Protein Synthesis in Sendai Virus-infected Cells

By V. M. ZAIDES, L. M. SELIMOVA, O. P. ZHIRNOV AND A. G. BUKRINSKAYA

The D. I. Ivanovsky Institute of Virology, the U.S.S.R. Academy of Medical Sciences, Moscow, U.S.S.R.

(Accepted 29 January 1975)

SUMMARY

The rate of protein synthesis in chicken embryo cells infected with Sendai virus 18 to 20 h previously was about two times greater than in mock-infected controls. At this time of infection six stable virus-induced proteins, four major structural proteins (P, NH, NP and M) and two non-structural proteins (28K and 61K), were identified by electrophoresis in SDS-polyacrylamide gel of total cell extracts. The structural glycopeptide F was not detected in the infected cell extracts. Pulse-chase experiments showed that P, NP, M and 28K proteins either did not undergo any post-translational processing or the processing occurred very rapidly. By contrast, a glycopeptide NH was apparently derived from one of two unstable precursors, 69K or 63K, which were revealed only after a short pulse. The synthesis of virus-specific proteins appeared to be regulated since its rate varied for individual classes of proteins.

In nucleocapsid-like particles isolated from infected cells two major structural proteins (P and NP) were found. A minor component with a very large mol. wt. was revealed in these particles as well as in the virus particle.

INTRODUCTION

Studies on protein metabolism in paramyxovirus-infected cells have been limited to Newcastle disease virus (NDV) systems (Lomniczi, Meager & Burke, 1971; Alexander & Reeve, 1972; Samson & Fox, 1973, 1974; Hightower & Bratt, 1974). Considerably less is known about another paramyxovirus, Sendai virus. Stone, Kingsbury & Darlington (1972) using the method of SDS-polyacrylamide gel electrophoresis (SDS-PAGE) have shown the accumulation of two structural proteins (largest and nucleoprotein) in infected chicken cells. However the other proteins were not identified.

The present studies were undertaken to follow the synthesis and processing of virus-specific proteins in Sendai virus-infected cells and the effects of virus infection on host cell protein metabolism.

METHODS

Virus and cells. 960 strain of Sendai virus was used. The cultivation and purification of virus were described earlier (Zaides et al. 1974a). Primary chick embryo cells were grown in the standard vials for scintillation counting (Packard). Monolayer cultures were infected with Sendai virus at an input multiplicity of 50 to 100 ID_{50}/cell and incubated in 0.5 % lactalbumin hydrolysate (LH) at 37 °C (Zaides et al. 1973).
Radioisotopic labelling. Cells were labelled 18 h after infection, the time when the synthesis of virus-specific RNA reached the maximum (Blair & Robinson, 1968). The maintenance medium was removed, monolayers were washed thoroughly with pre-warmed Hank's balanced salt solution (BSS) and cells were incubated with 2 to 40 μCi/ml (0.4 ml/vial) of pre-warmed [14C]-amino acid mixture (CFB-104, Amersham, England, containing 14 [14C]-amino acids at similar molar concentrations) in BSS. For chasing the radioactive media was removed and cells were washed by, and incubated in, 0.5% LH. In these conditions the incorporation of [14C]-amino acids in polypeptides was stopped in 2 min. After incubation the labelled (or unlabelled) cells were washed with a buffer (0.14 M-NaCl; 0.01 M-tris-HCl, pH 7.4) and, further: (i) lysed in dissociation buffer (2% SDS; 5% 2-mercaptoethanol; 6 M-urea; 0.01 M-tris-HCl, pH 7.6) and used for electrophoresis (Skehel, 1972); (ii) washed with 5% trichloracetic acid (3 times), ethanol and, after drying, standard toluene based scintillation fluid was put into the vial and acid-insoluble radioactivity was determined in a liquid scintillation counter (Ershov et al. 1973), or (iii) used for preparation of cytoplasmic extracts (in this case the cells were grown in the 250 ml bottles; for details see legend to Fig. 5).

