continued to increase with time and finally 31 polypeptide bands were

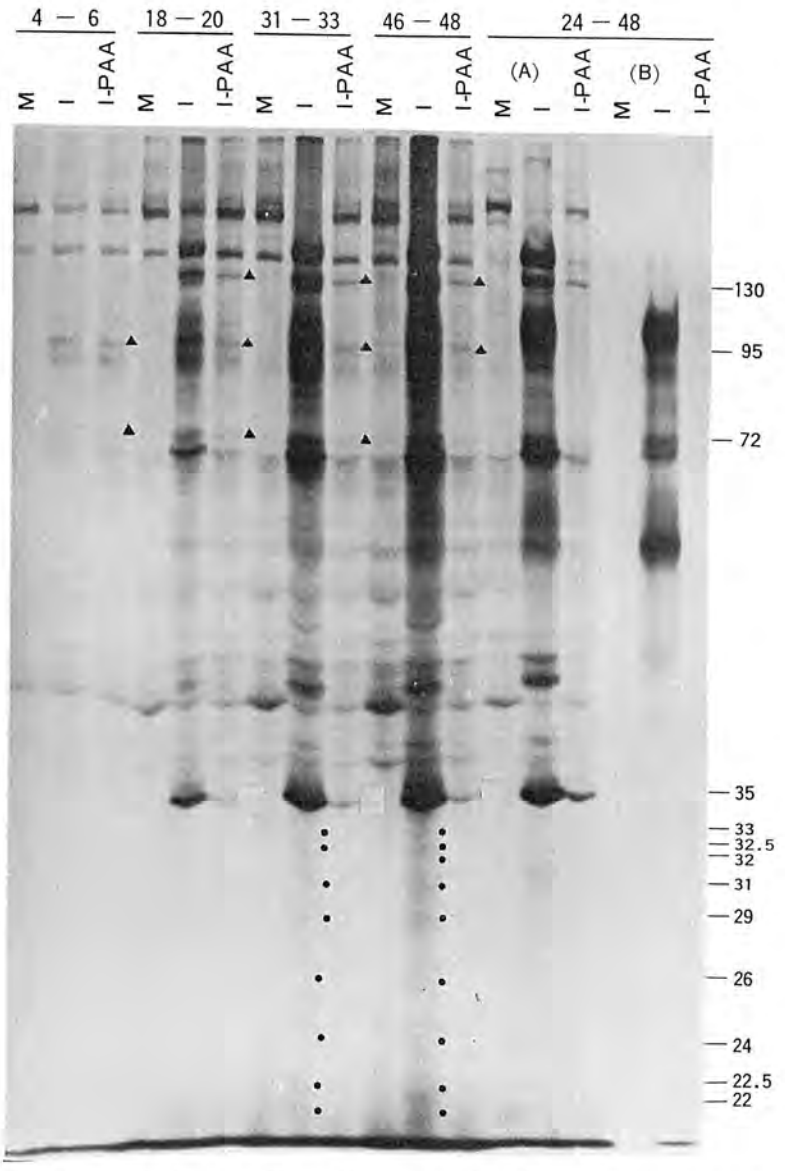

FIGURE 5. Time course of polypeptide synthesis in infected cells assessed by immunoprecipitation with hyperimmune green monkey antiserum. HEL cells were inoculated with cells infected with the Oka strain of varicella virus at a ratio of about 1 infected cell to 5 uninfected cells, in the absence (I) or presence (I-PAA) of PAA, or were mock-infected (M). The cells were labelled with 10 μCi/ml of ^{35}S-methionine for 2 h at various times after infection. Then the cells were fractionated and TD-lysates were prepared. The TD-lysates were immunoprecipitated with anti-VZV green mokey serum, and the immunoprecipitates were analyzed by SDS-PAGE and fluorography. Samples prepared from the cells labelled from 24 to 48 h after infection with 2 μCi/ml of ^{35}S-methionine (A) or 1 μCi/ml of ^{14}C-glucosamine (B) were run simultaneously on the right of the same gel. The gel was exposed to a film for 1 week.

