VIRUS-SPECIFIC PROTEINS ASSOCIATED WITH RIBOSOMES OF KREBS-II CELLS INFECTED WITH ENCEPHALOMYOCARDITIS VIRUS

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1. Introduction

Infection of cells with picornaviruses is accompanied by the selective inhibition of host cell protein synthesis [1]. The mechanism of this inhibition is obscure. One of the possible explanations may consist in modification of the cellular protein-synthesizing machinery by virus-specific protein(s) in such a way that translation of host cell mRNA is preferentially inhibited. It was suggested, for example, that such a role may be played by the proteins of poliovirus procapsids, VPO, VP 1 and VP 3, after complexing with the smaller ribosomal subunit [2]. In this connection, we have attempted to determine virus-specific proteins bound to the ribosomes of Krebs-II cells infected with encephalomyocarditis (EMC) virus.

In this letter we show that three labelled non-structural EMC virus-specific proteins are associated with the ribosomes isolated from the infected cells which are chased over a relatively long period of time after a pulse with radioactive amino acids. Molecular weights of these proteins were estimated to be 55–58, 22 and 16–18 kdalton. Labelled proteins with similar electrophoretic mobilities are absent from the ribosomes isolated from the uninfected cells, although these ribosomes also contain several labelled polypeptides.

2. Methods

Krebs-II cells were infected with EMC virus as described previously [3] and after overnight incubation in the presence of actinomycin D (5 μg/ml) at 4°C the suspension was warmed up and incubated at 37°C. Labelled amino acids ([^{14}C]valine, 225 mCi/mmole, 4 μCi/ml or [^{3}H]valine, 27 Ci/mmole, 50 μCi/ml) were added after 3.5 hr incubation at 37°C for 5–60 min and the suspension was chased in the presence of 100-fold excess of unlabelled valine for 30–120 min.

Cells were disrupted in a Dounce homogenizer in a buffered solution of the following composition: 10^{-2} M KCl, 10^{-3} M Mg acetate, 10^{-2} M triethanolamine, pH 7.45. The supernatant obtained after centrifugation of this homogenate at 15 000 *g* for 15 min, was run in a linear 15–30% sucrose concentrations gradient, prepared in the same buffer, in a Beckman SW 25.1 rotor at 21 000 rpm, 4°C for 14 hr.

Ribosome-containing fractions from the gradient were treated with formaldehyde and centrifuged in a preformed CsCl-density gradient essentially as described by Belitsina et al. [4].

Samples for electrophoresis were prepared as described by Butterworth et al. [5]. Electrophoresis was performed in neutral, pH 7.1, sodium dodecyl sulfate-containing 10% polyacrylamide gels 25 cm long [6].

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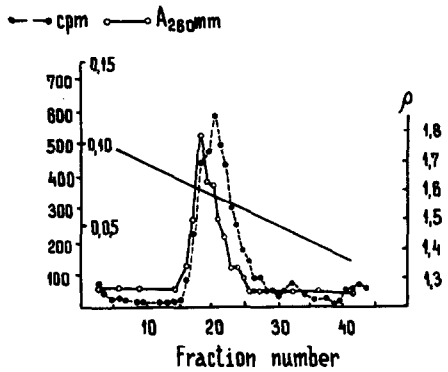

Fig. 1. Banding in CsCl of a ribosomal preparation from the infected cells. The infected cells were labelled with [^{14}C] valine for 60 min at 3.5 hr after infection and then chased for 40 min in the presence of a 100-fold excess of unlabelled valine. The ribosomal fraction obtained and treated as described under Methods was centrifuged in a preformed CsCl-density gradient in a Beckman SW-39 rotor for 14 hr at 36 000 rpm and 21°C.

3. Results and discussion

3.1. *Ribosome-bound labelled proteins in homogenates of the virus-infected cells*

Sucrose-density gradient analysis of extracts from cells labelled and chased at the time of active synthesis of virus-specific proteins, has shown that some radioactivity was always present in the ribosomal region as well as in the regions of ribosomal subunits. This ribosome-associated radioactivity in experiments with 5 min pulse – 40 min chase, comprised from 4 to 9% of the total radioactivity recovered from the gradients. Fractions containing ribosomes and ribosomal subunits retained radioactivity upon recentrifugation in sucrose-density gradients.

Fractions from the ribosomal region of the gradient were pooled, treated with formaldehyde and subjected to centrifugation in a CsCl gradient (fig. 1).

