Description of Cell Adhesion by the Langmuir Adsorption Isotherm

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Z. Naturforsch. **43c,** 769–776 (1988); received August 18, 1987/June 10, 1988

Adhesion, Granulocytes

The adhesion of granulocytes to a glass surface is both theoretically and experimentally investigated. The basic results are: (i) The adherence process of cells to a surface can be described by the Langmuir adsorption isotherm which also holds for the adsorption of molecules to a substrate. (ii) The granulocytes do not interact with each other during the adsorption/desorption process. (iii) The equilibrium constant at 37 °C for granulocytes adsorption to the glass surface is 170 cells/mm^3 = 1.7×10^5 cells/ml. (iv) The equilibrium constant increases with decreasing temperature. (v) The equilibrium constant is a function of the chemokinetic/chemotactic stimulus f-Met-Leu-Phe. (vi) The desorption rate is also a function of the chemokinetic/chemotactic stimulus (k_{de}^{-1} = 71.5 min for 1 nM and 330 min for 1 μM f-Met-Leu-Phe). (vii) The adsorption rate is a function of the chemokinetic/chemotactic stimulus and the cellular bulk concentration ($k_{ad}^{-1} \cdot c_v^{-1}$ = 104 min for 1 nM and 8 min for 1 μM f-Met-Leu-Phe, and c_V = 4000 cells/mm^3 = 4×10^6 cells/ml). (viii) The total number of cells which can bind to the surface is independent of temperature (and of the chemokinetic/chemotactic stimulus). (ix) The high affinity receptor site is responsible for the adherence ($K_{f\text{-}Met\text{-}}$ = 1.8 nM f-Met-Leu-Phe). (x) The chemokinetic/chemotactic stimulated adherence is a cooperative process on a molecular level. (xi) The cellular adsorption/desorption process is a rate controlled process. The thermodynamic description of the adsorption/desorption process failed.

Introduction

A local increase of polymorphonuclear leukocytes at inflammatory sites is essential to the host in its defence against invading microbial organisms [1]. Although knowledge of the direction-finding mechanisms of granulocytes has increased tremendeously [2], other mechanisms, additional to the directional migration, must exist which are responsible for the ability of granulocytes to accumulate [3]. The directed locomotion of granulocytes can only occur if the cells are attached to a surface (2 dimensional space). Therefore, the cells have to leave the three-dimensional aqueous phase and come to surfaces where they can move. In order to reveal the cellular mechanisms involved in the adhesion behaviour, it is useful to describe the experimental results mathematically such that the mathematics may be used to uncover information from the data which is not immediately evident.

How can we proceed? Cellular adhesion is stimulated by external factors such as, for example, molecules in the bulk phase, molecules on the plane of migration, etc. It is not surprising that considerable efforts have been made to understand the whole system on a molecular level [3]. One can speculate that cellular adhesion is the result of a sequence both of biochemical reactions and of biophysical events. Following the ideas of Laplace [4], one could imagine that the cellular behaviour of the cell could be predicted if all the motions and all the reactions of the molecules, atoms, ions, etc. of the cell were known. The number of particles which need to be handled is enormous since the cell alone is composed of about 10^{13} atoms. Even assuming that all the details of the biochemistry and the biophysics were known, we could still not make correct predictions since no computer is available to handle 10^{13} coupled differential equations.

Instead of the necessity for knowing all of the "atomic" coordinates of many degrees of freedom, we need only to know a few parameters. Using this concept, one does not start from first principles, but it is still possible to look for fundamental laws such that the behaviour of the cell can be predicted in a known environment. The adhesion is described in a phenomenological way, without considering the source of that process. The aim is to find an unifying law which can be applied to different cell types and which can even be applied to inert particles such as, for example, atoms, molecules, etc. The knowledge of the law has the advantage of allowing one to predict how a cell will behave under certain boundary conditions.

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Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/88/0900–0769 $ 01.30/0

Materials and Methods

Granulocytes

Heparinized human blood of healthy donors was allowed to sediment by gravity for approximately 1 h. The supernatant with the buffy coat was then collected and 7 ml of the leukocyte enriched solution was deposited on top of 3 ml Ficoll. The tube was centrifuged at 400 g for 20 min. After centrifugation, the granulocytes were enriched in the Ficoll. This fraction was washed 5 times in Hanks' solution. A fixed granulocyte concentration (typical values are $\approx 10^7$ cells/ml (= 10^4 cells·mm^{-3}) solution) was established by using the Neubauer counting chamber (three independent measurements were performed).

