JOURNAL OF VIROLOGY, June 1983, p. 956–963
0022-538X/83/060956-08$02.00/0

Vol. 46, No. 3

Virus Receptor Interaction in the Adenovirus System
II. Capping and Cooperative Binding of Virions on HeLa Cells

ROBERT PERSSON,* ULLA SVENSSON, AND EINAR EVERITT
Department of Microbiology, University of Lund, S-223 62 Lund, Sweden

Received 13 December 1982/Accepted 17 February 1983

Adenovirus type 2 attachment to HeLa cells was analyzed under controlled conditions. The temperature-dependent attachment kinetics revealed an inflection point at around 20°C, and above this temperature the increase of the rate was doubled. In multiplicity dependence experiments, the attachment exhibited positive cooperative binding at 37°C. This binding pattern was inhibited by low temperatures and prefixation of cells with 0.015% glutaraldehyde. Attachment of rhodamine-labeled virions revealed capping of the particles on 15% of the cells at 37°C. Capping was inhibited by low temperatures, glutaraldehyde fixation of cells, and treatment with cytochalasin B, azide, and 2-deoxyglucose. Consequently, we propose that the adenovirus type 2 attachment to cells leads to rearrangements in the plasma membrane, resulting in cooperative binding and capping of the virus particles.

The important first step for a successful replication of animal virus includes attachment, penetration or internalization, and uncoating. Attachment to cells is believed to involve an interaction between viruses and specific components of the plasma membrane. Lonberg-Holm and Philipson (18) have outstandingly surveyed the early interactions between animal viruses and cells, and review articles which focus on recent achievements within the field continually appear (3, 5, 19).

For clarity, we have adopted the nomenclature defined by Lonberg-Holm (17): virus attachment proteins are the structural components of the virion recognizing a specific cellular receptor, cellular receptor units (CRU) are cellular molecules recognizing one virus attachment protein, and cellular receptor sites (CRS) are cellular structures composed of one or more CRU involved in the binding of a virus particle.

In the adenovirus system, the well-characterized fiber protein has been identified as the virus attachment protein in the sense that isolated fibers adsorbed to cells efficiently block a subsequent virion attachment (26). Moreover, antibodies against purified fiber antigen quantitatively precipitate virions (27) and consequently inhibit infection as assayed by the fluorescent focus technique, but curiously not by the plaque assay (23, 24). The maximum number of virions which can attach to a cell is limited by the number of available CRS. Since in the adenovirus system the CRU/CRS ratio is in the range of 10 to 100 (26), either the isolated fiber molecules may recognize attachment units unavailable for the binding of larger virions, or virion attachment implies the need for engagement of several CRU. This multivalency of CRS may be achieved during the mere process of virion attachment, which might cause physical alterations of the lipid bilayer, as well as provoke changes in the lipid composition within the microenvironment of the CRS (17).

Kohn (15) has suggested that attachment of viruses or ligands to possibly cytoskeletally anchored CRU cross-links the receptors and thus modifies the interaction of the latter with the lipid phase of the membrane, and this is expressed as a change in fluidity. Interestingly, it has been shown for some nonenveloped viral systems that a decrease in microviscosity is induced upon virus attachment, an attachment-specific phenomenon since it only appeared in cells with intact receptors and in cells permissive with regard to the attachment step (16). As a consequence of virion adsorption to KB cells at low temperatures, Hennache et al. (10, 11) demonstrated that adenoviruses induce a rearrangement and clustering of intramembranous particles and a change in the distribution of sterols.

In light of the preceding information, we have in this study made attempts to unravel the mechanisms behind the adenovirus type 2 (Ad2) attachment to HeLa cells by manipulating the permissive system through changing a physical parameter such as the temperature, chemically stabilizing the cell surface by fixation, and adding physiologically interacting drugs and reagents.

MATERIALS AND METHODS

Cells and viruses. HeLa cells were maintained in suspension cultures at cell densities of 2×10^5 to 6×10^5 cells per ml in Eagle minimal essential medium supplemented with 5% fetal calf serum and 5 μg of gentamicin per ml. Adenoviruses were propagated, radioisotope labeled, and purified as described previously (7).

Attachment studies. The general procedure of virion attachment was as described earlier (35). HeLa cells were washed in phosphate-buffered saline (PBS) once or three times for experiments in unfixed or glutaraldehyde (GA)-fixed series, respectively. [^{3}H]thymidine-labeled virions (3.0×10^6 cpm per 10^{12} virions) were allowed to attach to cells in PBS at a density of 5×10^7 cells per ml.

In temperature dependence experiments, cells and viruses were incubated in PBS at temperatures between 2.8 and 37°C. In all experiments, cells were equilibrated at the relevant temperature for 5 min before viruses were added. The number of virions per cell was kept at a constant multiplicity of infection (MOI) of 340. Samples were withdrawn at 5, 10, 20, 30, 45, and 60 min after the addition of virus, diluted 10 times in PBS, and gently sedimented for 25 s. The radioactivity confined to cell pellets and supernatants was measured and used for calculating the degree of virion attachment.

