Purification of Attachment Moiety: A Review

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Mycoplasma pneumoniae and *M. gallisepticum* possess binding sites of protein nature which mediate attachment to neuraminidase-sensitive regions on both respiratory epithelium and red blood cells. The binding sites of these organisms are similar though not identical. Several approaches were applied for the isolation of the binding sites. Of these, the use of affinity chromatography yield the least complex protein fraction. We have recently been using sialo-glycopeptides as the ligands in affinity chromatography. The availability of monoclonal antibodies which inhibit the attachment of *M. pneumoniae* to host cells should provide a very specific tool for the isolation of the attachment moiety. It should be mentioned that not all mycoplasmas adhere to host cells via sialic acid specific receptors, and other approaches should be developed to study these mycoplasmas' attachment moieties.

INTRODUCTION

Several reasons underlie the efforts to isolate and characterize the attachment moieties (binding sites or adhesins) of the mycoplasma membranes. In addition to the basic knowledge of these membrane components, one may expect that this knowledge will lead to the development of a drug and/or a vaccine which will prevent attachment of the mycoplasmas to the host cells. Lastly, the isolation of the binding sites will clarify whether the binding sites are directly involved in the mechanism of pathogenicity of the mycoplasmas, e.g., the inhibition of host cell catalase [1].

Various approaches can be applied for isolation of the attachment moieties. These depend primarily on our knowledge of the biochemical nature of the binding sites and also may take advantage of their localization. For example, since electron microscopy studies have indicated that *M. gallisepticum* and *M. pneumoniae* attach to host cells via tip structures, on which the binding sites are presumably concentrated, one may aim to isolate these structures as a source for the binding sites.

Isolation of Attachment Organelles

The most detailed work of this approach is by Quinlan and Maniloff [2], conducted on mildly sonicated *M. gallisepticum*. This treatment released blebs from part of the cells. By density gradient centrifugation, a fraction enriched with blebs was obtained. The authors characterized several properties of the fraction but did not report on its attachment capacity to host cells. With that aim and by using a very similar procedure, we were unable to obtain such a fraction from *M. gallisepticum* [Kahane I, Reisch-Saada A: unpublished results]. In another approach *M. pneumoniae* were used to test the working hypothesis that tip structures are present

only in organisms which are able to attach. The hemadsorbing *M. pneumoniae* strains were, therefore, compared to their non-hemadsorbing mutants. The tip structures were easily demonstrated in the "Trition-shell" preparation of mycoplasma [3]. The shells were obtained by exposing the organisms for a short time to a solution of 0.5 to 1 percent Triton X-100. Using this procedure, we have observed the tip structures and associated cytoskeletal network in both the hemadsorbing (virulent) and non-hemadsorbing (avirulent) mutants of *M. pneumoniae* [Kahane et al: in preparation]. This suggests that binding sites are not always associated with the tip structure, or that the binding sites on the tip structures of the non-hemadsorbing mutants are deformed. It should, however, be noted that the "Triton-shells" of *M. pneumoniae* may be a good starting material for the isolation of binding sites [Kahane I, Reisch-Saada A: in preparation].

Evaluation of the Nature of Binding Sites

There are various indications for the protein nature of the binding sites of *M. pneumoniae* and *M. gallisepticum*. Thus, proteolytic treatment of these mycoplasmas considerably decreased or abolished their attachment to erythrocytes (RBC). Additional data reviewed recently by Kahane et al. [4] as well as the heat sensitivity or inactivation of the binding sites by glutaraldehyde may be added as further indications of the protein nature of the binding sites. However, the attachment capacity of *M. dispar* [5] and of *M. pulmonis* [Kahane et al: unpublished data] was not affected by these proteolytic treatments. Hence, it appears that the binding sites of these mycoplasmas are other cell membrane constituents (e.g., lipids or glycoconjugates) or consist of proteins that resist the proteolytic digestion applied. Examples of such proteins do exist (e.g., the chemotactic receptor of neutrophils). Therefore, in order to adapt the optimal isolation procedure, as much information as possible should be acquired on the nature of the binding sites prior to attempts at their isolation.

Procedure Proven to Be Successful in Isolation Attachment Moieties

Solubilization by high salt concentration   Chandler and Barile [6] have exposed *M. pneumoniae* organisms to 2 M NaCl concentrated solution. They obtained a cell extract which was free of membrane fragments. The fraction exhibited ciliostatic, hemagglutinating, and proteolytic activities. The chemical characteristics of these activities are not yet defined. The electrophoretic profile of the fraction is complex and it is composed of most of the organism's polypeptides.

Isolation of the binding sites by affinity chromatography   This approach may be attempted if the binding is to a specific, identified compound. *M. pneumoniae* can serve here as an example for the application of such a method. This stems from data obtained from *in vitro* systems for *M. pneumoniae* infections, including tracheal organ cultures and erythrocytes (RBC). In these systems it was indicated that surface components containing sialic acids serve as specific receptor sites for *M. pneumoniae* (for review, see [4]). In this context one should add that although the data for attachment of *M. pneumoniae* to RBC seemed complex for a while, recent studies [7] using a more sensitive system for assessment of *M. pneumoniae* attachment indicate that in human RBC, as in sheep RBC, sialic acid-containing regions are responsible for the majority of *M. pneumoniae* binding (Table 1 and Fig. 1).