FIGURE 6. Fluorographic patterns of polypeptides precipitated with hyperimmune green monkey antiserum. The same slab gel as that used in Fig. 6 was exposed to a film for 3 weeks.

found in precipitates 46 to 48 h after infection. The molecular weight of these polypeptide ranged from 145,000 to 22,000. A capsid protein (145 k) was prominent at 18 to 20 h after infection, and its density increased strikingly until 46 to 48 h after infection. When the gel was exposed for a longer period, low molecular weight polypeptides of 33,000 to 22,000 were clearly identified. The relative amounts of these polypeptides also increased with time after infection, as shown in Fig. 6. In infected cells with PAA, in which the syntheses of most virus-induced polypeptides

were inhibited, some polypeptides with molecular weight of 130,000, 95,000, 72,000 could be detected. These polypeptides were identified in the early stage of protein synthesis and their density later decreased.

Table 1 shows the radioactivities of immunoprecipitates with green monkey antiserum and guinea pig antiserum. The syntheses of virus-specific protein and glycoprotein increased with time in infected cells in the absence of PAA. In cells treated with PAA, a considerable amount of protein was synthesized in the early stage of infection, when very few glycoproteins were synthesized. Therefore, protein synthesized in the presence of PAA appeared to be early protein synthesized before viral DNA synthesis, which was supposed to correspond to the polypeptide bands detected in infected cells treated with PAA.

In the fluorographic patterns of immunoprecipitates obtained with guinea pig antiserum, the time course and the rate of protein syntheses were almost the same as those seen with green monkey antiserum. As has been reported previously (Asano and Takahashi, 1979), 30 polypeptides with molecular weights of about 145,000 to 22,000 were detected in the late stage of infection. Four polypeptides (125,000, 115,000, 48,000 and 44,000) precipitated with green monkey antiserum were not detected in immunoprecipitates with guinea pig antiserum, while 3 polypeptides (46,000, 42,000 and 25,500), not precipitated with green monkey antiserum, were precipitated by guinea pig antiserum.

Glycoprotein synthesis was followed using green monkey antiserum (Fig. 7). Glycoprotein synthesis became detectable 18 to 20 h after infection, and then gradually increased, and 13 glycoproteins were identified 46 to 48 h after infection. None of the glycoprotein bands were detectable in infected cells treated with PAA under the conditions used in this

TABLE 1. *The time course of incorporation of ^{35}S-methionine and ^{14}C-glucosamine into VZV-infected HEL cells assessed by the precipitation with anti-VZV monkey or guinea pig serum*[a]

	Time of pulse labelling (hours after infection)	^{35}S-Methionine		^{14}C-Glucosamine	
		(I)[e]-(M)[f]	(I+PAA)[g]-(M)	(I)-(M)[h]	(I+PAA)-(M)[i]
(A)[b]	4-6	198.0[d]	113.8	0.4	0
	18-20	296.0	237.2	37.2	1.4
	31-33	1175.6	80.0	50.0	0
	46-48	2533.8	0	103.6	0
(B)[c]	4-6	394.2	338.6	10.6	7.6
	18-20	594.8	338.0	47.2	2.4
	31-33	1196.0	0	96.8	0.6
	46-48	2579.4	0	206.8	0

[a] TD-lysates of infected or mock-infected cells labelled with ^{35}S-methionine of ^{14}C-glucosamine were treated with anti-VZV monkey or guinea-pig serum and the radioactivities of the precipitates were counted.
[b] (A): Precipitation with anti-VZV green monkey serum.
[c] (B): Precipitation with anti-VZV guinea pig serum.
[d] Expressed as cpm/10 μl of sample.
[e] (I): Infected.
[f] (M): Mock-infected.
[g] (I+PAA): Infected in the presence of PAA.
[h] (I)-(M): Radioactivity of infected cells minus that of mock-infected.
[i] (I+PAA)-(M): Radioactivity of infected cells in the presence of PAA minus that of mock-infected.

work.

4. *Fluorographic patterns of ^{35}S-labelled polypeptides and ^{14}C-labelled glycoproteins of partially purified virion*

^{35}S-Methionine or ^{14}C-glucosamine labelled lysates of HEL cells infected with VZV were immunoprecipitated with antiserum against partially purified virions of VZV, and the immunoprecipitates were analyzed by SDS-PAGE and fluorography (Fig. 8).

At least 12 polypeptides with the molecular weight of 145,000 to 35,000 and 7 glycoproteins with the molecular weight of 115,000, 90,000, 84,000, 72,000, 70,000, 60,000 and 56,000 were detected. An ^{35}S-labelled polypeptide band with a molecular weight of 145,000 (145 k) was prominent. Since no ^{14}C-glucosamine labelled band corresponding to the band was found in the virions, this 145 k plypeptide may be the main capsid protein.