Adhesion test

The adherence of granulocytes to glass was investigated. First of all, the granulocytes were allowed to equilibrate to the temperature of the experiment for 30 min. Secondly, glass spheres (1.5 mm in diameter) were filled into a siliconized glass tube, 1 ml of the granulocyte suspension was added and the whole sample was kept at constant temperature for 30 min. Then, the cell concentration in the liquid fraction was measured in the Neubauer counting chamber. In order to obtain the cell concentration in the liquid fraction, the cells were firstly washed out. This experimental procedure works quite well. It means that the cell cannot respond very quickly to the altered experimental conditions since, if that were the case, all the cells would be washed out from the test tube. The washing-out time must be very small compared to the characteristic time constant of the desorption process. We determined the time constant of the desorption process from the experimental data of Fehr and Dahinden [3] and found 71.5 min for non-stimulated cells and 330 min for stimulated cells (1 μM f-Met-Leu-Phe). More details can be found in the section "Rise and Decay Time for the Adherence" in this publication.

Experimental considerations: at the beginning of the experiment, the initial bulk cell concentration is c_V^0, and at steady state, after the experiment has finished, the final bulk cell concentration is c_V. These bulk concentrations, c_V^0 and c_V, are connected with the surface concentration, c_S, by

$$c_V^0 = c_V + c_S \cdot L^{-1} \tag{1}$$

since the total number of granulocytes is conserved in the test tube. The characteristic length, L, of the assay is the ratio of the volume, V, which is accessible to the cells, divided by the surface, A, on which the cells can adhere (see Fig. 1).

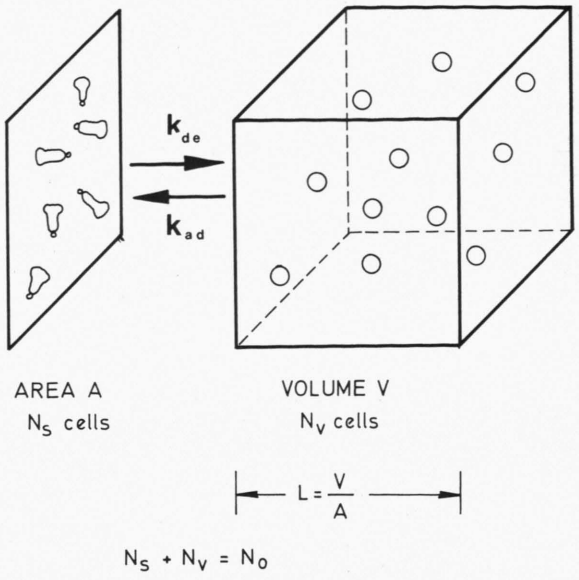

Fig. 1. Schematic representation of the adsorption process.

$$L = V/A. \tag{2}$$

Two types of experiments were performed: (i) the characteristic length of the assay was constant but the total number of cells was variable (L = const, N_0 = variable). (ii) The total number of cells was constant but the characteristic length of the assay was variable (L = variable, N_0 = const). The experiments were carried out in duplicates.

Physical analysis of cell adhesion

The phenomenology of the cell adhesion is simply described. The cells can change their physical state by going from the 3-dimensional space (= bulk phase) to the 2-dimensional space (= surface) and back again as shown in Fig. 1. One assumes in this model that the whole surface consists of N_S^0 surface sites to which the cell can bind.

$$\mathrm{CELL_{BULK}} + \mathrm{SURFACE\ SITE} \underset{k_{de}}{\overset{k_{ad}}{\rightleftharpoons}} \mathrm{CELL_{SURFACE}}$$

The same phenomenological description holds for molecules adsorbed onto a surface [5]. Therefore, it

is likely that the Langmuir adsorption isotherm [5] will hold for cells as well as for inert particles.