In multiplicity dependence experiments, ratios of viruses over cell numbers were between 50 and 20,000. Incubation periods for cell and virus mixtures were 45 min for unfixed and GA-fixed cells at 37°C and 3 h for unfixed cells at 2.8°C. After incubation, samples were diluted six times in PBS, sedimented, and assayed for radioactivity as described above.

The possible effect of cytochalasin B (15 to 60 μg/ml) colchicine (0.1 to 0.4 mM), sodium azide (10 to 50 mM), and 2-deoxyglucose (10 to 50 mM) on the virion attachment was studied. Before virus addition, cells were preincubated for 10 min at 37°C with the appropriate reagent. Ad2 was added at an MOI of 340, and incubations were continued for 60 min. Samples were withdrawn at intervals, and virus attachment was assessed as above. The same reagents were used in the fluorescence studies.

GA fixation of cells. Various concentrations of GA were tested to obtain an optimal stabilization of cells with a concomitant minimal influence on the properties of the CRS. Cells, washed three times in PBS, were suspended in PBS at a concentration of 10^6 per ml. Freshly prepared 2.5% GA in PBS was added to yield final concentrations of 0.001 to 0.6%. Cell suspensions were incubated at 4°C for 30 min under slow agitation, after which the cells were sedimented and resuspended, at the density above, in PBS containing 0.05 M neutralized ethanolamine. After a 30-min incubation, cells were washed three time in PBS and finally suspended at a density of 5×10^7 per ml. The status of the cells was studied accordingly.

Virion attachment at 37°C revealed that cells fixed with 0.01% GA were almost unaffected, with a reduction in attachment of less than 10%, whereas at 0.06% GA the reduction amounted to 90%. Swelling of cells in hypotonic buffer (10 mM Tris-hydrochloride buffer containing 10 mM KCl, 2 mM $MgCl_2$, and 2 mM β-mercaptoethanol, pH 7.3) for 15 min at 22°C, detergent treatment (0.1% Triton X-100 in PBS) of cells for 60 min at 22°C, and ultrasonic treatment of cells for 5 s on ice demonstrated that cells stabilized with 0.01% GA appeared to be unaffected by the various treatments, as judged from microscopic examination. A concentration of 0.015% GA was chosen in our subsequent fixation studies of cells, and at this concentration virus adsorption was reduced at the most by 30%.

Rhodamine labeling of Ad2 virions. Tetraethylrhodaminylisothiocyanate (TRITC) was covalently linked to Ad2 virions as follows. Immediately before conjugation, highly purified virions were filtered through a PD-10 column, equilibrated in 0.1 M $NaHCO_3$, pH 8.7. The concentration of recovered viruses was 3.6 optical density units per ml, corresponding to 1.01 mg of virus protein (22). TRITC in 0.1 M $NaHCO_3$–ethanol (1:1) at a concentration of 1.0 mg/ml was added to the virus suspension at a ratio of TRITC to virus protein of 0.05. The mixture was incubated at 26.5°C for 3 h, after which the conjugated virions were separated from free TRITC in a PD-10 column equilibrated in PBS, with a recovery of 75%. Spectrophotometric measurements at 560 nm revealed a number of TRITC molecules per virion in the range of 5×10^3 to 7×10^3. No disintegration of conjugated virions was discernible after sedimentation analyses in glycerol gradients. The capacity of TRITC-labeled Ad2 to attach to cells was reduced by 10 to 15%, and unconjugated Ad2 particles competed quantitatively with TRITC-labeled Ad2 for receptor sites on the cells.

Attachment studies with TRITC-conjugated Ad2. Cells were carefully washed and suspended in PBS at a density of 5×10^7 per ml. TRITC-conjugated Ad2 was added at an MOI of 3,000 and adsorbed at 2.8°C for 60 min. After attachment, cells were washed with PBS and further processed in one of the following ways.

(i) Cells were fixed with GA directly after the initial attachment step. (ii) cells were incubated further at 37°C for 0.5, 1, 2, 5, 10, 20, 40, and 60 min and subsequently fixed. (iii) Cells were fixed and further incubated at 37°C for 60 min. TRITC-conjugated Ad2 was also added to prefixed cells and incubated at 2.8°C for 60 min.

In addition, attachment of TRITC-conjugated Ad2 was studied in the presence of sodium azide (10 and 30 mM), cytochalasin B (15 μg/ml), colchicine (0.1 mM), and 2-deoxyglucose (10 mM). The virions were added to cells together with one of the reagents and incubated for 60 min at 2.8°C. After removal of unadsorbed virus with PBS, cells were suspended in PBS containing the indicated concentrations of reagents and further incubatd at 37°C for 10 min. Immediately after the second incubation, the cells were fixed with 0.015% GA. Analyses were made in a Zeiss fluorescence microscope by using epifluorescence with BP 546, FT 580, and LP 590 filters. For photography, Ilford HP 5 films were used.