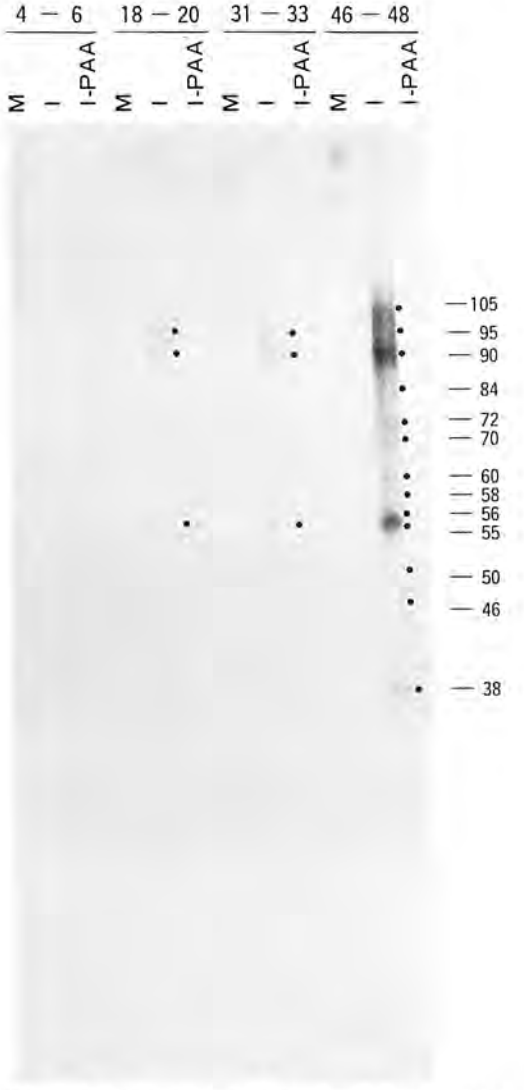

FIGURE 7. Time course of glycoprotein synthesis in infected cells assessed by immunoprecipitation with hyperimmune green monkey antiserum. The experimental conditions were similar to those for Fig. 5, except that ^{14}C-glucosamine was used.

DISCUSSION

We examined the synthesis of VZV-induced proteins and glycoproteins by immunoprecipitation, SDS-PAGE and fluorography. As it was not possible to obtain high concentration of cell-free virus, cells were inoculated with VZV infected cells at a ratio of about 1 infected cell to 5 uninfected cells, conditions which gave the maximal yield of infectious cell-free virus 44 to 48 h after infection.

It was not easy to distinguish virus-induced polypeptide bands clearly by SDS-PAGE and radioautography because of the high background of host cells. Therefore, we used immunoprecipitation by hyperimmune monkey antiserum or guinea pig antiserum followed by SDS-PAGE and fluorography in this study. For this purpose, we first examined whether there was any non-specific interaction of immunogloblin G with FC receptor protein in VZV-infected cells, as reported with other human herpesvirus infections (Westmoreland et al., 1974; Furukawa et al., 1975). Our results showed that no such reaction occurred in our system.

About 12 polypeptides were identified 18 to 20 h after infection, and virus protein synthesis increased to a maximum at 46 to 48 h after infection. A polypeptide with a molecular weight of 125,000 that was not detected

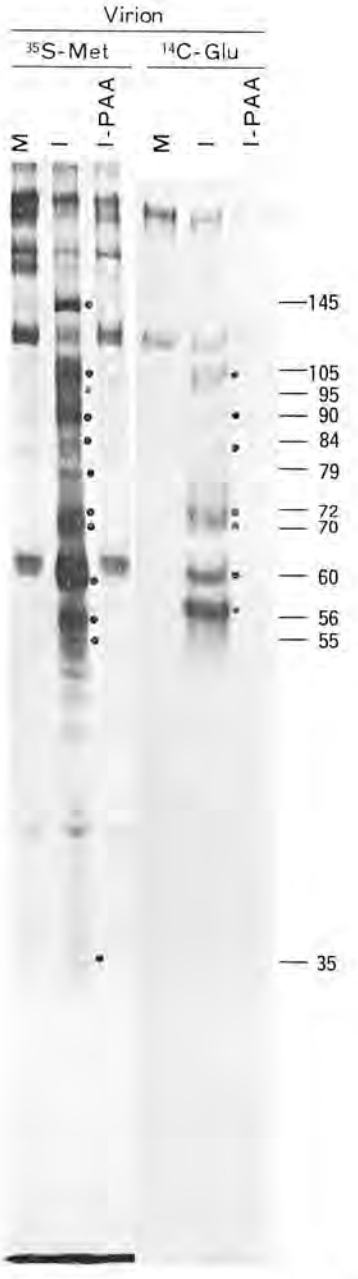

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FIGURE 8. Fluorographic patterns of polypeptides and glycoproteins of labelled VZV-infected cells immunoprecipitated with antiserum against partially purified VZV virion.

