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Adenovirus-Dependent Release of Choline from Plasma Membrane Vesicles at an Acidic pH Is Mediated by the Penton Base Protein

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It has been suggested that during receptor-mediated endocytosis of human adenovirus (Ad) type 2 into cells, Ad disrupts the membrane of endocytic vesicles to enter the cytosol. To understand the mechanism of Ad-mediated disruption of the endocytic vesicles, I exposed Ad to plasma membrane vesicles derived from KB cells. Ad caused release of choline from the plasma membrane vesicles preloaded with [^{3}H]choline. The efflux of choline was dependent on (i) the concentration of Ad, with a half-maximal effect at 0.5 μg/ml; (ii) the pH of the buffer, with the optimum pH of the reaction ranging from 5.5 to 6.0; (iii) the length of the incubation, with a half-maximal release at 2 min; and (iv) the temperature of the incubation, with the optimum temperature being 37°C. The Ad-dependent release of choline was inhibited by anti-penton base, while antihexon did not block the effect. These results suggest roles for a low-pH environment and the penton base protein in the Ad-dependent efflux of choline from plasma membrane vesicles.

Human adenovirus (Ad) type 2 is a nonenveloped virus which enters cells through receptor-mediated endocytosis (4, 10, 12, 18). This process involves the binding of Ad with its cell receptor, followed by its movement into the endocytic vesicles (4, 6). It has been shown that Ad disrupts the membrane of endocytic vesicles to escape into the cytoplasm and thereby release cointernalized molecules such as proteins and nucleic acids into the cytoplasm (3–7, 10, 12, 20). Disruption of the vesicles is favored by the low pH of the vesicles (13) and a viral capsid protein; the penton base plays an important role in this process (11). Ad can also increase cell membrane permeability of small molecules when cells are placed under mildly acidic conditions (12, 14–17) and can cause the lysis of liposomes (1). In this communication, I now show that plasma membrane vesicles made from KB cells can be used to study Ad-dependent changes in plasma membrane permeability.

Plasma membrane vesicles were made from KB cells by the sucrose density gradient method as published previously (2). To evaluate the effect of Ad on the permeation of choline across the membrane vesicles, vesicles were loaded with [^{3}H]choline (2 μCi/ml) for 15 min at room temperature. After the vesicle suspension was diluted 50-fold with phosphate-buffered saline (PBS), the vesicles were incubated with different concentrations of Ad for 10 min at 37°C in a buffer at pH 6.0 (20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES], 120 mM NaCl). Following incubation, the vesicles were rapidly filtered through nitrocellulose membranes (0.45-μm pore size) with a Millipore filtration device and washed four times with cold PBS. Vesicle-associated choline in the membrane filters was counted with a beta scintillation counter. The results of this experiment, as shown in Fig. 1, indicate that Ad causes the release of choline. The amount of choline released as a result of incubation with Ad depended on the concentration of virus used (Fig. 1). Half-maximal release of choline occurred at an Ad concentration of about 0.5 μg/ml, and maximum choline release occurred at 1 to 10 μg/ml. This amount of choline represented nearly 60% of the maximum releasable choline from the vesicles. The control, Ad heated for 5 min at 100°C, failed to release choline (Fig. 1).

Ad-dependent release of choline was also time dependent. Figure 2 shows that at pH 6, Ad (1 μg/ml) caused a time-dependent release of choline from membrane vesicles. The release of choline was fairly rapid; the time required to release half of the maximal amount was about 2 min. The amount of choline released because of Ad was nearly fourfold greater than the amount released with buffer alone.

Ad-mediated release of choline was dependent on the pH of the buffer, with a pH optimum of about 5.5 to 6.0 (Fig. 3), although, there was some release of choline at a neutral pH. These results demonstrate that Ad-dependent release of choline from plasma membrane vesicles is favored in the acidic range.

Ad-dependent release of choline from the plasma membrane vesicles was also temperature dependent. Below 25°C there was no detectable release of choline, and maximum release was observed at 37°C. Ad-dependent release of choline was completely destroyed when the virus was preheated at 50°C for 30 min (data not shown). This result suggests that a protein with an enzymatic activity is responsible for Ad-mediated effects.

Ad effects on the plasma membrane vesicles likely involve one or more capsid proteins of Ad. The three major capsid proteins of Ad are the hexon, penton base, and fiber proteins (8, 9). To investigate whether any of these Ad proteins has any role in increasing the permeation of choline, I evaluated the effects of antibodies against the whole virus and the hexon and penton base capsid proteins. Ad was pretreated with preimmune serum, anti-Ad, anti-penton base, or antihexon for 30 min at room temperature. Plasma membrane vesicles preloaded with choline were exposed to pretreated Ad (1 μg/ml), and choline release was monitored for 10 min. The counts per minute under various preincubation conditions were as follows: with preimmune serum, 4,328; with anti-Ad (1:100), 15,439; with anti-penton base (1:100), 14,585; and with anti-

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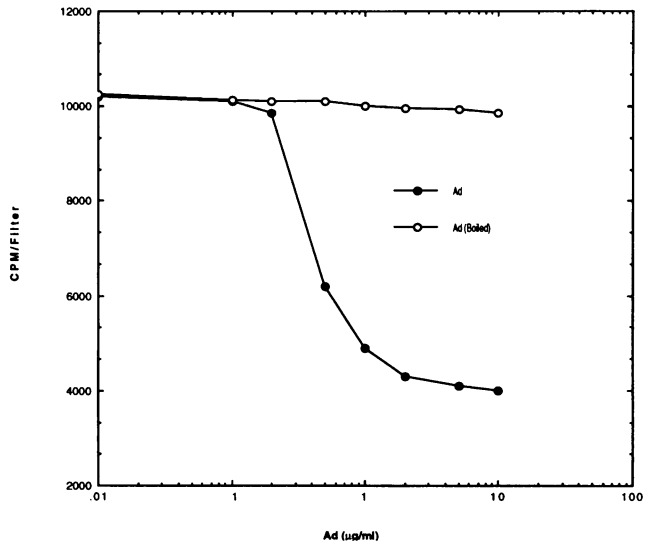

