Sindbis virus-induced inhibition of protein synthesis is partially reversed by medium containing an elevated potassium concentration

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Infection of cultured vertebrate cells by Sindbis virus, an alphavirus, results in a reduction in the overall rate of protein synthesis and in selective termination (shutoff) of host-specified protein synthesis. The shutoff of host protein synthesis by Sindbis virus has been temporally correlated with a decrease in intracellular K⁺ concentration (and an increase in intracellular Na⁺ concentration) which occurs as a consequence of virus-mediated inhibition of the plasma membrane-associated Na⁺/K⁺ ATPase. Incubation of Sindbis virus-infected cells in medium containing an elevated concentration of K⁺ resulted in an increase in the intracellular concentration of K⁺, an increase in the overall rate of protein synthesis, and in partial reversal of the virus-induced termination of cell-specified protein synthesis. These results suggest that the virus-induced decrease in intracellular K⁺ concentration is required for efficient shutoff of host protein synthesis by Sindbis virus.

Shutoff of host macromolecular synthesis by certain lytic viruses is important in the sequence of events leading to cell death. In the case of cytopathic infection of vertebrate cells by Sindbis virus, an alphavirus, subordination of cellular protein synthesis may be, in part, an indirect result of virus-induced plasma membrane modification(s). An early event during lytic infection by Sindbis virus is inhibition of the plasma membrane-associated Na⁺/K⁺ ATPase (Garry et al., 1979; Ulug et al., 1984, 1989; Munoz et al., 1985). This ion transport system is largely responsible for maintaining high intracellular concentrations of K⁺ ([K⁺]ᵢ) and low intracellular concentrations of Na⁺ ([Na⁺]ᵢ), relative to the extracellular fluid. Sindbis virus-induced inhibition of the Na⁺/K⁺ ATPase begins at 2 to 3 h post-infection (p.i.) and is temporally correlated with an increase in [Na⁺]ᵢ and a decrease in [K⁺]ᵢ. Like other lytic viruses, such as influenza virus (Pinto et al., 1992) and human immunodeficiency virus (Miller et al., 1991; Garry et al., 1991; Mobley et al., 1992), alphaviruses encode envelope proteins that can function as ion channels (Schlegel et al., 1991). Alphaviruses have also been reported to activate membrane-associated phospholipases that could alter ion permeability (Perez et al., 1993).

The intracellular ionic changes induced by Sindbis virus are temporally correlated with the virus-induced shutoff of protein synthesis (Garry et al., 1979; Ulug et al., 1984, 1989; Munoz et al., 1985). In the case of Sindbis virus, shutoff involves two effects: (i) a reduction in the overall rate and (ii) a selective termination of cellular protein synthesis. Sindbis virus mRNAs are able to initiate translation efficiently under altered ionic conditions such as those found in the infected cell, whereas most cellular mRNAs are not efficiently translated. Therefore, we postulated that the Sindbis virus-induced alterations in [Na⁺]ᵢ and/or [K⁺]ᵢ may be involved in the shutoff of protein synthesis (Garry et al., 1979).

If the alterations in intracellular monovalent ion concentrations that occur in Sindbis virus-infected cells are involved in shutoff, then conditions which restore intracellular ion concentrations to those found in uninfected cells would be expected to increase protein synthesis. Alonso & Carrasco (1981) demonstrated that incubation of encephalomyocarditis (EMC) virus-infected cells in medium containing a low concentration of Na⁺ resulted in a complete reversal in the shutoff of host protein synthesis. However, in Sindbis virus-infected cells, previous studies have demonstrated that low Na⁺ medium, which lowers [Na⁺]ᵢ to levels found in uninfected cells incubated in normal medium, failed to restore host-specified protein synthesis (Garry et al., 1979). However, in addition to elevating [Na⁺]ᵢ, Sindbis virus inhibition of the Na⁺/K⁺ ATPase also decreases [K⁺]ᵢ. Therefore, the present studies were undertaken with media containing elevated concentrations of K⁺, which increases [K⁺]ᵢ (Garry & Bostick, 1986), to define the possible role of K⁺ in regulation of protein synthesis in Sindbis virus-infected cells.

BHK cells were infected with a low multiplicity of Sindbis virus (5 p.f.u./cell) or mock-infected and at 2 h.p.i. the normal medium containing approximately
5 mM-KCl was removed and replaced with media containing various concentrations of added KCl between 50 and 200 mM (final concentrations of approximately 55 to 205 mM). Proteins synthesized in the cells were labelled for 1 h beginning at 4 h p.i., by incubating the cultures in media that contained altered KCl concentrations, lacked unlabelled methionine, and were supplemented with 25 μCi/ml [35S]methionine. After the labelling period, cell extracts were prepared, proteins were separated by SDS–PAGE, visualized by autoradiography, and quantified by scanning densitometry. Incubation of mock-infected cells in medium containing 100 or 200 mM of

added KCl inhibited protein synthesis by more than 90% compared to cells incubated in normal medium (Fig. 1a, e). Similar results were obtained by quantitative analysis of [35S]methionine incorporation into TCA-precipitable radioactivity.