We will now derive the Langmuir adsorption isotherm. It is assumed that the surface has a maximum number of binding sites, N_S^0, where each binding site can be occupied by one single cell. The adsorption rate, dc_S^{ad}/dt, is proportional to the number of free sites, $N_S^0 - N_S$, and to the cell density in the bulk phase, c_v.

$$dN_S^{ad}/dt = k_{ad} \cdot (N_S^0 - N_S) \cdot c_V. \tag{3}$$

k_{ad} is the adsorption rate constant. The desorption rate, dc_S^{de}/dt, is proportional to the number of occupied sites, N_S.

$$dN_S^{de}/dt = k_{de} \cdot N_S. \tag{4}$$

k_{de} is the desorption rate constant. The total change in the surface concentration is then

$$dc_S/dt = -k_{de} \cdot c_S + k_{ad} \cdot (c_S^0 - c_S) \cdot c_V \tag{5}$$

where the binding site density on the surface is c_S^0. The steady state condition ($dc_S/dt = 0$) leads directly to the Langmuir adsorption isotherm (Eqn. (6))

$$c_S \cdot (c_S^0 - c_S)^{-1} = c_V/K \tag{6}$$

with the equilibrium constant, K,

$$K = k_{de}/k_{ad}. \tag{7}$$

The ratio c_V/K is a dimensionless number which reflects the natural range of the cellular sensitivity to the surface. If the bulk concentration, c_V, equals K then 50% of the binding sites are occupied by cells.

The adsorption-desorption process of cells to a surface can be treated by analogy with a chemical reaction,

$$A + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C$$

with which everyone is familiar. The ratio of the reaction rates, k_1 and k_2, yields the equilibrium constant K in the same way as described by Eqn. (7). At equilibrium, the concentrations of the molecules A, B, and C are related as

$$c_C = c_A \cdot c_B/K. \tag{8}$$

If the number of molecules of type B are conserved in the reaction volume ($= c_B^0 \cdot V$), one obtains an equation which has the same structure as Eqn. (6)

$$c_C \cdot (c_B^0 - c_C)^{-1} = c_A/K. \tag{9}$$

The Langmuir adsorption isotherm of cells has to be verified by experiments in which the equilibrium constant, K, and the cell site density, c_S^0, are fitting parameters. Eqn. (6) can be rewritten in such a way that the Langmuir adsorption isotherm is a straight line if c_S^{-1} is plotted *vs.* c_V^{-1} (Linwaver-Burk-plot).

$$c_S^{-1} = (c_S^0)^{-1} + (K/c_S^0) \cdot c_V^{-1}. \tag{10}$$

The cell site density, c_S^0, can be obtained by the extrapolation, $c_V^{-1} \to 0$, and the slope of the straight line is the equilibrium constant, K, divided by the cell site density, c_S^0.

An adsorption order parameter, Θ, can be introduced as

$$\Theta = c_S/c_S^0 = N_S/N_S^0. \tag{11}$$

The fundamental equation (6) can be rewritten to

$$\Theta = \{1 + (K/c_V)\}^{-1}. \tag{12}$$

The order parameter is unity when all the binding sites are occupied and zero when no cell is adhering to the surface.

The temporal change of the surface concentration will now be discussed. In the case of a constant bulk concentration, one obtains from Eqn. (5) (c_V = const):

$$c_S(t) = c_S^0 \cdot \{1 + (K/c_V)\}^{-1} \cdot (1 - e^{-t/\tau}) \tag{13}$$

with the rise time τ

$$\tau^{-1} = k_{de} + k_{ad} \cdot c_V = k_{de} \cdot \{1 + c_V/K\}. \tag{14}$$

It is worthwhile to note that the rise time for the cell adherence is, as expected, a function of the adsorption constant, k_{ad}, but is also a function of the desorption constant, k_{de}, and of the bulk concentration of the cells, c_V.

In the experimental situation, the number of cells in the test tube is constant (N_0 = const). Solving Eqn. (5) for this boundary condition unfortunately leads to very complex expressions:

$$t = \frac{L}{k_{ad} \cdot \delta} \cdot \ln \frac{1 + 2 \cdot c_S/(A - \delta)}{1 + 2 \cdot c_S/(A + \delta)} \tag{15}$$

with $A = -(K \cdot L + c_S^0 + c_V^0 \cdot L)$ (16)

$$\delta = \{A^2 - 4 \cdot c_S^0 \cdot c_V^0 \cdot L\}^{1/2}. \tag{17}$$

The next question concerns the equilibrium constant K. Let us again consider a system consisting of the volume, V, and the area, A, as shown in Fig. 1. N_0 particles as molecules or cells are introduced into this system. In thermal equilibrium, the particles are arranged in such a way that the entropy of the whole