SDS-PAGE. The Laemmli method was used (Laemmli, 1970). The samples were solubilized, and, prior to electrophoresis, heated in dissociation buffer at 100°C for 3 min. Electrophoresis was performed in 7.5% acrylamide gels at 5 mA/gel for 3 h. After electrophoresis the gels were either stained with amido black or sliced longitudinally and after drying exposed for 1 to 7 days to X-ray film (Kazan Chemical Factory, U.S.S.R.) which was subsequently developed.

Quantitative determination of autoradiographic data. Fragments of the gel corresponding to the strips of reduced silver grains on film were cut out and their radioactivity was counted (Hay, Skehel & Burke, 1968). To determine the 'true' radioactivity of virus-specific proteins in the gel the total radioactivity corresponding to virus-specific bands was counted and then a correction on contaminating labelled cellular proteins was made by the formula:

\[ q_3 = q_1 - \frac{q_2}{l_1} \times l_0, \]

where \( q_3 \) = true total radioactivity of virus-specific proteins; \( q_1 \) = revealed total radioactivity of virus-specific bands; \( q_2 \) = total radioactivity of the gel minus radioactivity of the virus-specific bands and radioactivity migrated with the front of the gel; \( l_1 \) = total length of gel fragments used for determination of \( q_2 \); \( l_0 \) = length of gel fragments used for determination of \( q_1 \).

Then the relative amount of virus-specific proteins in the gel was determined by the formula:

\[ S = \frac{q_3}{q_0} \times 100 \%, \]

where \( q_0 \) = total radioactivity of the gel (without radioactivity migrating with the front of the gel). These calculations assumed that virus infection had a uniform effect on the synthesis of all cell proteins. While this assumption may not be completely true, it is useful and allows for a more convenient presentation of the data.
**RESULTS**

**Polypeptides in the virus particles**

Fig. 1 shows the electrophoretic patterns of Sendai virus particle polypeptides. In all, 5 major and 5 minor bands in purified preparations were routinely revealed. In the present paper the major proteins are designated according to the recommendations of Scheid & Choppin (1974): P (largest protein), NH (glycopeptide), NP (nucleoprotein), F (lower mol. wt. glycopeptide) and M (membrane protein). In previous communications they were designated L, GP₁, NP, GP₂ and M respectively (Zaides et al. 1974a, b). All minor proteins were designated as m proteins (from m₁ to m₅) in accordance with their electrophoretic mobility. The more detailed description of 960 strain Sendai virus particle polypeptides was published earlier (Zhirnov et al. 1974). Relative molar ratios of virus particle polypeptides are presented in Table 2.

**Effect of virus infection on total protein synthesis in the cells**

Table 1 shows that [¹⁴C]-amino acid incorporation into acid-insoluble material of infected cells 18 h after infection increases about twice as much as mock-infected control cells. The stimulation of incorporation did not depend upon the period of labelling, 15 or 60 min, suggesting that the rate of protein synthesis increases about two times.

**Accumulation of virus-specific proteins in infected cells**

Fig. 2 shows an electrophoretic pattern of cell polypeptides obtained at various times after infection. The accumulation of virus-specific proteins was sufficient to allow their detection by staining with amido black. A distinct band of virus-specific protein which
Table 1. Incorporation of \([^{14}C]\)-amino acids into intracellular proteins*

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Infection</th>
<th>Acid-insoluble radioactivity (ct/min/vial)</th>
<th>(\frac{b}{a} \times 100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Mock (a)</td>
<td>23 863</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>21 443</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 683</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ (b)</td>
<td>45 832</td>
<td>222</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 353</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>39 132</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Mock (a)</td>
<td>19 625</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>23 464</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 1375</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ (b)</td>
<td>43 305</td>
<td>260</td>
</tr>
<tr>
<td></td>
<td></td>
<td>58 412</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>65 797</td>
<td></td>
</tr>
</tbody>
</table>

* 18 h after infection the cells in vials were incubated in BSS with \(3 \mu\text{Ci/ml}\) of \([^{14}C]\)-amino acids mixture for 30 min. After incubation acid-insoluble radioactivity was determined as described in Methods.