Liquid scintillation spectrometry. Direct radioactivity measurements of water-containing samples were done in a cocktail of toluene-methanol (1:1) with 0.4% Omnifluor. A Mark II liquid scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill.) was used to determine radioactivity.

Chemicals and reagents. Eagle minimal essential medium, L-glutamine, gentamicin, and fetal calf serum were obtained from Flow Laboratories Ltd., Irvine, Scotland. [^{3}H]thymidine (74 Ci/mmol) and Omnifluor

were purchased from New England Nuclear Chemicals GmbH, Dreieich, West Germany. Cytochalasin B, colchicine, sodium azide, and 2-deoxyglucose were from Sigma Chemical Co., St. Louis, Mo. Rhodamine B isothiocyanate and ethanolamine were obtained from BDH Chemicals Ltd., Poole, England, and GA (25% for electron microscopy) was from E. Merck AG, Darmstadt, West Germany. PD-10 columns with Sephadex G-25M were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden.

RESULTS

Temperature dependence of Ad2 attachment. The attachment of Ad2 to cells was analyzed at several temperatures ranging between 2.8 and 37°C. To minimize fluctuation effects due to the cellular status, all samples were analyzed within 1 day and with material obtained from the same cell harvest. The virus attachment was highly dependent on the incubation temperature (Fig. 1). Also depending on the incubation temperature, maximum values of attachment were reached after different periods of incubation. These maximum values varied from around 80% attachment after 30 to 40 min at 37°C to around 45% after 3 h at 2.8°C (data not shown).

From the slopes of the curves in Fig. 1, the initial rates of viral attachment were calculated. Figure 2 shows that the attachment rates were strongly dependent on the incubation temperatures, with a shift at around 20°C, leading to a doubling of the rate increase above this temperature. For comparison, an analogous curve was made for cells fixed with 0.015% GA. The slope of this curve resembled the one for unfixed cells below 20°C, but revealed no shift in the increase in attachment rates above 20°C.

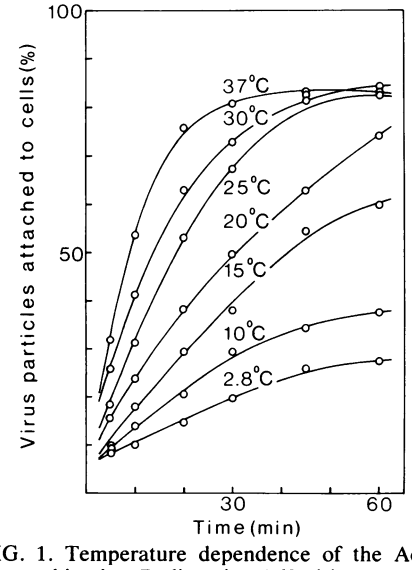

FIG. 1. Temperature dependence of the Ad2 attachment kinetics. Radioactive Ad2 virions were added to cells equilibrated at the appropriate temperature and further incubated. Samples from each incubation mixture were withdrawn at the indicated times, and the attachment of virions was determined as described in the text. The circles represent the mean values of attached viruses in two separate experiments.

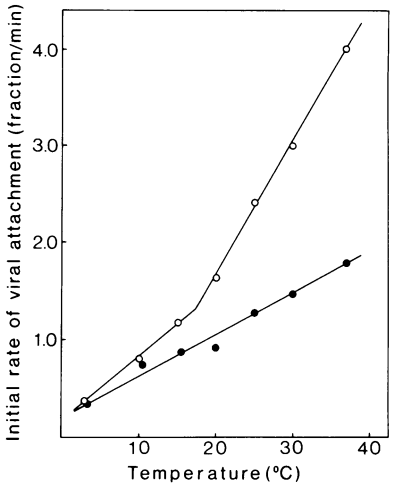

FIG. 2. Temperature dependence of the initial rate of adenovirus attachment. The initial rates of viral attachment at the different temperatures were calculated from the slopes in Fig. 1 to give the fraction of adsorbed virions per minute (○). ●, Attachment data from an analogous experiment with GA-fixed (0.015%) cells.

Temperature dependence of attachment of rhodamine-labeled Ad2. Ad2 particles conjugated with the isothiocyanate derivative of rhodamine were employed to visualize the early events of virion attachment. Due to the high specific fluorochrome labeling of virions, MOIs of 3,000 particles per cell were used to give a moderate number of attached virions (ca. 20%). Cells incubated with TRITC-conjugated Ad2 at 2.8°C for 60 min and subsequently fixed with 0.015% GA demonstrated a granular and patchy fluorescence pattern, uniformly distributed over the cell surface (Fig. 3A). This general pattern was not altered if fixed cells were transferred to 37°C and further incubated for 60 min.