system is maximum. If we consider only a part of our closed system, then we have an open system where the particles can exchange with another subsystem (– cells on the surface can go to the bulk phase and vice versa). Gibbs [6] showed that such an open system is in equilibrium when the chemical potential, μ, is the same in all subsystems. This means

$$\mu_{\text{VOLUME}} = \mu_{\text{SURFACE}}. \tag{18}$$

The chemical potentials, μ_{VOLUME} and μ_{SURFACE}, can be derived from the free energies, F_{VOLUME} and F_{SURFACE}, of the subsystems as

$$\mu_{\text{VOLUME}} = \{\delta F_{\text{VOLUME}}/\delta N_{\text{VOLUME}}\}_T. \tag{19}$$

$$\mu_{\text{SURFACE}} = \{\delta F_{\text{SURFACE}}/\delta N_{\text{SURFACE}}\}_T. \tag{20}$$

The free energies can be calculated from the grand partition function which is a special sum of all configurations of the particles. We do not want to go into further details here because these are described in many physical textbooks. The final result is the Langmuir adsorption isotherm (Eqn. (6)). This time, however, we also obtain an expression for the equilibrium constant

$$K = K_0\, e^{-E/(kT)}. \tag{21}$$

The equilibrium constant is a function of a dimensionless number which is the cellular binding energy, E, divided by the thermal energy, kT. The equilibrium constant decreases as expected if the adsorption energy is increased. K_0 of Eqn. (21) depends on the cellular movements, which can be translocational as well as intrinsic movements.

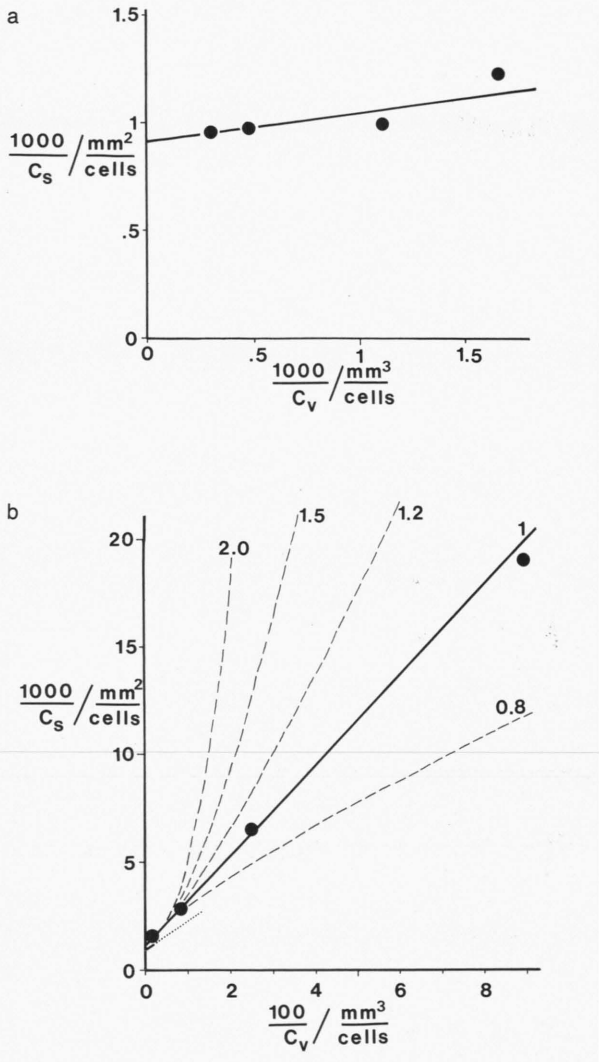

Fig. 2. Linwaver-Burk-Plot: Inverse of the surface concentration *versus* the inverse of the bulk concentration. a) N_0 = const and L varied and b) N_0 varied and L = const. The dashed lines are theoretical predictions for cooperative adsorption processes ($h \neq 1$). The bar close to the ordinate represents the data of Fig. 2a. The dotted line is the extrapolation of these data.

Results and Discussion

Langmuir adsorption isotherm

Case 1 (N_0 = const, L = varied): the adsorption process was investigated by varying the characteristic length of the assay. In this case, the number of glass spheres in every test tube was different (V = const, A = varied). The Linwaver-Burk-plot of the experimental data is shown in Fig. 2a. The experimentally determined dots follow the straight line predicted by the Langmuir adsorption isotherm quite well. The binding site density, c_S^0, was 1090 ± 100 cells $\cdot$ mm^{-2} and the equilibrium constant was 145 ± 30 cells $\cdot$ mm^{-3} = $1.45 \cdot 10^5$ cells/ml.

