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NOTES

Transcription of Vesicular Stomatitis Virus Activated by Pardaxin, a Fish Toxin That Permeabilizes the Virion Membrane

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The toxic protein, Pardaxin, of the Red Sea flatfish *Pardachirus marmoratus* readily induced transcription of vesicular stomatitis virus by making the virion membrane permeable to nucleoside triphosphates in the absence of nonionic detergents. Virion transcription was activated over a wide range of Pardaxin concentrations, but at optimal concentrations, the rate of transcription exceeded that induced by Triton X-100. The inhibitory effect of M protein was manifested for both Pardaxin-induced and Triton-induced transcription at high concentrations of vesicular stomatitis virions; however, unlike the Triton-induced reaction, the inhibitory effect of M protein was not reversed by polyglutamic acid added to the Pardaxin-induced transcription reaction. We propose that activation of virion transcription by Pardaxin resembles more closely intracellular transcription initiated by virion penetration than does detergent-activated transcription of vesicular stomatitis virus.

The virion of vesicular stomatitis (VS) virus consists of a nucleocapsid core and a limiting membrane derived from the plasma membrane of the infected host cell (13, 17). The virion membrane contains two proteins, an externally oriented, intrinsic glycoprotein (G) (16) and a matrix (M) protein which apparently lines the inner surface of the lipid bilayer (13, 18) in close proximity to the nucleocapsid (8). The three other viral proteins comprise the nucleocapsid and collectively serve as the RNA-dependent RNA polymerase (9), which sequentially transcribes the five mRNA's that are then translated into the five viral proteins (1, 6). In their original description of the VS virion transcriptase, Baltimore, Huang, and Stampfer (2) clearly demonstrated that the virion membrane is impermeable to nucleoside triphosphates and that induction of transcription requires nonionic detergents. The only role of the detergents is to solubilize the limiting membrane since nonenveloped VS viral nucleocapsids isolated from infected cells function equally well or better in the transcription reaction in the absence of detergents (12).

For many years, we and other investigators have tried unsuccessfully to activate VS virion transcription in vitro in the absence of detergents. Attempts to disrupt the VS virion membrane by simple physical means, such as ultrasonic vibration, ionic shock, or freeze-thawing, have failed to induce transcription (B. S. Fong, Ph.D. dissertation, University of Virginia, Charlottesville, 1977; E. J. Patzer, unpublished data). Very recently, the cationic bee venom protein, melittin, has been found to render the membrane of avian retroviruses permeable to deoxynucleoside triphosphates, thereby allowing the reverse transcriptase to synthesize authentic cDNA in the absence of detergents (3–5). Melittin also promotes transcription by VS virions in detergent-free suspensions (3; A. K. Banerjee, personal communication), an observation that we have confirmed.

When enveloped viruses, such as VS virus, infect a host cell, the transcribable nucleocapsid is uncoated and released into the cytoplasm either as a result of fusion of the virion membrane with cytoplasmic membrane (10) or by interaction with coated phagocytic vesicles and lysosomes (11). We have continued to pursue our search for nondetergent substances which, by physical means, would make the membrane permeable or dissociate it from the nucleocapsid in a manner that simulates VS virion penetration into host cells and that initiates the transcriptional event.

In this communication, we report the action

of such a substance, Pardaxin, the hydrophobic, hemolytic toxin secreted from the skin of the Red Sea flatfish *Paradachirus marmoratus* (14, 15). Pardaxin is an acidic protein of molecular weight ≃17,000 that is composed of 162 amino acids and is devoid of arginine, histidine, and trytophan but is rich in serine, glycine, and phenylalanine (14). Preliminary studies in our laboratories have shown that Pardaxin exerts a profound effect on the phase separation behavior of membrane lipids and on the morphology of artificial lipid vesicles and VS virions (unpublished data). Pardaxin also makes membranes permeable, as demonstrated by enhanced leakage of 6-carboxylfluorescein entrapped in lipid vesicles (E. Zlotkin, personal communication).

The ability of Pardaxin to induce transcription by VS virions (0.2 mg/ml) was tested at 31°C in a transcription reaction mixture which, as described previously (7, 9), contains all four nucleoside triphosphates and Mg^{2+} in a pH 8.0 buffer, except that Pardaxin was substituted for Triton X-100. Synthesis of RNA was measured at 60 min by incorporation of [^{3}H]uridine into acid-precipitable material. No RNA synthesis was detected in the absence of Pardaxin or Triton. Figure 1 shows that RNA transcription by VS virions can be readily induced by various amounts of Pardaxin present in the reaction mixture in the absence of detergent. The amount of RNA synthesized was a function of Pardaxin concentration; transcriptase activity was detected with as little as 2 μg of Pardaxin but optimal transcription was observed at a level of 10 μg of Pardaxin. It was found that larger amounts of Pardaxin were inhibitory, resulting in gradual decline in transcriptase activity of the same concentration (0.2 mg/ml) of virions. Similar dose-dependent effects were noted for activation of retrovirus reverse transcriptase by melittin (3), but in this case, optimal activity was manifested over a much narrower range than was the case for Pardaxin activation of VS virion transcription.