HEL cells were infected with the Oka strain of varicella-zoster virus in the absence (I) or presence (I-PAA) of PAA, or were mock-infected (M). The cells were labelled with 10 μCi/ml of ^{35}S-methionine or 6 μCi/ml of ^{14}C-glucosamine for 6 h at 40 h after infection. Then TD-lysates were prepared from the infected cells and incubated with antiserum for partially purified virions of VZV and the immunoprecipitates were analyzed by SDS-PAGE and fluorography.

in previous work (Asano and Takahashi, 1979) was newly detected 18 to 33 h after infection using green monkey antiserum, and in all, 34 polypeptides were identified during infection. The major capsid protein, with a molecular weight of 145,000, was prominent 18 to 20 h after infection, and its synthesis increased up to 46 to 48 h after infection. Glycoprotein synthesis was also followed by immunoprecipitation, SDS-PAGE and fluorography. About 3 glycoproteins were detected 18 to 20 h after infection, and synthesis increased to a maximum 46 to 48 h after infection, when 13 glycoproteins were identified.

PAA is a specific inhibitor of several herpesvirus-induced DNA polymerase (Overby et al.; 1974, Huang, 1975), including that induced by VZV (May et al., 1977). The effect of PAA (200 μg/ml) on virus-induced protein and glycoprotein syntheses in infected cells during the course of infection was examined by comparison of PAA-treated and untreated cells. The syntheses of virus-specific proteins were detected by immunoprecipitation 4 to 20 h after infection of cells treated with PAA, when expressed as incorporation of ^{35}S-methionine, but not as incorporation of ^{14}C-glucosamine, as shown in Table 1. On SDS-PAGE and fluorography, a few polypeptides (MW 130,000, 95,000 and 72) were identified in infected cells treated with PAA at an earlier stage of infection. These observations suggest that 3 polypeptides detected in the presence of PAA might be virus induced early proteins synthesized before viral DNA synthesis. However, under the present experimental conditions in which

infected cells were used as inoculum, it was not possible to identify virus-induced early proteins exactly. Recently, Yamanishi et al. (1980) detected VZV antigen in the nucleus of infected cells treated with PAA as early as 2 to 4 h after infection using cell-free virus as inoculum, and this was supposed to be virus-induced early antigen. It would be interesting to determine the molecular weight of this early protein and to compare it with those of the proteins detected in the present work. Studies along these lines are now under way.

Labelled lysate of VZV-infected cells was also incubated with the antiserum against partially purified virions, and the immunoprecipitate was analyzed by SDS-PAGE and fluorography. We used lysates of similarly labelled mock-infected cells and infected cells in the presence of PAA as controls to check the specificity of the reaction. At least 12 polypeptides with the molecular weights ranging from 145,000 to 35,000 were identified in the virion, and 4 of them appeared to be glycoproteins. The polypeptide with a molecular weight of 145,000 detected in the virions seemed to be the main capsid protein, because it had high density and no corresponding band was detected when ^{14}C-glucosamine was used for labelling. Consistent with this, there are reports that several other herpes viruses, namely herpes simplex virus type 1 and 2, EB virus, bovine mammallitis virus, pseudorabies virus and equine abortion virus have a protein with molecular weight of 140,000 to 160,000 as a major capsid protein (Gibson and Roizman, 1972; Perdue et al., 1974; Powell and Watson, 1975; Stevely, 1975; Dolyniuk et al., 1976; Killington et al., 1977).

NOTE

During preparation of this manuscript, a paper entitled " The synthesis of glycoproteins in human melanoma cells infected with varicella-zoster virus " by C. GROSE, Virology 101, 1–9, (1980) appeared, in which five infected-cell specific glycoproteins (45K, 62K, 88K, 98K, 118K) were identified.

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