Long-Range Interactions in Adsorbed Layers of Virus Particles

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The spatial distribution of adsorbed virus particles on a quartz surface was measured by electron microscopy. For low surface concentrations of adsorbed particles (around 10^8 particles/cm^2) the distribution was found to deviate from a random distribution. The particles with a diameter of 54 nm were found to interact at distances up to 0.7 μm. These interactions showed up both in the pair distribution function $g(r)$ and in the nearest-neighbor distance distribution.

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There are only a few experimental systems available giving direct information of intermolecular forces acting at surfaces. One method is to measure forces between mica sheets with adsorbed layers of macromolecules.[1] With this method an averaged value of forces is measured and only indirect information of the organization of the molecules can be obtained. Measuring compression isotherms of monolayers at interfaces is another method giving information of the thermodynamic state.[2] Also with this method no direct information is obtained concerning the actual conformation or organization of macromolecules.

For liquids, x-ray studies can be performed to give experimental determinations of the intermolecular forces. Light scattering and neutron scattering are also methods giving information about intermolecular forces. However, a direct measure of intermolecular forces may be obtained from an analysis of the spatial distribution. In order to study lateral interactions via the spatial distribution an investigation was carried out to study adsorbed layers of virus particles. The virus particles were chosen as a model substance because of their size and protein surface characteristics.

The virus adsorption was examined by means of a newly developed staining technique.[3] With this technique the virus particles, phage λ, were adsorbed on a thin (40 nm) quartz membrane supported by a silicon grid. The silicon grids were incubated in a moist chamber with sample solutions of different particle concentrations for different times. The incubation was interrupted by pouring an excess of uranyl acetate (2%–4%) over the grid surface. Then the staining solution was blotted by suction with a filter paper. The remaining staining solution was left to dry at room atmosphere and the grids were examined in a Jeol 100 CX electron microscope. A typical example of the resulting particle distribution is shown in Fig. 1. The virus particles were found to be 54 nm in diameter with a tail 140 nm long, in accordance with literature values.[4]

The particle distribution and surface concentration were found to depend on incubation parameters such as bulk concentration of virus particles, incubation time, rinsing time, and surface energy of the quartz membrane. At low surface concentrations of virus particles, the adsorption follows diffusion-limited adsorption kinetics.[3] At higher surface concentrations of particles the adsorbed amount is lower than expected from a diffusion-limited association and also sensitive to surface energy (Fig. 2).

Cluster formation could be seen at higher concentrations especially on hydrophilic substrates indicating that there are lateral interactions in the adsorbed layers. For statistical evaluation of the distribution of particles the micrographs were digitized and analyzed by a technique similar to the technique used for analyzing computer-simulated aggregates.[5,6] This method has also been used to study experimental two-dimensional fractal structures like electrodeposited zinc metal leaves[7] and percolation in thin gold films.[8] By use of a fast-Fourier transform to

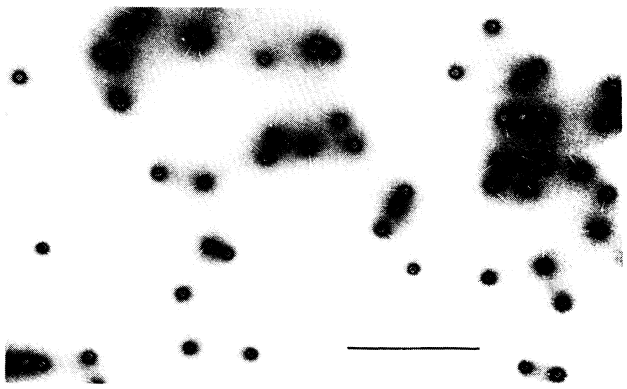

FIG. 1. Micrograph of adsorbed virus particles (bar = 1 μm). Incubation time was 20 sec and the relative concentration $c = 1$. The substrate was a hydrophilic quartz membrane.