The same pattern was also observed if cells were fixed before virion attachment and incubated as above. However, if cells with virions adsorbed at 2.8°C were transferred to 37°C, without prior fixation, the fluorescence became clustered and polarized to one end of the cells at a frequency of around 15% (Fig. 3B and C). Capping of labeled virus was evident as early as 30 s after the temperature shift. Approximately 20 min after the shift, the fluorescent clusters appeared less pronounced and became somewhat diffuse (Fig. 3D), possibly due to translocation of virions into the cytoplasmic region of the cells.

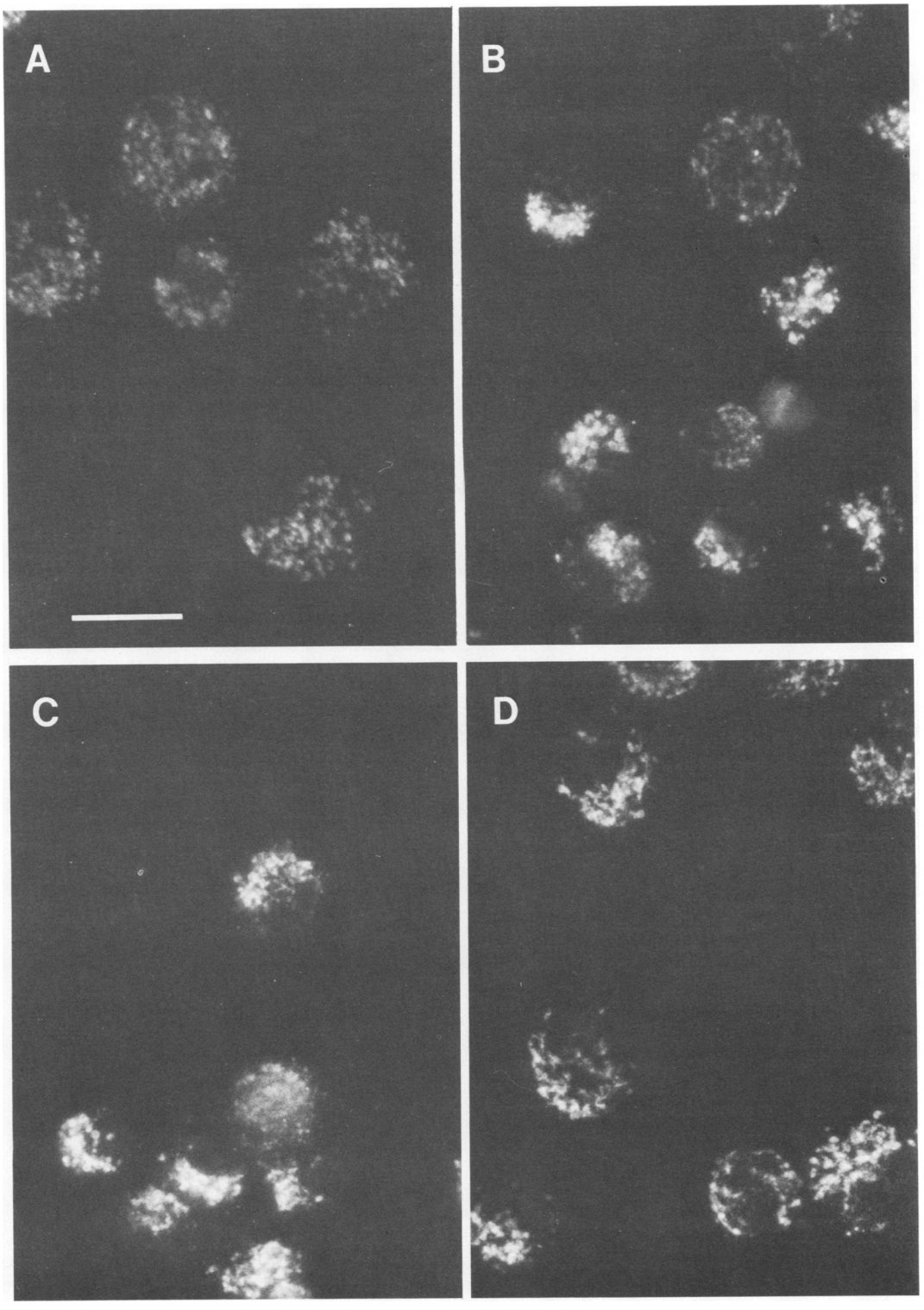

FIG. 3. Attachment of rhodamine-labeled Ad2 virions. Rhodamine-labeled virus was added to cells at an MOI of 3,000 at 2.8°C for 60 min. After removal of unadsorbed virus (80%), cells were transferred to 37°C and incubated for increasing periods of time. Samples were removed, subsequently fixed with GA (0.015%), and

Influence of drugs and metabolic inhibitors on virion attachment. Metabolic inhibitors (NaN_3 and 2-deoxyglucose) and drugs interfering with the cytoskeletal elements (cytochalasin B and colchicine) were analyzed with respect to possible effects on virion attachment. The reagents were added to cells 10 min before the addition of radiolabeled virus, and attachment was continually measured during a period of 60 min at 37°C. Azide, cytochalasin B, and colchicine were negative or reduced the final attachment by less than 10%, whereas 2-deoxyglucose at 50 mM reduced attachment by 20% as compared with control cells.