Fig. 2. Accumulation of virus-specific proteins in infected cells. Confluent monolayers in the vials were infected and the samples (2 vials per sample) were obtained at intervals (monolayers were washed and cells were lysed and heated in dissociation buffer as described in Methods). After electrophoresis the gels were stained with amido black: (a) mock-infected sample; (b) 8 h post infection (p.i.); (c) 18 h p.i.; (d) 23 h p.i.; (e) 42 h p.i.; (f) purified virus.

Figures show proteins migrating similarly to NP is seen in the gel as early as 18 h after infection. Later on the other virus-specific proteins, which correspond in their electrophoretic mobility to the major virus particle proteins P, NH and M, are also detected in stained gels. Meanwhile, one of the major structural proteins (F) was never seen. A distinct band ahead of NP (Fig. 2, b to i) was probably a cellular component since the electrophoretic mobility of this protein was some-
Sendai virus protein synthesis

Fig. 3. Electrophoretic pattern of [14C]-proteins after cumulative labelling of infected and uninfected cells. 18 h after infection the cells in the vials were exposed to a [14C]-amino acid mixture in BSS (2 μCi/ml) for 4 h. Then the cells were lysed and electrophoresed and the gels were cut longitudinally and dried. X-ray film was exposed to the gels for 3 days and subsequently developed: (a) mock-infected cells; (b) infected cells.

Fig. 4. Pulse-chase experiment. 18 h after infection the cells in 4 vials were exposed to a [14C]-amino acid mixture in BSS (40 μCi/ml). After 5 min the cells from 2 vials were lysed in dissociation buffer (a) and the cells from other 2 vials were chased for 2 h and then lysed (b). The results of 2 independent experiments are presented.

what smaller than that of the F protein and strongly corresponded to the uninfected cell band in Fig. 2a (see also Fig. 3).

When [14C]-labelled proteins were analysed by autoradiography the same virus-specific polypeptides were detected (Fig. 3). In a special experiment their electrophoretic identity to P, NH, NP and M polypeptides was shown in co-electrophoresis of [14C]-cellular proteins...
Table 2. Molecular weights and molar ratios of virus particle and virus-specific proteins

<table>
<thead>
<tr>
<th>Designation of protein</th>
<th>Presence in virus particle</th>
<th>Presence in infected cells</th>
<th>Mol. wt.*</th>
<th>Virus particle 5 min pulse</th>
<th>Infected cells 5 min pulse + 2 h chase</th>
</tr>
</thead>
<tbody>
<tr>
<td>$m_1$</td>
<td>+</td>
<td>+ (?)</td>
<td>$\sim 100000$</td>
<td>0.026</td>
<td>ND†</td>
</tr>
<tr>
<td>P</td>
<td>+</td>
<td>+</td>
<td>71400</td>
<td>0.19</td>
<td>0.30</td>
</tr>
<tr>
<td>69K</td>
<td>−</td>
<td>+</td>
<td>68700</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>NH</td>
<td>+</td>
<td>+</td>
<td>67500</td>
<td>0.20</td>
<td>—</td>
</tr>
<tr>
<td>63K</td>
<td>−</td>
<td>+</td>
<td>62600</td>
<td>—</td>
<td>0.37</td>
</tr>
<tr>
<td>61K</td>
<td>−</td>
<td>+</td>
<td>61400</td>
<td>—</td>
<td>0.35</td>
</tr>
<tr>
<td>NP</td>
<td>+</td>
<td>+</td>
<td>59000</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>F</td>
<td>+</td>
<td>−</td>
<td>52700</td>
<td>0.57</td>
<td>—</td>
</tr>
<tr>
<td>M</td>
<td>+</td>
<td>+</td>
<td>42400</td>
<td>0.97</td>
<td>0.85</td>
</tr>
<tr>
<td>28K</td>
<td>−</td>
<td>+</td>
<td>$\sim 28000$</td>
<td>ND†</td>
<td>ND†</td>
</tr>
</tbody>
</table>

* Mol. wt. of structural proteins, averaged from 7 experiments using bovine serum albumin (68000), pyruvate kinase (57000) and ovalbumin (43000), were used as standards; corresponding bands from infected cells mol. wt. were taken to be identical; mol. wt. of the non-structural bands were estimated similarly (average from 3 experiments).

