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Mycoplasma Attachment to Solid Surfaces: A Review

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Mycoplasma attachment to glass in a protein-containing environment requires energization of the cells, probably to provide more accessibility of binding sites.

The substance mediating attachment is of protein nature. Studies with monoclonal antibodies on *M. pneumoniae* suggest a concentration of the binding sites at the tip structure.

INTRODUCTION

Attachment to solid surfaces is a long-known property of mycoplasmas [1,2,3]. Motile species in particular—*Mycoplasma pneumoniae, Mycoplasma pulmonis,* and *Mycoplasma gallisepticum*—efficiently attach to glass and plastic. These species are equipped with specialized structures which are involved in motility [4]. Attachment to solid surfaces provides a useful model for more detailed studies, especially on the metabolic aspects of adherence. Only qualitative data were available [5], until recently when methods were developed to investigate quantitatively the interaction between some organisms (*M. pneumoniae* and *M. gallisepticum*) and glass surfaces.

METHODS

Basically two methods were used: *(i)* sedimentation of the ^{3}H-fatty acid-labeled cells on to a glass cover slip during two to three hours of incubation and measurement of the activity attached to the glass; this method was mostly used for *M. pneumoniae* [6,7,8,9], and *(ii)* incubation of the labeled mycoplasma suspension in a glass vial for 30 minutes and determination of the activity bound to the glass; this method was mostly used for *M. gallisepticum* [10].

RESULTS AND DISCUSSION

Phenomena

Two types of attachment can be observed, depending on the presence or absence of protein in the buffer. In buffer without protein, two negatively charged surfaces—mycoplasma and glass—are interacting directly, probably by electrostatic and hydrophobic forces [7,11]. This assumption is supported by the pH optimum between 5 and 6, the decreasing effect of higher ionic strength, and the increased attachment after neutralization of carboxyl groups. This type of interaction may be

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termed "non-specific," because it can be observed, under similar conditions, with many other kinds of particles.

A second type of attachment, probably more important *in vivo,* can be observed in the presence of protein in the environment. The non-specific attachment is considerably reduced, to 25 percent or less, by as little as 1 mg per ml of BSA [7]. Fatty acid-free BSA is even more effective, causing reduction to about 7 percent [11]. This effect seems to be explained by the binding of BSA to fatty acids, thereby changing the properties of the membrane surface [12]. However, this BSA-induced low level of attachment improved considerably (about tenfold) when these cells were transformed to an energetically active state by the addition of a metabolizable sugar, e.g., glucose or mannose [6,8]. These studies, performed with *M. pneumoniae*, revealed a distinct optimum at 0.25 and 0.5 mg per ml of glucose or mannose, respectively [8]. Higher concentrations inhibited attachment; however, this effect could be overcome by the addition of 0.1 mM of cyclic AMP, indicating cAMP-dependent catabolite repression [9]. The glucose effect could be prevented specifically by glucose analogues, e.g., 3-O-methyl-D-glucopyranoside, ionophores like carbonylcyanide m-chlorophenylhydrazone (CCCP), or inhibitors of Mg^{2+}-ATPase like dicyclohexylcarbodiimide (DCCD). The ATP content of the cells reacted according to the respective mechanisms [9]. Whereas the ATP content was reduced by the glucose analogue and the ionophore to 61 percent and 25 percent, respectively, it was increased by DCCD to 347 percent.

Structures

The components potentially involved in glass attachment are the glass surface and the structures of the mycoplasma membrane, especially its surface configuration. In the experiments using BSA buffer or growth medium, the glass surface is probably coated with protein, either with BSA or with components of the serum included in the medium. Experiments with pretreated cover slips suggested that a BSA layer may have an attachment-reducing effect [11], perhaps by saturating potential "non-specific" adsorption sites at the glass surface. Another possible explanation would be the mobilization of protein molecules from the glass which subsequently bind to the mycoplasma surface, acting like protein-containing buffer. Pretreatment with serum, on the other hand, improves attachment considerably [6], perhaps because of components with affinity to both surfaces. The extent of the involvement of the glass surface charge is not exactly known, but the superiority of pyrex glass, with its higher negative charge, over soda glass [3] suggests some influence by this factor.

The second component, the mycoplasma surface itself, presents a more complicated pattern. Only experiments with BSA buffer will be considered here. Surface charges are certainly one factor: attachment increased, if the negative charge was reduced by blocking of carboxyl groups or by neutralizing the negatively charged lipids by tetracaine [11]. The accessibility of the potential binding sites also seems to be of importance; if a membrane hyperpolarization was produced by the ionophore valinomycin, attachment was increased, possibly by exposing larger areas of the surface polypeptides [8]. The binding sites were further characterized by the attachment-reducing effect of trypsin, which suggested a polypeptide nature [6]. Recently further information was obtained by using a monoclonal antibody which primarily inhibited adherence to red blood cells. We found that it also inhibited attachment to glass. This antibody reacts specifically with the tip structure of *M. pneumoniae* [13], and we must therefore assume that this area of the mycoplasma cell is at least partly responsible for its binding to glass.

Energy Requirement

Energy may be required for synthesis or for structural functions. The minor effect of antibiotics which inhibit protein synthesis excludes a major role of production and release of a polypeptide in short-time attachment. Synthesis and action of polysaccharides or fatty acids cannot be completely ruled out. The correlation between attachment and energy metabolism suggests a more functional role of the energized state of the cells. Several mechanisms can be discussed: *(i)* effects on the vertical disposition of the binding sites, resulting in an increased exposition on the surface—This assumption is supported by the effect of valinomycin. Furthermore a recent paper reported increased exposure of amino groups on the membrane surface in the presence of glucose [14]. *(ii)* effects on the lateral disposition of the binding sites resulting in the formation of patches [15]—However, the preformed concentration of binding sites at the tip as shown by the antibody does not support this otherwise attractive possibility. *(iii)* effects on the cytoskeleton—The presence of contractile material in *M. pneumoniae* has been confirmed by several methods [16,17]. The action of the cytoskeleton might be necessary to shape the cell body, which might result in protrusion of the tip structure so that the binding sites were able to penetrate the charge barrier [11] and to facilitate bridging to the glass surface [18].

Elements of all three mechanisms may be involved in completing attachment. The phenomenon of attachment to inert or substance-covered surfaces, together with motility, is probably of importance for the first step of infection, the mucus penetration, and for the further approach of the mycoplasma toward the cell surface itself, where more specific interactions lead to final settlement.

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