The effect of high KCl medium on mock-infected cells contrasts directly to the effect on Sindbis virus-infected cells. At 4 to 5 h after Sindbis virus infection of BHK cells, the overall rate of protein synthesis was reduced by over 75% and cell protein synthesis was selectively reduced over 95% (Fig. 1b, lane 1). Incubation of Sindbis virus-infected cells in medium containing 100 mM
of added KCl resulted in an increase in the overall rate of protein synthesis at 4 to 5 h p.i. (Fig. 1b, lane 3). The synthesis of both cellular proteins and virus-specified proteins was increased by the altered media, although the synthesis of certain cellular proteins was increased to a greater extent than that of other cell proteins (for example, those labelled ‘a’ and ‘t’). KCl medium (200 mM) also increased total protein synthesis in Sindbis virus-infected cells, although to a lesser extent than medium containing 100 mM-KCl. Osmotic effects other than those related to modulation of intracellular ion concentration could contribute to the lower overall rate of protein synthesis in cells exposed to medium containing this high KCl concentration. Nevertheless, the increase in cell-specified protein synthesis relative to that which was virus-specified was higher in infected cells incubated in medium containing 200 mM of added KCl than in infected cells incubated in medium containing 100 mM of added KCl (Fig. 1b, compare lanes 3 and 4).

We have also examined the effect of high KCl medium at various other times following Sindbis virus infection. By 6 to 7 h p.i., Sindbis virus infection of BHK cells incubated in normal medium reduced overall protein synthesis to less than 10% of the rate in mock-infected cells (Fig. 1c, lane 1). As at 4 to 5 h p.i. (Fig. 1b), incubation of infected cells in medium containing added K+ (beginning at 4 h p.i.) resulted in increased synthesis of both virus- and cell-specified protein synthesis at 6 to 7 h p.i. (Fig. 1c, lanes 2 to 4). The increase in protein synthesis was largely due to virus-specified protein synthesis. Sindbis virus is known to inhibit cellular mRNA synthesis, and therefore, relative to earlier times after infection, at this later time fewer cellular mRNAs may be available for translation. During a period after infection preceding Sindbis virus-induced inhibition of the Na+/K+ ATPase and shutoff of host protein synthesis, protein synthesis in Sindbis virus-infected cells was inhibited by medium containing 100 or 200 mM of added KCl.

Incubation of mock-infected cells in medium containing elevated concentrations of KCl resulted in an increase in [K+]i from approximately 150 mM in normal medium to over 250 mM in 200 mM-KCl medium as determined by quantification of the equilibrium distribution of 86Rb+, a potassium tracer. This increase in [K+]i, above the physiological range partly accounts for the inhibition of protein synthesis in mock-infected cells incubated in medium containing excess KCl. This could also involve the increase in the counterion. As previously observed (Garry et al., 1979; Ulug et al., 1984, 1989), the [K+]i of mock-infected BHK cells incubated in normal medium was approximately 140 mM, and Sindbis virus infection of BHK cells resulted in an approximately 40% decrease in [K+]i by 5 h p.i. Media containing elevated concentrations of KCl also increased [K+]i in Sindbis virus-infected cells. However, under these conditions of elevated KCl medium, [K+]i was similar to that in uninfected cells incubated in normal medium (a maximum [K+]i of approximately 170 mM in 200 mM-KCl medium).

To determine whether the effects of the increased KCl medium were due to the increase in K+ or to the increase in the counterion, Cl−, experiments similar to those conducted with altered KCl media were conducted with media formulated with potassium acetate to increase the concentration of K+. These high K+ media were also capable of partly reversing the shutoff of host protein synthesis by Sindbis virus and were inhibitory to protein synthesis in mock-infected cells. In contrast, media containing elevated concentrations of sucrose which elevates medium osmolality failed to reverse the shutoff, although at high concentrations high sucrose medium did inhibit protein synthesis in mock-infected cells. These results suggest that the reversal by high KCl medium of Sindbis virus-induced shutoff of host protein synthesis is specific to the K+ content of the medium and does not depend on the specific counterion or on the osmolality of the medium.

One possibility to account for the increase in cellular protein synthesis in Sindbis virus-infected cells incubated in high KCl medium is that the altered medium increased the level of translatable cellular mRNA. To determine whether the increase in cellular protein synthesis observed in Sindbis virus-infected cells incubated in high KCl medium required de novo synthesis of mRNA, Sindbis virus- and mock-infected cells were incubated in the presence of actinomycin D (AD), an intercalating agent which inhibits DNA-directed RNA synthesis. The normal medium was then replaced with media containing increased concentrations of KCl (in the presence of AD) at 2 h p.i., and the cells were labelled between 4 and 5 h p.i. as in Fig. 1(b). Incubation of the Sindbis virus-infected, AD-treated cells in medium containing increased KCl also resulted in an increase in the total rate of protein synthesis (Fig. 1d). Both the synthesis of cellular and viral proteins was increased, although the relative synthesis of cellular proteins was less than in the absence of AD (Fig. 1, compare b and d). This is apparently due to a decrease in the levels of cellular mRNA as a result of AD inhibition of mRNA synthesis. The cellular proteins which were synthesized in Sindbis virus-infected cells incubated in high KCl medium in the presence of AD, such as the protein designated ‘a’, may have mRNAs which possess longer half-lives than the mRNAs for proteins, such as ‘t’, which were synthesized under these medium conditions in the absence of AD. Consistent with this result, high KCl medium failed to increase the level of functional mRNA in Sindbis virus-
or mock-infected cells which could be translated in a wheatgerm in vitro protein synthesizing system (data not shown). Collectively, these results suggest that the increase in protein synthesis in Sindbis virus-infected cells exposed to high KCl medium (Fig. 1) was not due to increased synthesis of functional mRNA.