Case 2 (L = const, N_0 = varied): the adsorption process can also be investigated by altering the total number of cells in the test tube but keeping the characteristic length of the assay constant (V = const, A = const). The Linwaver-Burk-plot of this experimental data is shown in Fig. 2b. Again, the experimentally determined dots fit the predicted straight line behaviour quite well. The fitting parameters are the binding site density and the equilibrium constant ($c_S^0 = 910 \pm 100$ cells $\cdot$ mm^{-2}, and K = 190 ± 35 cells $\cdot$ mm^{-3} = $1.9 \cdot 10^5$ cells/ml).

We obtained similar results for both types of experiments. However, one should point out that experiment II (L = const, N_0 = varied) is easier to perform than experiment I (L = varied, N_0 = const). We have also to remark that the two types of experiments are complementary since case one (L = varied and N_0 = const) is easy to perform at high cell concentrations (bulk) and case two (L = const and N_0 = varied) at low cell concentrations. This is evident when the data of Fig. 2a are plotted into Fig. 2b. The case one data are very close to the ordinate but we are only able to show them schematically as a bar. The dotted line is the extrapolation of the data of Fig. 2a to lower cell concentrations. The Langmuir adsorption isotherm can thus investigated in a concentration range from 3000 cells·mm^{-3} (= $3 \cdot 10^6$ cells/ml) to 10 cells·mm^{-3}.

The first basic result of this report is that the adsorption process can be quantified by the Langmuir adsorption isotherm. The assumption that the surface consists of binding sites is obviously correct.

The binding site density of our glass for granulocytes is 1000 ± 90 cells per mm^2 (obtained by averaging all our results). The area of one binding site is then 1000 μm^2: this is a square with a side length of 32 μm. The binding site density obtained from the data of Fehr and Dahinden [3] is 1500 cells·mm^{-2}. This value of the binding site density leads to a square with a side length of 26 μm. The size of the granulocyte determined from microscopic pictures is very small compared with the size of the binding site. This result, however, is not so surprising if we photograph the contact area of the cell [7]. Thin veils connected with the pseudopods of the cell can occupy large areas. The binding site density is also a function of the cell treatment since Fehr and Dahinden [3] found that c_S^0 increases for PMA stimulated cells.

The large size of the binding site could also be explained if the cells were interacting with each other during the adsorption process in such a way that the cells repel one another. In the case of a cell-cell interaction, the Langmuir adsorption isotherm has to be altered by means of a cooperativity coefficient, h.

$$c_S \cdot (c_S^0 - c_S)^{-1} = (c_V/K)^h. \tag{22}$$

For $h = 1$, there is no cell-cell interaction except for steric hindrance. For $h > 1$, the cells interact in such a way that they attract one another; for $h < 1$, the cells repulse each other. In Fig. 2b, cooperative Langmuir adsorption isotherms for h = 0.8, 1.0, 1.2, 1.5, and 2.0 are shown. The best fit to the experimental points is obtained for $h = 1.0$. Here we have to remark that the most data points (see Fig. 2b) are in a region which is not very sensitive to a variation in the cooperativity parameter. Therefore the accuracy of h is 1 ± 0.2.

The equilibrium constant, K, for granulocytes adsorption to the glass surface is 170 ± 20 cells·mm^{-3}. If the bulk concentration, c_V, equals K then the adsorption order parameter is 0.5 when 50% of the binding sites are occupied by the cells.