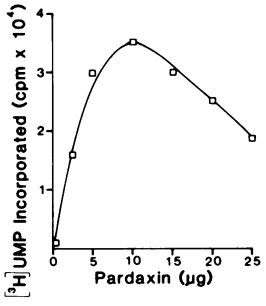

FIG. 1. *Activation of transcription of VS virions as a function of Pardaxin concentration. The RNA-dependent RNA polymerase was assayed in vitro as previously described (7, 9). Reaction mixtures (250 μl) were prepared with VS virions at final protein concentrations of 0.2 mg/ml, and each sample was treated with various amounts of Pardaxin for 10 min. The other components of the final reaction mixture were 7.4 × 10^{-4} M dithiothreitol, 5 × 10^{-3} M magnesium acetate, 1.44 × 10^{-1} M NaCl, 5.87% glycerol, 7 × 10^{-4} M each of ATP, CTP, and GTP, and 6.7 × 10^{-5} M [^{3}H]UTP (230 μCi/μmol) in 3 × 10^{-2} M HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer (pH 8.0). The samples were incubated in stoppered tubes at 31°C for 60 min. The reaction was terminated by adding 0.6 ml of 0.06 M sodium pyrophosphate with 200 μg of yeast carrier RNA and placed in an ice water bath. Ice-cold 25% trichloroacetic acid (0.5 ml) was added to each tube, and the acid-insoluble RNA was measured by scintillation spectrometry.*

In Fig. 2, the kinetics of the transcriptase reaction of VS virions activated by optimal concentrations of Pardaxin or Triton X-100 are compared. As noted, both of the reactions were linear over the 90-min period measured; however, Pardaxin clearly induced a higher rate of RNA synthesis than did Triton. Identical results were obtained when Nonidet P-40 was compared with Pardaxin. In fact, initial studies with fresher batches of Pardaxin resulted in transcription levels 2 to 3 times higher than those in parallel studies with Triton X-100. It appears that the various properties of Pardaxin diminish upon

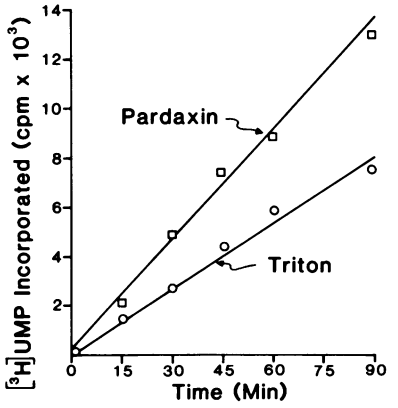

FIG. 2. *Comparative kinetics of VS virion transcription activated by Pardaxin or Triton X-100. Transcriptase activity was assayed at final viral protein concentrations of 0.2 mg/ml as described in the legend to Fig. 1. To each reaction mixture was added 10 μg of Pardaxin or Triton X-100 to a final concentration of 0.37%. At each time interval indicated, 50-μl samples were removed from each tube and placed in 0.067 M sodium pyrophosphate containing 200 μg of yeast carrier RNA. After 0.5 ml of 25% trichloroacetic acid was added, [^{3}H]uridine radioactivity was measured by scintillation spectrometry.*

storage, as exemplified by the gradual decline in its cytolytic and hemolytic activities with time (E. Zlotkin, personal communication). Although Pardaxin appears to augment the transcriptase activity of VS virions, an alternative explanation could be that nonionic detergents inhibit transcription by binding to nucleocapsids, which have been shown to have hydrophobic pockets of N protein that covalently links iodonaphthylazide (18). We had noted in previous experiments on in vitro transcription of intracellular VS viral nucleocapsids (12) that transcriptase activity was somewhat greater in the absence of nonionic detergents (R. L. Imblum and R. R. Wagner, unpublished data). The different mechanisms of action of Pardaxin and Triton X-100 were also suggested by the observation that VS virions exposed to Pardaxin exhibited no visible loss in turbidity, whereas Triton rapidly solubilized the turbid suspension of VS virions to a clear solution (data not shown). Electron microscopic examination of VS virions treated with Pardaxin (10 μg) revealed some intact virions and a certain proportion of extruded nucleocapsids, but the viral membranes retained the appearance of an intact bilayer despite marked alterations in the shape of the virion (Fig. 3).