Although exhibiting only minor effects on viral attachment kinetics, the above reagents were also used to study the possible influence on the distribution of virions attached to the cell surface. In these instances, the reagents were added to cells at 2.8°C and incubated along with the TRITC-labeled virions for 60 min. After removal of unadsorbed virus with PBS, reagents were added again before the cells were transferred to 37°C for 10 min. After the second incubation, all cells were fixed with 0.015% GA, and 300 cells from each series were examined. The already-mentioned extent of capping to around 15% in unfixed cells at 37°C was inhibited after the addition of sodium azide (10 and 30 mM), cytochalasin B (15 μg/ml), and 2-deoxyglucose (10 mM) and thus equalled the background level of 2% for cold-treated or GA-fixed cells. Cholchicine treatment of cells (0.1 mM) had no effect on capping.

MOI dependence of Ad2 attachment. Ad2 was added to HeLa cells at different MOIs ranging from 50 to 20,000 virions per cell. The virus-cell mixtures were incubated at 37 and 2.8°C for 45 min and 3 h, respectively. Virus attachment was studied on both GA-fixed and unfixed cells. Figure 4 shows the number of bound viruses at various MOI inputs. The curves reveal typical saturation patterns, demonstrating the virus-binding capacity of each cell. In this particular experiment, the GA fixation reduced the number of available CRS to 60% of the control cells. This was an exceptional outcome of the GA treatment, which normally did not display this CRS-reducing effect. However, these results are shown for the sake of comparison, since all temperature- and MOI-dependent experiments described in this communication were derived from the same cell harvest and performed within 1 day.

After conversion of the results into Scatchard plots, a positive cooperative binding at virion attachment was reproducibly discernible for untreated cells at 37°C (Fig. 5A). No such effect was revealed for the GA-fixed cells or for the attachment system at 2.8°C (Fig. 5B). A positive cooperative binding was detected in all experiments performed with untreated cells at 37°C. However, the position and width of the region of the function indicating this effect varied. An obvious explanation for this variation is the fluctuation in the status of the cells. From the Scatchard plots the numbers of CRS were estimated, and values ranging between 3,000 and 6,000 CRS per cell were obtained for all three series studied.

The Scatchard plots were further used for calculations of the association constants, and on a per-receptor-site (CRS) basis, a value between 2×10^6 and 3×10^6 M^{-1} was obtained for the virus-cell interaction at 37°C, whereas for the other two series the constants were in the range of 0.7×10^6 to 1.0×10^6 M^{-1}.

DISCUSSION

In this study we have specifically focused on the early interactions occurring between human Ad2 and CRS of the HeLa cell plasma membrane during the phase of virion attachment.

Experiments designed to study the temperature dependence demonstrated that the initial rates of viral attachment were strongly temperature dependent, as previously indicated by Philipson (25) and Inoye and Norrby (12). A shift in the rate increase by a factor of 2 was established for temperatures above 20°C. The reproducibly observed inflection point of the kinetics curve might be due to increased possibilities for the CRS to move laterally in the plasma membrane. This could be a consequence of alterations in the physical status of the lipid matrix, such as gel-to-liquid crystalline phase transitions or phase separations (2) or both. Besides an increased movement, alterations in the lipid moiety would allow a hypothetical unmasking of individual units within the receptor sites, further leading to higher affinity for virus particles. These ideas emerging from temperature dependence experiments are likewise supported by the results obtained with GA-fixed cells. The chemical fixation or cross-linking will lead to an increase in the rigidity of the membranes, where proteins no longer can undergo conformational changes or diffuse freely in the membrane (4, 21, 30). When our data were converted into Arrhenius plots (not shown), the activation energy of virus attachment was shown to be around 42 kJ/mol at temperatures above the inflection point, where-

studied in a fluorescence microscope. (A) cells fixed with GA directly after incubation at 2.8°C; (B to D) cells fixed with GA after transfer to 37°C and a further incubation for 0.5 (B), 5 (C), and 20 (D) min, respectively. All photographs are of the same magnification; bar = 25 μm.