We showed that it is possible to derive the Langmuir adsorption isotherm in two different ways. The theoretical framework of the first model is very general. The rate equation was established and the steady state condition lead to the Langmuir adsorption isotherm. The equilibrium constant is a fitting parameter. Within the framework of this model, there is no theoretical basis for the equilibrium constant. In the theoretical framework of the second model, it is assumed that the adsorption and the desorption process are in thermal equilibrium. This model also leads to the Langmuir adsorption isotherm. In addition, however, the model makes predictions for the equilibrium constant. The second model can be tested by a careful inspection of the equilibrium constant. The adsorption process of granulocytes is a function of external factors like chemokinetic/chemotactic stimuli. From an analysis of the data of Fehr and Dahinden [3] (see next section), the equilibrium constant of granulocytes exposed to low and to high concentrations of the chemotactic/chemokinetic molecules f-Met-Leu-Phe is 32,262 cells·mm^{-3} (= $3.22 \cdot 10^7$ cells/ml) and 72 cells·mm^{-3} (= $7.2 \cdot 10^4$ cells/ml), respectively. The difference in the binding energy between high and low concentration of the stimuli can be calculated by using Eqn. (21) and is about 6 times the thermal energy. This energy is much too small since *one* surface bound molecule requires about this amount of energy. The thermodynamic model of the adsorption is, therefore, not a good description for the cellular adsorption process: The adsorption process of cells to a surface is rate controlled as described by the first discussed model I.

There is a further hint which is not in agreement with the thermodynamic model. The adsorption process of granulocytes to a glass surface was investigated at different temperatures. The binding site density, c_S^0, is independent of temperature as shown

Table I. Temperature dependence of binding site density c_s^0.

Temperature [°C]	37	20	8
c_S^0/cells·mm^{-2}	769	952	780

Table II. Temperature dependence of the equilibrium constant K.

Temperature [°C]	37	30	25	20	15	10	5
K/cells·mm^{-3}	83	24	168	65	141	598	1270

in Table I. However, the equilibrium constant, K, is temperature dependent as shown in Table II: K increases with decreasing temperature. This means that the cells are more strongly absorbed at lower temperatures than at higher temperatures. If one describes the temperature dependence of the equilibrium constant with the thermodynamic model (Eqn. (21)), then one would obtain a negative value for the binding energy E. This is a surprising result since a negative binding energy would imply that the cells are repelled by the surface.

In summary, cellular adsorption to a surface is described by a steady state model. The thermodynamic equilibrium model fails to explain the experimental results.

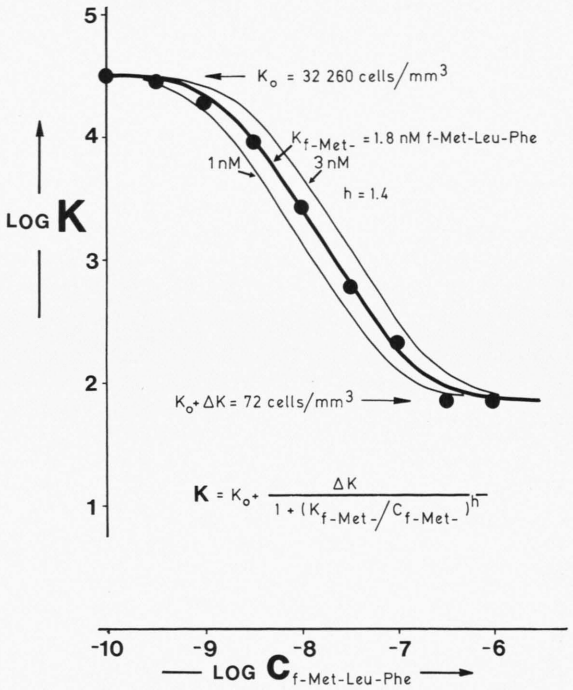

Fig. 3. Equilibrium constant K as a function of the chemokinetic/chemotactic stimulus f-Met-Leu-Phe (data from Ref. [3]).

Chemokinetic stimulus and the adsorption process

The adsorption process of cells to a substrate can be altered by external factors if the cellular state is influenced by such external factors. Fehr and Dahinden [3] investigated granulocytes in autologous heat-inactivated plasma and stimulated by the chemokinetic/chemotactic stimulus f-Met-Leu-Phe. We analyzed their data (Fig. 1 of Ref. [3]). The characteristic length, the initial bulk concentration and the binding site density was 0.93 mm, 4000 cells·mm^{-3}, and 1450 cells·mm^{-2}, respectively. By applying Eqns. (1) and (6), the equilibrium constant can be calculated. The results are shown in Fig. 3. The equilibrium constant decreases dramatically with increasing concentration of f-Met-Leu-Phe and the equilibrium constant can be described by the following equation which indicates that the adsorption process can be changed by chemical reactions.

$$K = K_0 + \delta K \cdot \{1 + (K_{\text{f-Met-}}/c_{\text{f-Met-}})^h\}^{-1} \qquad (19)$$

K_0, δK, $K_{\text{f-Met-}}$, and h are fitting parameters: K_0 = 32.262 cells·mm^{-3} ($c_{\text{f-Met-}} \to 0$). $K_0 + \delta K$ = 72 cells·mm^{-3} ($c_{\text{f-Met-}} \to \infty$). The cooperativity coefficient, h, is 1.4, and is determined by the middle part of the curve. The binding constant, $K_{\text{f-Met-}}$ = 1.8 nM f-Met-Leu-Phe, is obtained from the position of the curve. Two theoretical curves with different values of the binding constant ($K_{\text{f-Met-}}$ = 1 and 3 nM f-Met-Leu-Phe) are shown.