The size distribution of RNA transcripts resulting from the in vitro polymerase reactions of VS virions activated by either Pardaxin or Triton X-100 was found to be indistinguishable by polyacrylamide gel electrophoresis (data not shown). This finding further demonstrates that Pardaxin can render the otherwise intact membrane permeable to nucleoside triphosphates, resulting in authentic transcription by the virion genome. We also tested the capacity of an optimal concentration of melittin (3) to induce transcription of VS virions and found that the melittin-induced transcriptase reaction was about equivalent in efficiency to that induced by Triton but somewhat inferior to that induced by

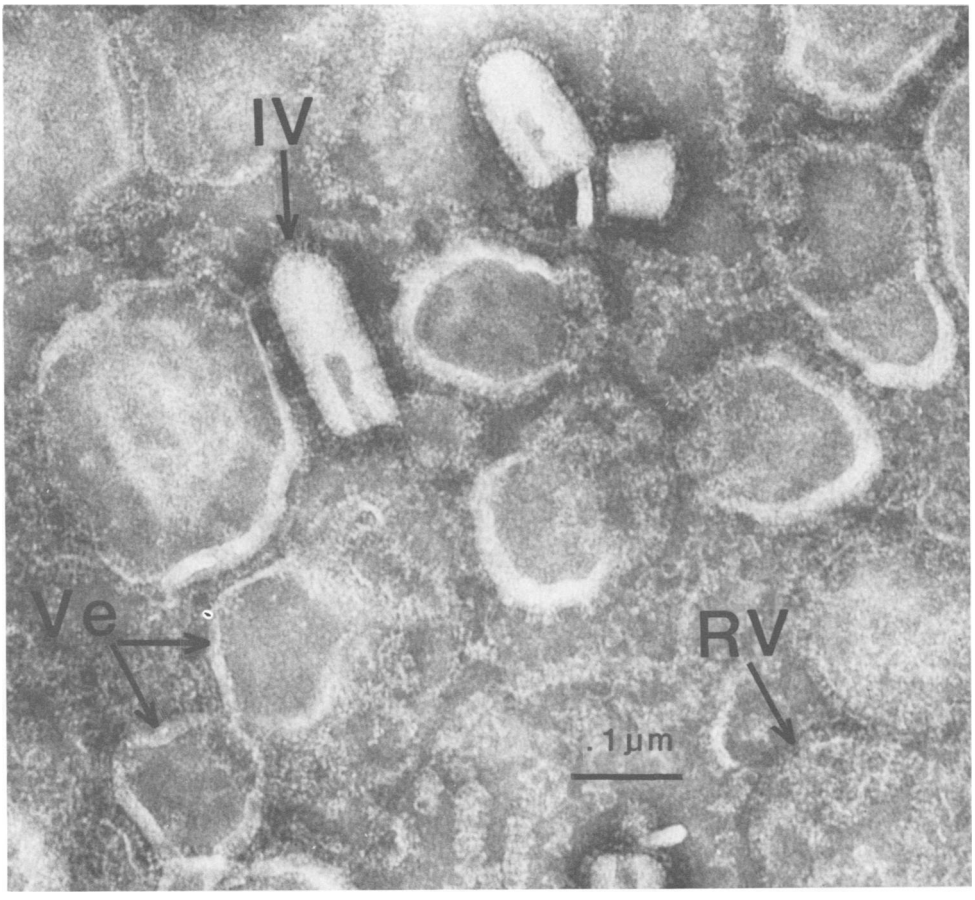

FIG. 3. *Negative-stain electron micrograph of VS virions exposed to 10 μg of Pardaxin for 10 min before mixing with 1% phosphotungstic acid. Shown are round virions (RV), vesicles (Ve), and intact virions (IV); ×140,000. At lower doses of Pardaxin, round virions predominated.*

Pardaxin (data not shown). As is the case in activation of reverse transcription by retroviruses (3), melittin-induced transcription of VS virus occurred over a much narrower dose range than was the case for Pardaxin-induced transcription of VS virus.

The M protein serves as an endogenous inhibitor of the transcription of VS virus (7). At high concentrations of VS virions in a reaction mixture containing Triton X-100, RNA synthesis is markedly suppressed. This inhibition by the positively charged M protein (pI ≃ 9.0) is readily reversed by certain polyanions, such as polyglutamic acid (7). It was of interest, therefore, to determine whether the M protein serves as an endogenous inhibitor of VS virion transcription activated by Pardaxin, in which the virion membrane is rendered permeable rather than being solubilized by detergent. To this end, the effects of Triton and Pardaxin were compared under conditions of optimal transcription occurring at low concentrations of VS virions (0.2 mg of viral protein per ml) and under conditions of transcription inhibition by M protein, which occurs at high concentrations of VS virions (0.8 mg of viral protein per ml). Also tested in each inhibited reaction was the capacity of polyglutamic acid (Sigma Chemical Co., St. Louis, Mo.) to reverse inhibition of transcription caused by M protein (7).