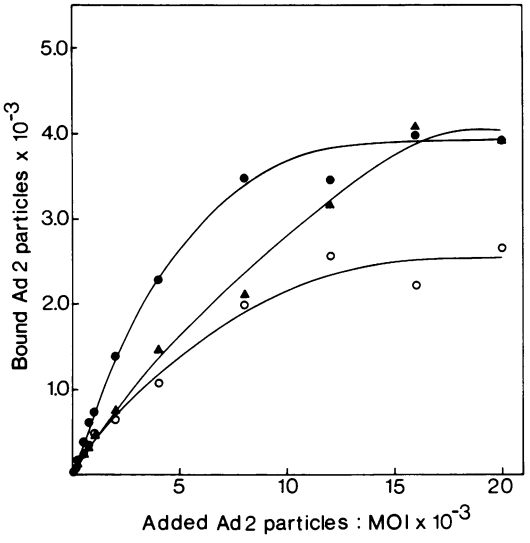

FIG. 4. Multiplicity dependence of the adenovirus attachment. Ad2 particles were added to cells at different MOIs, and the number of attached virions was estimated after different periods of incubation: ●, at 37°C for 45 min; ▲, at 2.8°C for 3 h; and ○, GA-fixed cells at 37°C for 45 min. For details, see the text.

as about 59 kJ/mol was obtained for the curve below this point, again indicating a temperature-dependent alteration of the system. In analogous attachment experiments with human rhinovirus type 2 and poliovirus type 2, linear Arrhenius plots were obtained, with activation energies of around 54 kJ/mol (20). Provided that the cells were pretreated at 0°C for more than 3.5 h, the viral attachment kinetics revealed an inflection point at 18°C. An explanation suggested for this effect was the introduction of the receptor sites into a fluid surrounding, as a consequence of phase separations. In analogy to the above shifts in activation energies at distinct temperatures, comparable effects at around 25°C have been demonstrated for, e.g., lipid analog diffusion in plasma membranes of human fibroblasts (13).

In the experiments with different MOIs, we applied the analysis technique of Scatchard (29) to estimate the number of virus receptor sites per cell and the association constants between viruses and cells at steady-state conditions. The number of CRS for adenoviruses on the HeLa cells was in the range of 3,000 to 6,000, which is in good agreement with previous determinations (26). The number of CRS was not affected by different temperatures of the virus-cell mixtures, and fixation of cells with GA also displayed no or minor effects on CRS numbers. A positive cooperative binding is a well-established phenomenon when multivalent ligands are attached to cells (14, 28), and Scatchard plots for untreated cells and viruses at 37°C indicate such a cooperative binding at Ad2 attachment. Low temperatures and chemical stabilization by fixation of cells clearly inhibit this effect. In support of our latter finding, it has been reported for other systems that cells fixed with minute amounts of GA do not bind ligands in a cooperative way, as is the case otherwise (32). In addition to the viral multivalency, it is possible that the mechanisms to achieve the demonstrated binding pattern involve either an increase in the affinity of the CRS for viruses or an increase in the number of available binding sites within the CRS. Even though it is pointed out above that the number of CRS is unchanged whether a positive cooperative binding is obtained or not, there is a possibility for unmasking each unit within the CRS, leading to higher affinity of viruses for these sites. The association constants show that cooperative binding is a result of an increased affinity for viruses. The constant for untreated cells at 37°C is two to three times higher than that obtained for cells incubated at 3°C or for GA-fixed cells.

Schlessinger et al. (31) have suggested that the lateral aggregation of membrane components forming the caps and the subsequent immobilization are caused by a ligand-induced conformational change of the receptor molecules or by receptor-ligand complexes that in turn increase the binding affinity between mobile receptors. Our experiments with rhodamine-labeled Ad2 show that adsorbed viruses redistribute in the membrane. Thus, after 10 min at 37°C, about 15% of the cells displayed capping of viruses. Azide, cytochalasin B, and 2-deoxyglucose treatment of cells inhibited the capping, as did low incubation temperatures and prefixation of cells with GA. The methods employed to obtain inhibition of the capping effect have been described for several other model systems (33), and we therefore conclude that the phenomenon studied in our system is a true capping. The observation that only 15% of the cells exhibited caps in our experiments might be explained by the fact that cells with high rates of endocytotic activity have a lower tendency to form caps than do cells with lower degrees of endocytosis (34). Thus, capping might be regarded as a consequence of competition between a lateral aggregation in the membrane and internalization of the ligand. Capping of other nonenveloped animal viruses has been reported for the systems of mengovirus (8), frog virus type 3 (1), reovirus type 3 (6), and also for the mycoplasma-specific bacteriophage L3 (9). Mengoviruses capped on Erlich ascites tumor cells are rapidly internalized (8), and reports concerning other model systems clearly demonstrate that capped ligands are subsequently internalized (33). However,