† The area of each peak for virus particle proteins (Fig. 1b) or corrected radioactivity in the autoradiograph bands see (Methods) was divided per corresponding mol. wt.; the value for the NP band was taken as 1.

‡ ND, not done.

(20 μg of protein) and unlabelled virus particle proteins (200 μg of protein; not shown).

Beside structural virus particle polypeptides the non-structural polypeptide 28K was revealed (Fig. 3). (In the experiment shown in Fig. 2, the 28K protein appears to have migrated with the front of the gel).

We estimated what part of overall [14C]-amino acid incorporation is contributed by virus-specific proteins. The calculations (see Methods) showed that the contribution of virus-specific [14C]-proteins 18 h after infection was 35 to 50%, independently of the time of labelling (5 min to 5 h).

**Pulse-chase experiments**

The data of two experiments with 5 min pulse labelling followed by 120 min chase are represented in Fig. 4. The following virus-induced proteins are revealed after a 5 min pulse: (i) virus structural proteins P, NP and M as well as 28K; (ii) 69K and 63K virus-specific proteins which were not revealed among virus particle proteins and in infected cells after a long period of labelling. Meanwhile, no protein with the mobility of NH or only a very faint band in its position is seen in these gels. After the chase all four major virus particle proteins are again revealed. In the autoradiographs, where the band corresponding to NP was a narrow one, an additional band of virus-specific protein (61K) very close to NP but with a somewhat slower mobility was observed (Fig. 4, exp. 1). In spite of very small differences in electrophoretic mobility of 69K and NH as well as 63K and 61K these classes were regularly revealed in a number of experiments. Electrophoretic patterns of virus-specific proteins were similar after different chase periods (from 1 to 24 h).

Table 2 shows mol. wt. and rough estimation of molar ratios of virus particle proteins and intracellular virus-specific proteins. It can be seen that the molar ratio in both cases is similar, being due to predominant amounts of NP and M proteins. P and NH in virus particles and...
Sendai virus protein synthesis

Fig. 5. Electrophoretic analysis of polypeptide from NLP. Monolayer cultures were grown in 250 ml bottles and infected. 18 h after infection cells were exposed to a [14C]-amino acid mixture in BSS (3 μCi/ml) for 4 h. Then the cells were removed, pelleted and disrupted in a buffer solution (0.01 M-NaCl; 0.003 M-MgCl₂; 0.01 M-tris-HCl, pH 7.4). Cell homogenate was centrifuged at 15000 g for 15 min, and the supernatant fluid was fractionated in a velocity sucrose gradient as described (Zaides et al. 1974a). After centrifuging, acid-insoluble radioactivity was determined in samples, the fractions with a peak at 200S were pooled and the material (≤ 5 μg of protein) was precipitated with 5% trichloracetic acid in the presence of bovine serum albumin (50 μg/ml). The precipitates were washed with acetone (twice), dried and used for electrophoresis. (a) 200S material from infected cells; (b) corresponding material from mock-infected cells.

Proteins of cytoplasmic nucleocapsid-like particles (NLP)

It has been shown previously that NLP are accumulated in the cytoplasm of Sendai virus-infected cells (Robinson, 1971), being involved in virus genome transcription (Bukrinskaya, Zaides & Zhdanov, 1972; Stone et al. 1972; Zaides et al. 1972, 1974a; Bukrinskaya, 1973). Two major virus particle proteins, NP and P have been identified in NLP (Stone et al. 1972; Zaides et al. 1974a). We found in NLP the same two major polypeptides (Fig. 5), corresponding to P and NP virus particle proteins, this being shown by co-electrophoresis with unlabelled virus particle proteins (not shown). One additional polypeptide which migrated in the gel similarly to the minor virus particles protein m₁ was also revealed in NLP.