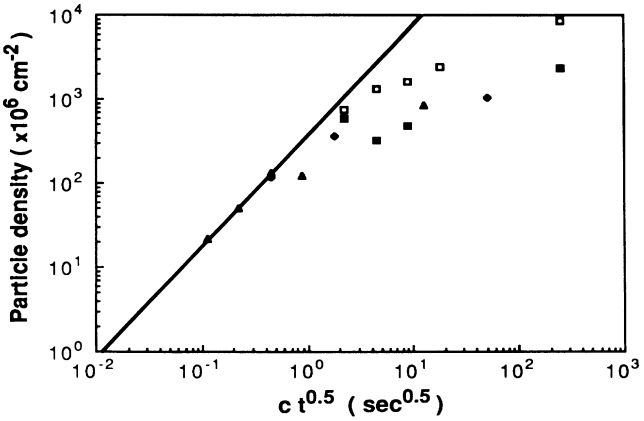

FIG. 2. Number of virus particles adsorbed to a quartz-membrane surface. Data are shown for different concentrations in the virus solution. The concentration c is given relative to the concentration in the stock solution. Hydrophilic surface: $c=1$ (filled squares), $c=0.2$ (filled lozenges), $c=0.05$ (filled triangles). Hydrophobic surface: $c=1$ (open squares). For low surface concentrations the amount bound is limited by diffusion (solid line) and thus proportional to $ct^{0.5}$. For higher surface concentrations the adsorption is not limited by diffusion. In this regime the binding is influenced by the surface energy.

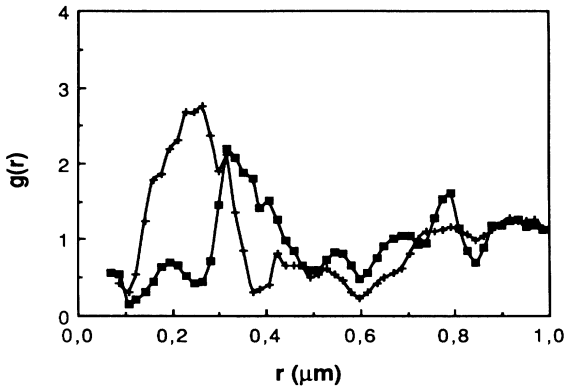

FIG. 3. Pair distribution function measured for particles adsorbed to a hydrophilic surface. The data are based on correlation analysis of 127 particles adsorbed from $c=0.2$ and a 320-s incubation time (squares), and 54 particles adsorbed with the same association parameters followed by a 22-h rinsing (plusses).

get the power spectrum and an inverse transform to give the correlation functions, both the density-density correlation function $c(r)$ and the pair distribution function $g(r)$ could be evaluated. The inverse transform was averaged over all directions to give the radial correlation functions. For analyzing clusters or aggregates the density-density correlation function $c(r)$ is used:

$$c(r)=N^{-1}\sum_{\mathbf{r}'}\rho(\mathbf{r}')\rho(\mathbf{r}'+\mathbf{r}), \quad (1)$$

where N is the number of particles in the cluster, $\rho(\mathbf{r})$ is the density, and $\mathbf{r}$ is the particle position vector. The density $\rho(\mathbf{r})$ is defined to be 1 for particles and 0 without particles. The computer program was checked by evaluation of $c(r)$ from computer-generated aggregates from the literature[6] and was found to give similar results. For low surface concentrations of particles the pair distribution function $g(r)$ can be evaluated as

$$g(r)=A[N(N-1)]^{-1}\sum_{\mathbf{r}'}\rho(\mathbf{r}')\rho(\mathbf{r}'+\mathbf{r}), \quad (2)$$

where A is the surface area. The pair distribution function $g(r)$ is a measure of the probability of finding a second particle at a distance r from a given particle and the function is defined so that $g(r)=1$ corresponds to a random distribution.[9] For samples with a low surface concentration of virus particles the pair distribution function $g(r)$ was used in order to see if there were any deviations from a random distribution of particles on the surface.

In Fig. 3 measured pair distribution functions are shown for particles adsorbed to hydrophilic surfaces. Data for $r<60$ nm are omitted since the evaluation technique according to Eq. (2) is not valid for distances shorter than the particle diameter. We can see several significant deviations from a random distribution. There is a region characterized by depletion of particles at a distance of approximately 0.6 μm and an accumulation of particles at a distance of approximately 0.3 μm. After a 22-h rinse there are still 50% of the virus particles left at the surface and we can see that the depletion region is more pronounced.