FIG. 1. Ad-dependent release of choline from plasma membrane vesicles. Plasma membrane vesicles were loaded with [^{3}H]choline as described in the text. The vesicles were incubated at 37°C with different concentrations of either Ad or Ad boiled for 5 min. Vesicle-associated choline was measured as described in the text. Results shown are actual counts which remained associated with the membrane vesicles incubated with different concentrations of Ad or boiled Ad.

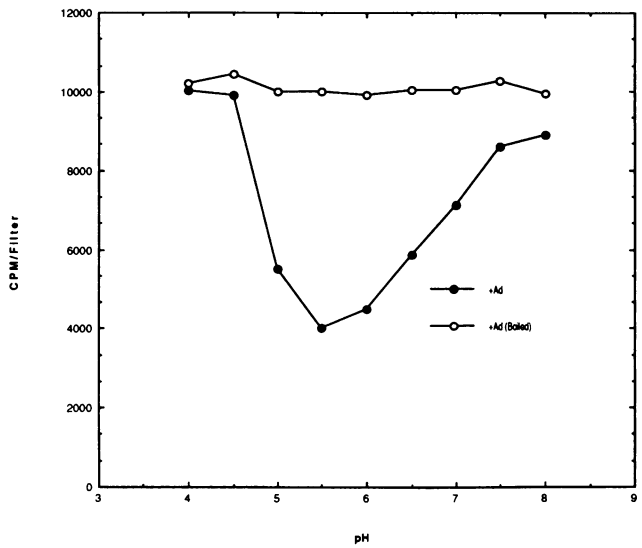

FIG. 3. pH optimum of Ad-dependent choline release. Plasma membrane vesicles were exposed to Ad in buffers with different pHs for 10 min at 37°C. Vesicle-associated radioactivity was measured as described in the text. Results shown are counts per minute obtained for vesicles treated with Ad or boiled Ad.

hexon (1:100), 5,846. Antibodies against Ad and the penton base neutralized the ability of Ad to cause the release of choline from the membrane vesicles. However, antihexon failed to block Ad-mediated choline release. Since the concentration of anti-penton base which neutralizes the Ad-mediated effects on choline release does not block the binding of virus with its receptor (11), it is possible that the penton base protein has a direct role in the Ad-dependent choline release from the vesicles. Interestingly, the penton base has been also postulated to be involved in disrupting endocytic vesicles (11) and more recently has been suggested to interact with integrin proteins, thereby allowing efficient internalization of Ad into cells (19). However, it remains to be established whether these effects of the penton base are caused by the same or different molecular domains of the penton base.

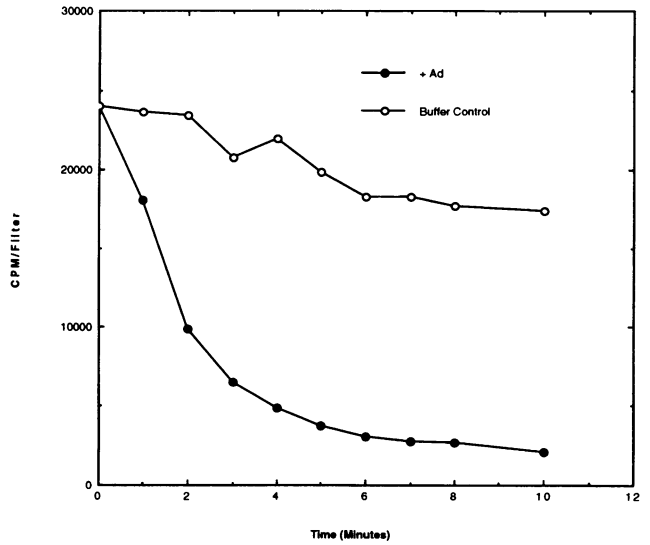

FIG. 2. Time dependence of Ad-dependent release of choline from plasma membrane vesicles. Plasma membrane vesicles preloaded with choline were exposed to either buffer alone or Ad for the shown lengths of time at 37°C. Vesicle-associated radioactivity was measured as described in the text. Results shown are the actual counts per minute associated with the vesicles treated with Ad or buffer alone.

The results presented here show that Ad bound to the plasma membrane vesicles can induce the leakage of choline from these vesicles and that these Ad effects are favored at an acidic pH. We have previously found that Ad-dependent disruption of intracellular endocytic vesicles is also favored by the acidic pH of vesicles (13). In this regard, the Ad effects described here are similar to the effects of Ad on intracellular endocytic vesicles. Therefore, these membrane vesicles represent an in vitro system which could be used to study the mechanisms of Ad-membrane interaction, particularly post-Ad-receptor-binding steps.

The results described in this communication suggest that during the interaction of Ad with plasma membrane vesicles, the penton base contacts the plasma membranes. However, it is known that Ad binding to the cell membrane receptor is mediated through the fiber protein (8, 9). This raises several questions, such as (i) how the penton base is involved in the Ad-dependent increase in permeability; (ii) whether fiber remains attached to the virus or is dissociated during the interaction of the penton base with the vesicles; and (iii) whether pentons, with or without fiber, are able to interact with plasma membranes and cause changes in the properties of plasma membranes. My future goal is to use the membrane vesicle system described here to answer these questions.

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