The results are remarkable. (i) The f-Met-Leu-Phe stimulated adherence is a huge effect. (ii) The f-Met-Leu-Phe stimulated adherence is obviously controlled by the high affinity receptor since $K_{\text{f-Met-}}$ is close to the binding constant of the high affinity receptor. In our receptor binding studies, we found a value of 1.8 nM f-Met-Leu-Phe. Snyderman [8] determined the binding constant of the f-Met-Leu-Phe receptor and found 0.53 nM. (iii) The f-Met-Leu-Phe stimulated adherence is a cooperative effect since h is greater than 1.

These results can be explained in the following way. The high affinity receptor stimulates the cellular adherence; the same receptor type also stimulates the chemokinetic activity. It is not the receptor alone, however, which provides the huge stimulation of cellular adherence. The cellular adherence must

be the result of biochemical and biophysical events which are stimulated by the receptor. This stimulation model is also supported by the observed cooperative effects. At low temperatures, when the cellular metabolism is switched off, the binding of f-Met-Leu-Phe to the membrane-bound receptor is a non-cooperative process [8]. Obviously the cellular metabolism is very important for cell adherence. This may be the reason why the thermodynamic model failed to explain the adsorption process.

The high affinity receptor sites are responsible for the cellular adherence process. But the surprise is that one finds at low temperature (4 °C) when the cellular metabolism is thermally blocked, only a few hundrets of high affinity binding sites available for the chemokinetic molecule f-Met-Leu-Phe [8]. The model which we have in mind for the adhesion is that the high affinity receptor after binding to a chemokinetic molecule, switches on the metabolism which changes the membrane composition. Obviously a small number of receptors is enough for this process.

Fehr and Dahinden [3] also measured the adherence of granulocytes in artificial media like Hanks' balanced salt solution or Gey's solution. The cells adhere strongly to the surface and the adherence could not be modified significantly by the addition of formylated peptides such as f-Met-Leu-Phe and f-Met-Phe. The equilibrium constant of the granulocytes for this adsorption process is around 100 cells $\cdot$ mm^{-3}. We studied the adherence of granulocytes in Hanks' buffered salt solution and obtained a similar value for the equilibrium constant (160 ± 20 cells $\cdot$ mm^{-3}). Thus, the adherence of granulocytes suspended in Hanks' buffered salt solution is comparable to the adherence of granulocytes suspended in autologous heat inactivated plasma containing about 100 nM of the chemokinetic/chemotactic stimulus f-Met-Leu-Phe.

Rise and decay time for the adherence

The steady state situation of the cell adherence is quite well described by the phenomenological picture. However, we do not know how fast the cells react to changed boundary conditions. The rate constants, k_{de} and k_{ad}, have to be measured in order to make predictions for the temporal change of the cell densities, c_V and c_S. Up to now, we only know the equilibrium constant, K, which is the ratio of these rate constants, k_{de} and k_{ad}.