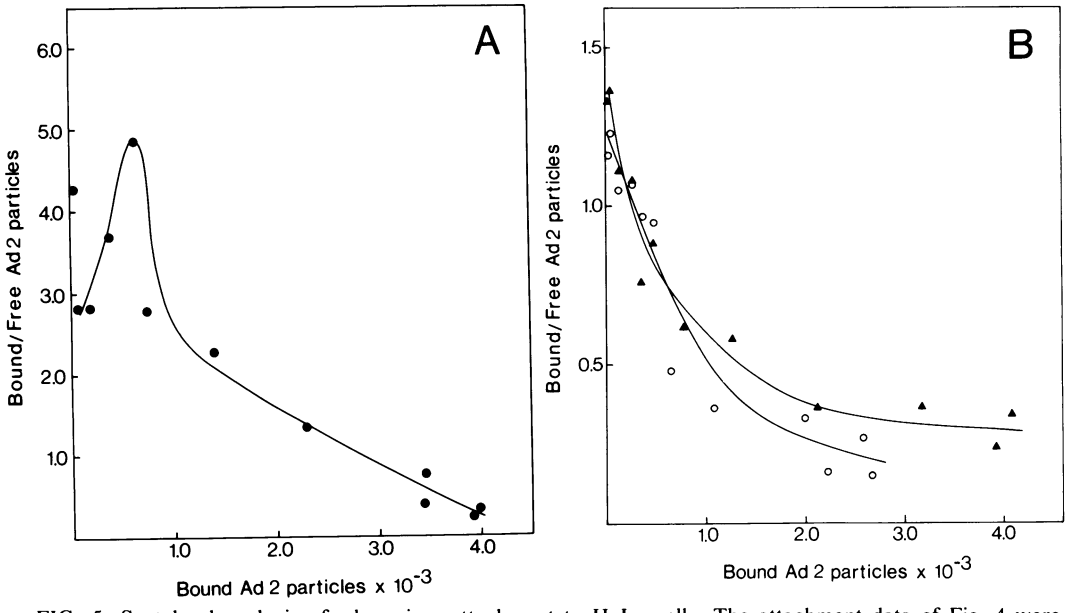

FIG. 5. Scatchard analysis of adenovirus attachment to HeLa cells. The attachment data of Fig. 4 were converted into Scatchard plots. Symbols: ●, virus-cell interaction at 37°C for 45 min; ▲, virus-cell interaction at 2.8°C for 3 h; and ○, virion attachment to GA-fixed cells at 37°C for 45 min. Note the different scales of the ordinates.

the relevance of capping for the following steps of internalization and uncoating in the adenovirus system is at this point speculative, but we are at present studying some aspects of these early events.

In this work, we present suggestive evidence that Ad2 attachment to HeLa cells leads to such alterations in the plasma membrane that redistribution of the viral receptors and possibly configurational changes of the entire receptor sites occur. These concomitant events result in cooperative binding and capping of attached virions.

ACKNOWLEDGMENTS

We thank Blanka Boberg and Inga Ohlsson for excellent technical and secretarial assistance, respectively.

This investigation was financially supported by the Swedish Cancer Society and the Swedish Medical Research Council (project no. 05976). Funds were also obtained from Carl Trygger's Foundation, Helge Ax:son Johnson's Foundation, Magn. Bergvall's Foundation, and Anders Otto Swärd's Foundation, all in Stockholm, Sweden.

LITERATURE CITED

1. **Aubertin, A. M., L. Tondre, and P. Descamps.** 1981. Capping of frog virus 3 antigen on the plasma membrane of infected cells. Ann. Virol. (Paris) **132E**:195–205.
2. **Boggs, J. M.** 1980. Intermolecular hydrogen bonding between lipids: influence on organization and function of lipids in membranes. Can. J. Biochem. **58**:755–770.
3. **Bukrinskaya, A. G.** 1982. Penetration of viral genetic material into host cell. Adv. Virus Res. **27**:141–204.
4. **Dalen, A. B.** 1976. The effect of glutaraldehyde on the stability of erythrocytes and on virus receptor substances. Acta Pathol. Microbiol. Scand. Sect. B. **84**:196–200.
5. **Dimmock, N. J.** 1982. Initial stages in infection with animal viruses. J. Gen. Virol. **59**:1–22.
6. **Epstein, R. L., M. L. Powers, and H. L. Weiner.** 1981. Interaction of reovirus with cell surface receptors. III. Reovirus type 3 induces capping of viral receptors on murine lymphocytes. J. Immun. **127**:1800–1803.
7. **Everitt, E., S. A. Meador, and A. S. Levine.** 1977. Synthesis and processing of the precursor to the major core protein of adenovirus type 2. J. Virol. **21**:199–214.
8. **Gschwender, H. H., and P. Traub.** 1979. Mengovirus-induced capping of virus receptors on the plasma membrane of Ehrlich ascites tumour cells. J. Gen. Virol. **42**:439–442.
9. **Haberer, K., M. Pfisterer, and H.-J. Galla.** 1982. Virus capping on mycoplasma cells and its effect on membrane structure. Biochim. Biophys. Acta **688**:720–726.
10. **Hennache, B., G. Torpier, and P. Boulanger.** 1979. Freeze-fracture study of adenovirus-induced KB cell surface alterations. Exp. Cell Res. **124**:139–150.
11. **Hennache, B., G. Torpier, and P. Boulanger.** 1982. Adenovirus adsorption and sterol redistribution in KB cell plasma membrane. Exp. Cell Res. **137**:459–463.
12. **Inouye, S., and E. Norrby.** 1973. Kinetics of the attachment of radioisotope-labelled adenovirus hemagglutinins to red blood cells and their detachment by antibody and by nonlabelled hemagglutinins of homologous and heterologous types. Arch. Gesamte Virusforsch. **42**:388–398.
13. **Jacobson, K., Y. Hou, Z. Derzko, J. Wojcieszyn, and D. Organisciak.** 1981. Lipid lateral diffusion in the surface membrane of cells and in multilayers formed from plasma membrane lipids. Biochemistry **20**:5268–5275.
14. **Ketis, N. V., J. Girdlestone, and C. W. M. Grant.** 1980. Positive cooperativity in a (dissected) lectin-membrane glycoprotein binding event. Proc. Natl. Acad. Sci. U.S.A. **77**:3788–3790.
15. **Kohn, A.** 1979. Early interactions of viruses with cellular membranes. Adv. Virus Res. **24**:223–276.
16. **Levanon, A., A. Kohn, and M. Inbar.** 1977. Increase in lipid fluidity of cellular membranes induced by adsorption of RNA and DNA virions. J. Virol. **22**:353–360.
17. **Lonberg-Holm, K.** 1981. Attachment of animal viruses to cells: an introduction, p. 1–20. *In* K. Lonberg-Holm and L. Philipson (ed.), Receptors and recognition, vol. 8,