Previously we proposed a model which suggested that the alteration in [K+]i may contribute to shutoff of protein synthesis by Sindbis virus in two ways: (i) by reducing the number of initiation complexes which may be formed and (ii) by providing conditions in which alphavirus mRNAs outcompete cellular mRNAs for a component(s) of the initiation complex (Garry et al., 1979). The results presented here contribute evidence for this model by demonstrating that medium alterations that increase [K+]i increase the overall rate of protein synthesis in Sindbis virus-infected cells without increasing the levels of translatable RNA. At times when the synthesis of cell proteins was almost entirely inhibited by Sindbis virus infection in normal medium, both cellular proteins and viral proteins were synthesized in infected cells incubated in high KCl medium. The increased cellular protein synthesis represents, at least in part, translation of cellular mRNAs present prior to infection. Translation of these mRNAs is blocked in Sindbis virus-infected cells incubated in normal medium. Thus, an important conclusion of the present study is that the competitive translational advantage of the viral mRNAs over cellular mRNAs is reduced when the [K+]i of infected cells is increased to values which are comparable to those of uninfected cells in normal medium.

The results presented here are consistent with previous studies suggesting that shutoff of host protein synthesis by alphaviruses is a consequence of translational competition and interactions of viral envelope proteins with the plasma membrane (Garry et al., 1979; Ulug & Bose, 1985; Schlesinger et al., 1993). For example, an in-frame insertion into the Sindbis virus gene for 6K, a highly palmitoylated, hydrophobic membrane protein, interferes with both virus glycoprotein processing and shutoff of host protein synthesis (Schlesinger et al., 1993). The possibility that Sindbis virus may employ mechanisms in addition to mRNA competition and alteration of intracellular [K+]i concentrations to inhibit cellular protein synthesis has not been excluded. The partial shutoff of host cell protein synthesis observed in Sindbis virus-infected cells incubated in high K+ medium might be due to virus-induced alterations of Na+ or other ions. It has been suggested that the capsid protein of alphaviruses, which has been found in initiation complexes, may induce an alteration in the protein synthesizing machinery which favours viral mRNA translation (van Steeg et al., 1984; Elgizoli et al., 1989). In this regard, the observation that the termination of cellular protein synthesis was partially reversed by medium containing increased K+ is of interest, since under these conditions the synthesis of the capsid protein was increased relative to the levels in infected cells incubated in normal medium (Fig. 1b, c). Wild-type levels of the capsid protein were also synthesized by Sindbis virus 6K insertion mutants defective in shutoff of host protein synthesis (Schlesinger et al., 1993).

As for Sindbis virus, cytopathic or fusogenic viruses of several other groups, including picornaviruses, induce alterations in intracellular cation concentrations (Alonso & Carrasco, 1981; Schaefer et al., 1984; Castrillo et al., 1987; Garry, 1989a; Garry & Waite, 1979; Carrasco & Esteban, 1982; Carrasco et al., 1989; Cloyd et al., 1989; Cloyd & Lynn, 1991). Incubation of EMC virus-infected HeLa cells in medium containing a reduced concentration of NaCl results in a reversal of the selective termination of host protein synthesis and an inhibition of the synthesis of EMC virus-specified proteins (Alonso & Carrasco, 1981). Shutoff by poliovirus is also reversed by low Na+ medium early after infection (Castrillo et al., 1987; Garry, 1989a). Incubation in low NaCl medium decreases intracellular concentrations of both Na+ and K+ (Garry et al., 1979). Under these conditions, picornavirus mRNAs do not appear to possess a competitive translational advantage over cellular mRNAs, and, in fact, appear to be at a competitive disadvantage when the intracellular concentrations of monovalent ions are severely reduced. In contrast, medium containing a low concentration of Na+ reduced [Na+]i in Sindbis virus-infected cells, but generally failed to restore host protein synthesis (Garry et al., 1979). In further contrast to our results in Sindbis virus-infected cells, high KCl medium failed to restore host protein synthesis in either EMC virus-infected cells (Alonso & Carrasco, 1981) or wild-type poliovirus-infected cells (Castrillo et al., 1987; Garry, 1989a). Among other shutoff mechanisms, different lytic viruses appear to employ alternative strategies involving monovalent ions to terminate cellular protein synthesis.

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References


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