The main pool of ribosomes at 3.5–4 hr after infection had the mean buoyant density of 1.57 g/cm^3, as judged by the optical absorption of the fractions. The peak of radioactivity, however, was located in a region with a somewhat lower density (1.54 g/cm^3). Treatment with 0.5–1.0% deoxycholate resulted in dissociation from the ribosomes of about 60% of the radioactivity. Deoxycholate treatment did not seem to affect significantly the relative proportions of different labelled polypeptides which remained ribosome-associated. Extracts from the infected cells mildly treated with pancreatic RNAase (7.5 μg/ml, 10 min, 0°C) contained essentially the same proportions of the radioactivity in the ribosomal region of the gradient. These results could be interpreted to mean that the radioactivity was associated with a fraction of ribosomes containing some additional proteins, including the translation factors, and active in protein synthesis (see [7]). On the other hand, the association of radioactive ribosomes with a lipid-containing component cannot at present be excluded.

3.2. *Nature of the radioactive polypeptides associated with ribosomes from infected cells*

Ribosome-bound labelled polypeptides were analysed by electrophoresis in polyacrylamide SDS-containing gels. The results presented in fig. 2, show that the radioactivity was mainly associated with three distinct polypeptides which comigrate with certain virus-specific proteins (fig. 2a). Among these were the following: polypeptide 5 [3] or E [5] with an estimated molecular weight of 53–58 kdalton, polypeptide 10 or G of 16–18 kdalton and a polypeptide with a molecular weight of about 22 kdalton (the latter could be resolved only in long gels and it was not listed in [3]). All these polypeptides are not structural components of the EMC virion [3, 5]. It was suggested [8, 9] that the polypeptide with a molecular weight of 22 kdalton (polypeptide 22) found in EMC virus-infected cells might be a host-specified product. Our preliminary observations showing the absence of this polypeptide from the infected cells incubated with some protease inhibitors, suggest, on the contrary, that polypeptide 22 is virus-specific. Elucidation of the exact origin of this protein requires further studies. As can be seen in fig. 2, a low radioactivity was also found in several other proteins, in particular in a polypeptide of molecular weight of about 46 kdalton and in some structural proteins of the virion.

3.3. *Comparison of ribosome-associated proteins in normal and EMC virus-infected cells*

Sucrose-density gradient centrifugation of extracts similarly prepared from the non-infected cells, also revealed some radioactivity apparently associated with

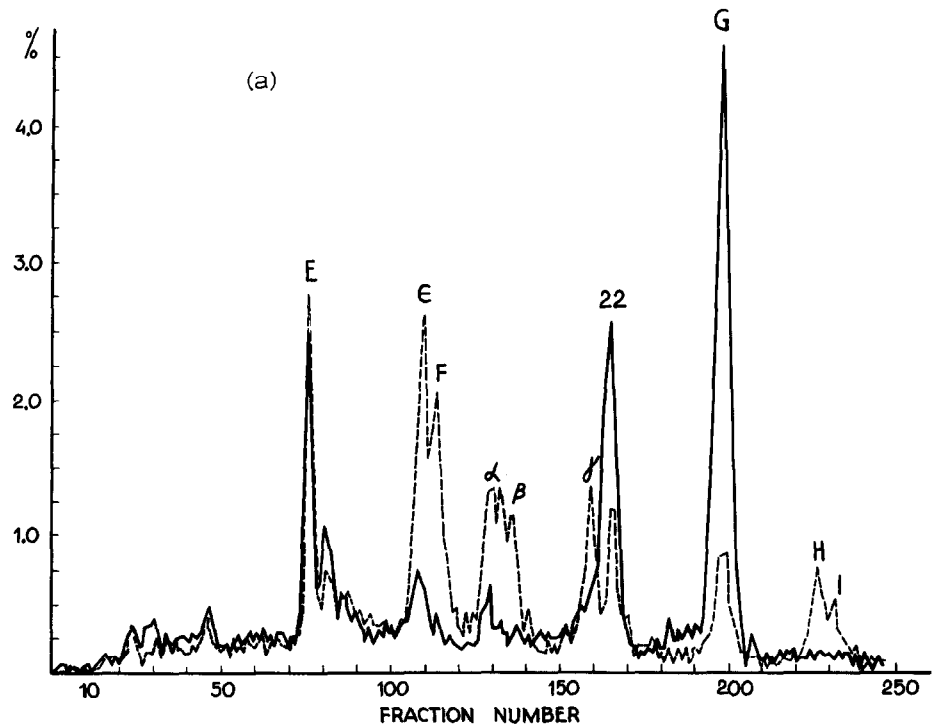

Fig. 2. Electrophoretic profiles of labelled proteins associated with ribosomes from infected and non-infected cells. Labelling of cells with either [^{14}C]- or [^{3}H]valine and chasing were performed as described in the legend to fig. 1, (both non-infected and infected cells were treated identically): (a) (——) ^{14}C-labelled proteins associated with ribosomes from the infected cells; (– – – – –) ^{3}H-labelled proteins from unfractionated extract of the infected cells.

ribosomes. Polyacrylamide gel electrophoresis of the material from the corresponding fractions of the gradient indicated the presence of three major labelled polypeptides (fig. 2b), their molecular weights (89–90, 43–48 and 39–40 kdalton, respectively) being different from those of the major radioactive polypeptides bound to ribosomes from the infected cells. It is not yet known whether one of the former proteins is identical with a minor labelled polypeptide of about 46 kdalton bound to the ribosomes from infected cells. In any case, the presence of polypeptides E, G and 22 is specific for ribosomes isolated from the cells infected with EMC virus.