DISCUSSION

It is evident from the data presented here that major structural proteins P, NH, NP and M and two non-structural proteins 61K and 28K are accumulated in Sendai virus-infected cells. Since neither synthesis nor accumulation of major virus particle protein F was regis-
tered in infected cells, 61K may be regarded as a possible product of the gene for F. This suggestion is in accordance with the data of Homma & Ohuchi (1973) and Scheid & Choppin (1974) who have shown that Sendai virus glycopeptide with a lower mol. wt. (F) is apparently a product of the specific cleavage of a larger precursor and such cleavage appears to take place within virus particles. However, more detailed investigation is required to prove the relationship between 61K and F protein.

The polypeptide with a very large mol. wt., m1, was regularly detected in virus particles and a similar band was detected in intracellular NLP. Similar polypeptides were revealed earlier in Sendai virus particles (Hosaka & Shimizu, 1972; Homma & Ohuchi, 1973; Marx, Portner & Kingsbury, 1974) and in NDV systems both in the virus particles and in infected cells (Hightower & Bratt, 1974). In our experiments m1 protein was revealed in NLP where the concentration of the other [14C]-proteins (and apparently respective unlabelled molecules) is rather small. For this reason m1 protein may not be a product of the aggregation of polypeptides with lower mol. wt. as it was proposed by Marx et al. (1974). It is worth mentioning that the protein with similar electrophoretic mobility in vesicular stomatitis virus was shown to be a unique class (Stampfer & Baltimore, 1973) which was necessary for virus genome transcription in vitro (Emerson & Wagner, 1973). Since NLP are involved in Sendai virus genome transcription in cells (Stone et al. 1972; Bukrinskaya, 1973; Zaides et al. 1974a), the detection of m1 proteins in these particles seems especially essential.

As has been shown, a 5 min pulse of [14C]-amino acids allows detection of intracellular virus-specific proteins P, NP, M and 28K. Thus, these proteins apparently do not undergo a post-synthetic processing (or this process occurs very quickly). This point of view is confirmed by results reported by Kingsbury (1973), who performed the synthesis of non-glycosylated proteins of Sendai virus in cell-free system.

On the other hand we believe that, in the system used, the post-translational modification of some virus-specific proteins takes place. This point is not quite distinctly confirmed by the data shown in Fig. 4, and Table 2 since the differences in mol. wt. (69K > NH, 63K > 61K) falls inside experimental error. However this difference appears to be real since in a number of experiments [14C]-virus-specific bands near the NH position coincided with co-electrophoresed virus particle NH protein only after chasing and never before. As to the proteins in the positions of about 60 kilodaltons, 63K was distinctly seen only before and not after chasing, while 61K was found only after chasing (when the NP band was not too thick). Taking into account all these considerations it seems appropriate to suggest that 69K and 63K are unstable precursor proteins while NH and 61K are stable products of post-translational modifications. However, more precise data are needed to support the indicated precursor and product relationship.

The high rate of NP and M protein synthesis as compared with other virus-specific proteins and relatively small amount of M protein after chasing suggest that the synthesis and accumulation of virus-specific proteins is regulated in infected cells.

As to the total protein synthesis in infected cells, it increases about two times 18 to 20 h after infection. Since the synthesis of virus-specific proteins at this interval comprises no more than 50 % of the total protein synthesis it appears that the synthesis of cellular proteins at this time of infection is not inhibited (moreover, it cannot be excluded that it is somewhat stimulated). This observation is in contrast to the NDV system where the inhibition of host-cell protein accumulation has been demonstrated (Hightower & Bratt, 1974). The amount of virus-specific protein in the late stages of infection is so significant that it is comparable to the amount of the cellular proteins; thus, in this respect, the situation is unusual, at least for animal virus systems.
Sendai virus protein synthesis

REFERENCES


(Received 6 November 1974)