It is interesting that for decreasing distances between particles the interaction starts at approximately a 0.7-μm separation distance. At these separations the average particle density is 2×10^8 particles/cm^2 which coincides with the particle density at which the adsorption isotherm from Fig. 2 stops being diffusion limited for hydrophilic substrates. This indicates that the adsorption seems to be self-passivated at these surface concentrations of particles and this happens long before there is a dense layer of particles. Figure 1 shows the distribution and density of particles shortly after leaving the diffusion-limited kinetics (surface density $\approx 4\times10^8$ particles/cm^2).

The distribution of particles was also analyzed in terms of the nearest-neighbor distance distribution. For a complete random situation it can be shown that the area $A_r=\pi r^2$ within which the nearest neighbor is situated should be distributed according an exponential distribution:

$$P(0<A_r<A_1)=1-\exp[-(N/A)A_1], \quad (3)$$

where N/A is the number of particles per unit area.

In Fig. 4 measured relative frequencies f_n of the nearest-neighbor distance is shown together with a theoreti-

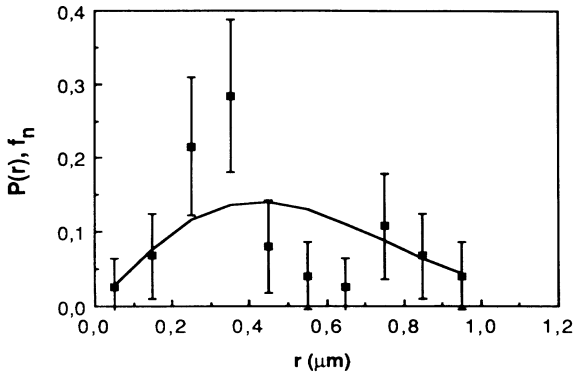

FIG. 4. Distribution of nearest-neighbor distance between adsorbed particles. The same experiment as in Fig. 3 at 22-h dissociation time. The solid line corresponds to a theoretical random distribution and the bars represent 95%-confidence limits in the statistical evaluation ($n=77$).

cal random distribution $P(r)$. Approximate 95%-confidence limits Δf_n were evaluated from

$$\Delta f_n = \pm 1.96[f_n(f_n-1)/n]^{0.5}, \quad (4)$$

where n is the number of particles in the sample. The data shown indicate that there are significant deviations from a random distribution at distances consistent with those derived from the pair distribution function $g(r)$.

Recent work on forces between mica surfaces with and without adsorbed macromolecules[1] have shown that in many systems interactions in water systems can extend over long distances (of the order of 0.5 μm). The interpretation of these long-range interactions between surfaces has been that the macromolecules were adsorbed end on. However, the present finding of interactions between virus particles over distances up to 0.7 μm indicates that there are long-range interactions present. The nature of these forces is unknown and the study of interactions in adsorbed layers of macromolecules and particles can be of importance in finding possible causes for different unpredicted experimental findings. So, for example, it is found that for protein adsorption at low bulk concentrations saturation in the adsorption may well be established long before there is a monolayer coverage.[10] This behavior resembles the adsorption seen in this work. Another experimental finding which could be related to lateral interactions in macromolecular layers is that the forward rate constant for immunochemical reactions carried out at surfaces depends on the amount bound even at low surface concentrations.[11]

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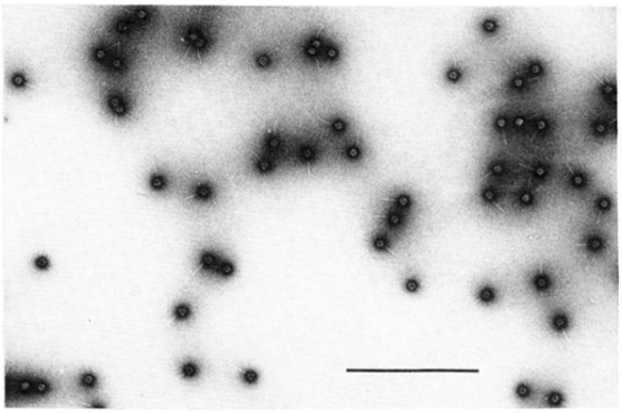

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