After establishing the affinity for sialic acid, we have used glycophorin, the major sialoglycoprotein of human RBC, as a ligand in affinity chromatography for the

TABLE 1

M. pneumoniae Adherence to Various Concentrations of Erythocytes (RBC)

Adherence (CPM)[a]

% RBC	HA[+b]		HA[-c]	
	Human	Sheep	Human	Sheep
0.5	1353 ± 241	758 ± 115	103 ± 10	72 ± 4
1.0	2470 ± 288	1728 ± 292	119 ± 18	107 ± 10
2.0	3527 ± 376	3448 ± 319	126 ± 13	130 ± 11
3.0	4913 ± 157	4657 ± 297	156 ± 17	167 ± 23
4.0	5035 ± 394	4811 ± 305	168 ± 20	173 ± 17

[a] Values are means ± standard error for four test samples from a representative experiment. Incubation was for 37°C.

[b] Hemadsorbing, virulent *M. pneumoniae* (specific activity was 2795 CPM/μg protein)

[c] Non-hemadsorbing, avirulent *M. pneumoniae* (specific activity was 2963 CPM/μg protein)

(From [7])

isolation of the binding sites from *M. pneumoniae* [8]. Membranes isolated from *M. pneumoniae* cells, radio-iodinated by the lactoperoxidase technique, were treated with 0.5 percent deoxycholate. The insoluble residue, exhibiting an increased capacity to bind to RBC, was solubilized by 0.1 percent sodium dodecyl sulfate. The solubilized material was subjected to chromatography-sepharose column. The fraction retained on the column was eluted with 0.2 percent sodium dodecyl sulfate. It lacked the high-molecular-weight polypeptides and was highly enriched with two polypeptides (apparent molecular weights, 45,000 and 25,000). The eluted fraction
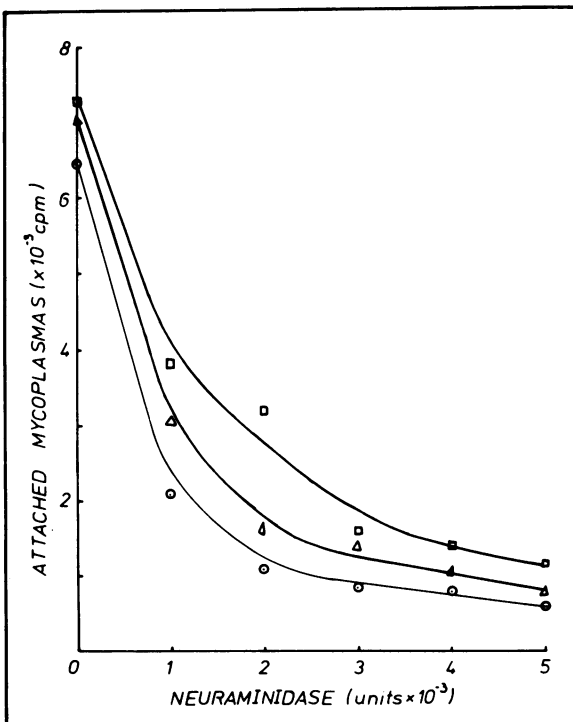

FIG. 1. Effect of neuraminidase pretreatment of human RBC of *M. pneumoniae* adherence. The percentages human RBC were exposed to different concentrations of neuraminidase for 0.5, 1.0, and 1.5 hours. These cells were washed, resuspended to 2 percent, and exposed to [³H]-labeled HA⁺ mycoplasmas (8 μg per sample; 332 CPM per μg protein) for 30 minutes at 37°C. Neuraminidase concentrates are presented as units per ml of 10 percent RBC □, 0.5 hour; △, 1.0 hour; ○, 1.5 hour. (From [7])

exhibited a high capacity to bind to glycophorin-sepharose beads and a lower capacity to bind to RBC. The binding of the eluted fraction to RBC was almost completely abolished by glycophorin, but not by its hydrophobic moiety. Binding of the fraction to glycophorin-sepharose beads was inhibited to about the same extent by both glycophorin moieties, suggesting that components of the eluted fraction are also capable of binding to the hydrophobic moiety of glycophorin, which is apparently exposed on the beads but not on the RBC surface. In order to circumvent this pitfall we have used the sialoglycopeptides of glycophorin as ligands. The affinity matrix prepared from it had a higher binding capacity. The fraction of the binding sites isolated from *M. gallisepticum* consisted primarily of one polypeptide [Kahane I, Granek J: in preparation]. Another point worth mentioning here is that efforts should be made to inhibit protease activity. This is important in general when attempting to isolate proteins, but also specifically in these studies, since the hemagglutinating activity may be associated with proteolytic activity [6].

Procedure Not Proved Successful

Although it is not common to discuss such an approach, here it seems important, as one should bear in mind that when dealing with binding sites of protein nature, one does not necessarily have to struggle with the solubilization of the entire membrane and its hydrophobic membrane protein, which usually requires the use of detergents. One may avoid it by cleaving a peptide from the binding site, which will retain its binding capacity. This approach failed, however, when applied to *M. pneumoniae* [8]. In these experiments, limited proteolysis was obtained by mild treatment with trypsin or pronase (in the presence of 0.15 M NaCl to reduce its activity). It seems, though, worthwhile to repeat these experiments with other proteolytic enzymes and under various conditions.

CONCLUDING REMARKS

The recent developments with the monoclonal antibodies to binding sites of *M. pneumoniae* [9,10] bring this high-power technology to our field and signal that these most specific ligands are at hand. One can easily foresee that, in addition to their use in morphological studies, the antibodies will be used for affinity chromatography. This will probably be the best approach for the isolation of quantities of binding sites and will enable their characterization. It will also answer the questions relating to be possibility of different types of binding sites occurring on a single organism.

Other approaches should be worked out for binding sites which do not seem to be of protein nature. The hydrophobic type of part of the attachment should draw our attention to interaction between proteins and lipids or lipids and lipids.

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