Thus, the results presented in this paper show that ribosomes from the cells infected with EMC virus specifically bind several polypeptides electrophoretic mobilities of which correspond to that of non-structural virus-specific proteins.

Cooper et al. [2] reported that some structural polypeptides of poliovirus were associated with the smaller ribosomal subunit. Our preliminary observations also indicate that the relative proportion of different labelled polypeptides bound to the smaller ribosomal subunit from EMC virus-infected cells is not the same as in either whole ribosomes or the larger subunit, the structural polypeptides of the virion being a much more prominent component in the former particles than in the latter.

It is not yet known whether the complexing of the virus-specific proteins with the ribosomes occurs after the completion of the synthesis of polypeptide chains or as a result of the impaired termination of the translation process. It should be noted that the current opinion [8, 9] places the cistrons coding for polypeptides E and G to distinctly different loci of EMC virus RNA.

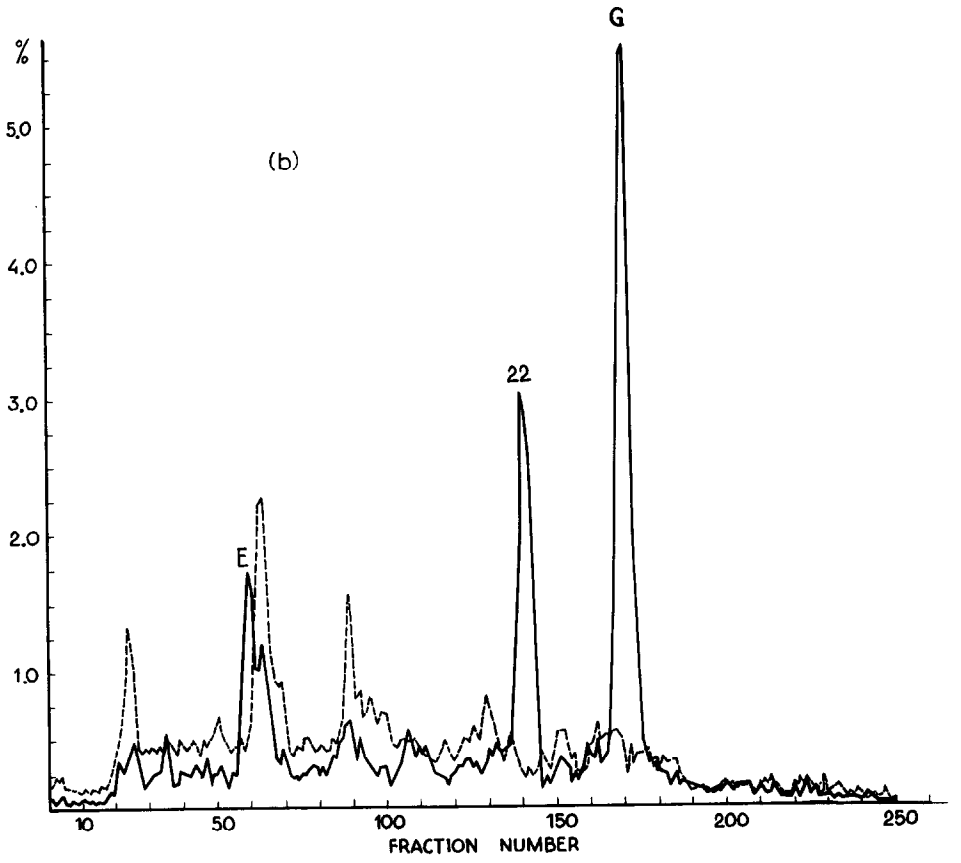

Fig. 2. (b) (——) Same as in (a); (– – – –) ^{3}H-labelled proteins associated with ribosomes from non-infected cells. The ordinate-radioactivity of a given fraction as per cent of the total radioactivity recovered from the gradient.

At present, we have no information indicating the functional role of the ribosome-associated virus-specific proteins in the control of translation in EMC virus-infected cells. But if we assume that the binding of the proteins is not an artifact arising after disruption of the cells (an assumption which is not as yet rigorously proven) then we are compelled to think over a functional significance of this binding. It may be suggested, for instance, that these proteins participate in ensuring the selective translation of virus-mRNA or in the cleavage of large precursor(s) of viral proteins.

The finding of several labelled proteins on the ribosomes from uninfected cells raises a separate problem which merits further investigation.

Acknowledgement

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