Data shown in Table 1 illustrate that transcription by VS virions was inhibited at high concentrations of virions when either Triton or Pardaxin was used to activate the transcription reaction. At high virion concentrations (0.8 mg/ml compared with 0.2 mg/ml), RNA synthesis was inhibited by 69% in the presence of Triton and by 80% in the presence of Pardaxin. Table 1 also shows that polyglutamic acid (2 mg/ml) completely reversed the inhibitory effect exhibited in the Triton-induced transcription reaction at high virion concentration (110% of the 0.2 mg/ml control level). In sharp contrast, polyglutamic acid had little or no effect on the suppressed level of RNA synthesis in the Pardaxin-induced transcription reaction at high concentrations of virions. We have no clear-cut explanation for this failure of polyglutamic acid to reverse the inhibition of transcription by M protein in the Pardaxin-induced reaction. One not very likely possibility is that the virion membrane made permeable to nucleoside triphosphates by Pardaxin is not permeable to polyglutamic acid. Another somewhat more likely possibility is that the acidic Pardaxin (14) binds tightly to the basic M protein (7), thus blocking the reaction of polyglutamic acid with the nucleocapsid-M protein complex. We have some preliminary evidence that negatively charged Pardaxin binds tightly to a nucleocapsid-M protein complex (unpublished data).

TABLE 1. *Comparative transcriptase activity of low (0.2 mg/ml) or high (0.8 mg/ml) concentrations of VS virions activated by Triton X-100 or Pardaxin and effect of polyglutamic acid on the inhibitory effect of M protein at high concentration*[a]

Transcriptase activator	[^{3}H]UMP incorporated (cpm/μg of protein per 60 min) at virion concn:		Ratio: 0.8 mg/0.2 mg[b]
	0.2 mg/ml	0.8 mg/ml	
Triton	204	64	0.31
Triton + polyglutamate	—[c]	226	1.11
Pardaxin	324	65	0.20
Pardaxin + polyglutamate	—[c]	89	0.27

[a] Transcriptase reactions were assayed by [^{3}H]uridine incorporation as described in the legends to Fig. 1 and 2 in mixtures containing VS virions at protein concentrations of either 0.2 or 0.8 mg/ml, samples of which were activated by either Triton X-100 (0.37%) or by pretreatment for 10 min with optimal Pardaxin (10 μg). Where indicated, polyglutamic acid dissolved in reticulocyte standard buffer (pH 8.0) was added to the reaction mixture at a concentration of 2 mg/ml; buffer alone was added to controls.

[b] Ratio of [^{3}H]UMP incorporated per milligram of viral protein under various conditions of activation, using 0.2 mg/ml of viral protein as the denominator.

[c] Not tested.

Although extremely useful for studying in vitro transcriptases of negative-strand viruses and retroviruses, nonionic detergents may inhibit faithful transcription by binding to hydrophobic sites of nucleocapsids. Certain events that occur during in vivo transcription (and replication) are difficult to imitate by in vitro transcription reactions. Moreover, the dissolution of virion membrane by nonionic detergents is not likely to mimic the dissociation of virion membrane that must occur when virions penetrate cells as infection is initiated. The use of melittin in the absence of detergents in the study of retrovirus transcription has already provided an important alternative means to study in vitro cDNA synthesis (3–5). We present here, in the potential use of the hydrophobic fish toxin, Pardaxin, an alternative means for studying in vitro transcription of VS virions which, hopefully, more closely mimics in vivo transcription than does detergent-activated transcription. The Pardaxin-induced transcription reaction is somewhat more efficient and can be induced with small doses of Pardaxin that do not dissolve the membrane. At doses of 1 to 2 μg (Fig. 1), Pardaxin activated

transcription without any dissociation of membrane from nucleocapsids, which occurs at higher doses (Fig. 3). In fact, in detailed studies to be reported later, we have found that nucleocapsids and intact envelopes of VS virions can be completely dissociated by 20 μg of Pardaxin and then separated by density centrifugation. Therefore, Pardaxin may provide a more physiological means for studying in vitro the dissociation and transcription of nucleocapsids of negative-strand viruses that more closely resemble the intracellular events that lead to initiation of infection.

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