Fehr and Dahinden [3] also measured the temporal change in the cell adherence. The characteristic length, the initial bulk concentration and the binding site density was 0.93 mm, 4000 cells $\cdot$ mm^{-3}, and 1550 cells $\cdot$ mm^{-2}, respectively. The equilibrium constant, K, was determined from the extrapolated value of the number of occupied binding sites. The equilibrium constants for non-stimulated, and for 1 nM f-Met-Leu-Phe treated cells are 5900 cells $\cdot$ mm^{-3}, and 104 cells $\cdot$ mm^{-3} for 1 μM f-Met-Leu-Phe treated cells. The equilibrium constant decreases with increasing concentrations of the stimulus. The equilibrium constant of the untreated and the 1 nM f-Met-Leu-Phe treated cells is, however, too small compared to the values which we have just discussed. If one inserts the value of the equilibrium constant obtained from the temporal adsorption measurements into Fig. 3, then one obtains a f-Met-Leu-Phe concentration of 5 nM. For this reason, one has to assume that the cells of this experiment were slightly stimulated. Now the logarithm in Eqn. (15) can be calculated if the cell surface concentration, c_S, obtained from Fehr and Dahinden's data, is inserted. The results are shown in Fig. 4. The measured values for "non-stimulated" and for "1 nM f-Met-Leu-Phe stimulated" cells follow one straight line. The slope of this straight line determines the rate constant, k_{ad}: one obtains $2.4 \cdot 10^{-6}$ mm^3 $\cdot$ cells^{-1} $\cdot$ min^{-1}. The value of the desorption rate constant, k_{de}, is $1.4 \cdot 10^{-2}$ min^{-1} or k_{de}^{-1} is 71.5 min. k_{de}^{-1} is the characteristic time for how long a cell will stay on the glass surface. The characteristic time for how long a cell will stay in the bulk phase is, in the case of a constant bulk concentration, given by $k_{ad}^{-1} \cdot c_V^{-1}$: one obtains 104 min for $c_V = 4000$ cells $\cdot$ mm^{-3}. The rate constants for 1 μM f-Met-Leu-Phe stimulated cells can be estimated from the data. k_{de}^{-1} is 330 min and $k_{ad}^{-1} \cdot c_V^{-1}$ is 8 min in the case of a constant cell concentration of 4000 cells $\cdot$ mm^{-3}. The chemokinetic/chemotactic stimulus f-Met-Leu-Phe obviously increases the mean duration time of the cells on the surface and decreases the mean duration time of the cells in the bulk phase.

Concluding Remarks

We have shown that the granulocytes behave like inert particles. The cellular adsorption process is a rate controlled process. The thermodynamic description of the adsorption process failed. The Langmuir

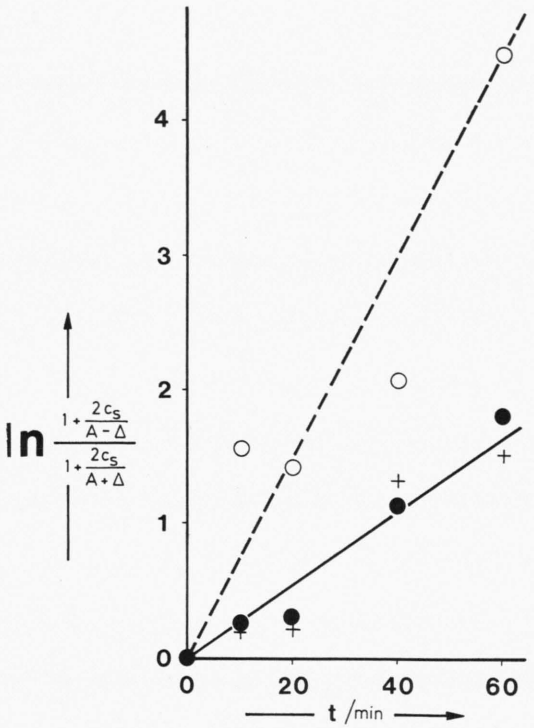

Fig. 4. Temporal behaviour of the adsorption process plotted accordingly to Eqns. (15), (16), and (17). (● 0 nM, + 1 nM, ○ 1 μM f-Met-Leu-Phe, data from Ref. [3]).

adsorption isotherm is a good description for both molecules and cells adhering to a surface. Detailed analysis of the adherence showed that the granulocytes do not interact with each other. Fehr and Dahinden showed that the adsorption process can be controlled by chemokinetic/chemotactic molecules. The influence of the stimulus on the adsorption process is cooperative. However, the binding of the stimulus to the membrane-bound receptor is a non-cooperative phenomenon. A further result of our detailed analysis is that the mean duration time of a cell in the two- and the three-dimensional space is altered by the chemokinetic/chemotactic stimulus.

The practical importance of this study lies in a detailed analysis of the experimental conditions and provides an objective description of cell adherence which may be useful in the definition of granulocytes dysfunctions. Furthermore, the advantage of the technique employed is that it is not restricted to the adherence studies of granulocytes alone and is likely to be useful for similar investigations on other cell types.

Acknowledgements

This work was supported by a NATO grant and by "Fond der Chemischen Industrie".

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