series B. Chapman & Hall, London.
18. **Lonberg-Holm, K., and L. Philipson.** 1974. Early interactions between animal viruses and cells. Monogr. Virol. **9**:1–148.
19. **Lonberg-Holm, K., and L. Philipson.** 1980. Molecular aspects of virus receptors and cell surfaces, p. 789–848. *In* H. A. Blough and J. M. Tiffany (ed.), Cell membranes and viral envelopes, vol. 2. Academic Press, London.
20. **Lonberg-Holm, K., and N. M. Whiteley.** 1976. Physical and metabolic requirements for early interaction of poliovirus and human rhinovirus with HeLa cells. J. Virol. **19**:857–870.
21. **Lyles, D. S., and F. R. Landsberger.** 1976. Virus and lectin agglutination of erythrocytes: spin label study of membrane lipid-protein interactions. Proc. Natl. Acad. Sci. U.S.A. **73**:3497–3501.
22. **Maizel, J. V., Jr., D. O. White, and M. D. Scharff.** 1968. The polypeptides of adenovirus. I. Evidence for multiple protein components in the virion and a comparison of types 2, 7A and 12. Virology **36**:115–125.
23. **Pettersson, U., L. Philipson, and S. Höglund.** 1968. Structural proteins of adenoviruses. II. Purification and characterization of the adenovirus type 2 fiber antigen. Virology **35**:204–215.
24. **Philipson, L.** 1961. Adenovirus assay by the fluorescent cell-counting procedure. Virology **15**:263–268.
25. **Philipson, L.** 1967. Attachment and eclipse of adenovirus. J. Virol. **1**:868–875.
26. **Philipson, L., K. Lonberg-Holm, and U. Pettersson.** 1968. Virus-receptor interaction in an adenovirus system. J. Virol. **2**:1064–1075.
27. **Prage, L., U. Pettersson, S. Höglund, K. Lonberg-Holm, and L. Philipson.** 1970. Structural proteins of adenovirus. IV. Sequential degradation of the adenovirus type 2 virion. Virology **42**:341–358.
28. **Prujansky, A., A. Ravid, and N. Sharon.** 1978. Cooperativity of lectin binding to lymphocytes, and its relevance to mitogenic stimulation. Biochim. Biophys. Acta **508**:137–146.
29. **Scatchard, G.** 1949. The attractions of proteins for small molecules and ions. Ann. N.Y. Acad. Sci. **51**:660–672.
30. **Schlessinger, J., D. Axelrod, D. E. Koppel, W. W. Webb, and E. L. Elson.** 1977. Lateral transport of a lipid probe and labeled proteins on a cell membrane. Science **195**:307–309.
31. **Schlessinger, J., E. Van Obberghen, and C. R. Kahn.** 1980. Insulin and antibodies against insulin receptor cap on the membrane of cultured human lymphocytes. Nature (London) **286**:729–731.
32. **Schmidt-Ullrich, R., D. F. H. Wallach, and J. Hendricks.** 1976. Interaction of concanavalin A with rabbit thymocyte plasma membranes. Distinction between low affinity association and positively cooperative binding mediated by a specific glycoprotein. Biochim. Biophys. Acta **443**:587–600.
33. **Schreiner, G. F., and E. R. Unanue.** 1976. Membrane and cytoplasmic changes in B-lymphocytes induced by ligand-surface immunoglobulin interaction. Adv. Immunol. **24**:37–165.
34. **Storrie, B., and P. J. Edelson.** 1977. Distribution of concanavalin A in fibroblasts: direct endocytosis versus surface capping. Cell **11**:707–717.
35. **Svensson, U., R. Persson, and E. Everitt.** 1981. Virus-receptor interaction in the adenovirus system. I. Identification of virion attachment proteins of the HeLa cell plasma membrane. J. Virol. **38**:70–81.