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Specific Monovalent Cation Effects on Modification of Reovirus Infectivity by Chymotrypsin Digestion In Vitro

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Specific monovalent cations control the modification of reovirus infectivity by chymotrypsin. Digestion in K^+, Rb^+, or Cs^+ reduces infectivity several logs, whereas in Na^+ or Li^+ digestion markedly enhances infectivity.

Recently, we demonstrated that specific monovalent cations have a remarkable influence on both the activation of reovirus transcriptase (2) and the conversion of intact reovirions to various subviral particles (1) by chymotrypsin (CHT) digestion in vitro. Digestion in the presence of facilitating monovalent cations (K^+, Rb^+, or Cs^+) leads to activation of the latent transcriptase activity, as determined by in vitro assay, and conversion of the ~75-nm diameter intact virions to ~50-nm diameter cores. Digestion in the presence of nonfacilitating monovalent cations (Na^+ or Li^+) leads to conversion of the intact virions to subviral particles (SVP's) which are ~65 nm in diameter and in which the transcriptase activity is retained in a latent state. Subsequent addition of sufficient facilitating monovalent cation permits the conversion to proceed from the intermediate SVP stage to the final core stage, with concomitant activation of the transcriptase. In this preliminary report we demonstrate that modification of the infectivity of reovirions by CHT digestion in vitro also is controlled by the species of monovalent cation present during the digestion.

Reovirus type 3, Abney strain, was grown in suspension cultures of L cells and purified by isopycnic and velocity centrifugation as previously described (2). After the final purification step, virus was dialyzed against 0.10 M Tris-Cl (pH 8.3), containing 0.10 M NaCl, and stored in this buffer at ~300 μg/ml. For CHT digestion, samples of the virus were diluted five times into 0.10 M Tris-Cl (pH 8.3), containing 0.20 M of the desired monovalent cation (chloride salt) along with a specified concentration of CHT, and incubated at 37 C for 1 h. Samples from the incubation mixture were sonicated and examined for RNA polymerase activity in vitro (2), particle diameter (electron microscopy; reference 1), and infectivity titer (2). The results are presented in Table 1. After incubation of the purified virus in either NaCl or CsCl without any CHT, analysis indicated that the particles were ~75 nm in diameter, the transcriptase was in a latent state, and the preparations had infectivity titers of 1.9×10^9 and 2.0×10^9 PFU per milliliter, respectively. After digestion with CHT in the presence of NaCl or LiCl, the particles had a diameter of ~65 nm, transcriptase was latent, and infectivity titers were markedly enhanced to 1.4×10^{10} and 2.1×10^{10} PFU per milliliter, respectively. These differences in titer were not altered significantly between 5 and 7 days of incubation of the monolayers, eliminating the possibility that slowly developing plaques were responsible for the differential titer. After digestion in the presence of KCl, RbCl or CsCl, the particles had a diameter of ~50 nm, transcriptase was active, and infectivity titers were markedly reduced to 2.3×10^7, 1.0×10^6, and 4.4×10^5 PFU per milliliter, respectively. The values in the table are for a particular preparation, but we have repeated this basic experiment several times with qualitatively identical results. Quantitatively, there is a fair degree of variation with infectivity enhancements ranging between 6-fold and 40-fold and infectivity reductions ranging between 2 and 4 logs in different experiments. Several experimental parameters including virus and CHT concentrations, in addition to the species of monovalent cation present, influence the results. These factors are being investigated in detail. Nevertheless, at this time it is clear from the above data that specific

TABLE 1. *Particle diameter, transcriptase activity, and infectivity titer of purified reovirus digested with CHT in the presence of specific monovalent cations*

Salt (0.2 M)	CHT[a] (μg/ml)	Particle diameter (nm)	Transcriptase activity[b]	Infectivity titer (PFU/ml)	Relative infectivity	Remarks
NaCl	0	75	0.5	1.9×10^9	1.00	Intact virions
CsCl	0	75	3.1	2.0×10^9	1.05	Intact virions
NaCl	40	65	0.7	1.4×10^{10}	7.38	Intermediate SVP
LiCl	40	65	2.2	2.1×10^{10}	11.0	Intermediate SVP
KCl	40	50	77.8	2.3×10^7	1.21×10^{-2}	Cores
RbCl	40	50	79.6	1.0×10^6	5.26×10^{-4}	Cores
CsCl	40	50	103.7	4.4×10^5	2.32×10^{-4}	Cores

[a] Virus concentration was 60 μg/ml in the digestion mixture.

[b] Picomoles of ^{3}H-UMP incorporated into trichloroacetic acid-precipitable form in 60 min at 37 C by 6 μg of virus.

monovalent cations play a major role in controlling the CHT-mediated modification of the infectivity of reovirions, as well as of particle structure and transcriptase activation.

Several authors (5, 6, 7, 8) reported enhancement of infectivity of reovirus preparations after digestion with proteolytic enzymes, whereas others (3, 4) reported a decrease in infectivity after digestion with CHT. We suggest that the monovalent cation involvement in this phenomenon which is reported here is one of the critical factors in its control.

A further point not made in the table is that, in addition to the enhanced infectivity titer of the (Na^+·CHT) and (Li^+·CHT) SVP's, the plaques arising from them develop earlier than do plaques from intact virions. Typically, with the intermediate SVP's, plaques of pin-head size are first visible on day 3 of the titration incubation, whereas with intact virions plaques of this size are first visible on day 4. Also, we have tested unpurified virus in cell lysates and found a qualitatively identical effect of the specific monovalent cations.

It is of interest to consider some of the implications of the above data. First, the marked loss of infectivity which is correlated with transcriptase activation suggests the necessity for a mechanism to prevent such activation until the particle is inside the host cell. Second, the enhanced infectivity of the intermediate subviral particles suggests a possible advantage to the virus if the intact virions were converted extracellularly to such intermediates. A simple model incorporating these suggestions is offered for speculation, as follows. (i) Intact virions, on being transmitted to a host animal, initially enter into the extracellular fluids which characteristically contain high Na^+ ion concentrations. (ii) Proteases present in the extracellular fluids convert the intact virions to the intermediate particles. Transcriptase activation is prevented by the Na^+ ion environment. (iii) The intermediate particles very efficiently gain entry into cells. (iv) Transcriptase activation occurs, mediated by the high K^+ ion concentration present intracellularly. (Other data [reference 2; manuscript in preparation] indicate that activation of transcriptase in intermediate subviral particles occurs upon addition of facilitating monovalent cations and does not require further digestion by chymotrypsin.) In such a hypothetical scheme the differential effects of the specific monovalent cations, (i) serve to insure that the virion transcriptase is not activated extracellularly; (ii) increase the probability that the particle does successfully enter a cell and; (iii) mediate activation of the transcriptase inside the host cell. Such interaction between viral particles and their environment, resulting in modification of the structural and functional characteristics of the virus particles in a manner determined by properties of the environment, would allow the virus a degree of positive control over its own transmission and propagation.

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