Glycosaminoglycans inhibit *Candida albicans* adherence to extracellular matrix proteins

Stephen A. Klotz a and Robert L. Smith b

a Departments of Medicine at the Veterans Administration Hospital, Kansas City, Missouri, USA, and at the Kansas University School of Medicine, Kansas City, Kansas, USA, and b Department of Biochemistry, Louisiana State University School of Medicine of Shreveport, Louisiana, USA

Received 15 July 1992
Revision received 15 September 1992
Accepted 17 September 1992

Key words: *Candida albicans*; Extracellular matrix; Fibronectin; Glycosaminoglycan; Heparin

1. SUMMARY

The ability of *Candida albicans* to adhere to subendothelial extracellular matrix (ECM) may be important in the pathogenesis of disseminated candidiasis. ECM proteins, such as fibronectin, laminin, and types I and IV collagen bind *C. albicans* avidly. These proteins all possess heparin-binding domains. The influence of the glycosaminoglycans (GAGS) including heparin, heparan sulfate and dextran sulfate on *C. albicans* adherence to subendothelial ECM and ECM proteins was studied. It was demonstrated that the GAGS inhibited *C. albicans* adherence to ECM and ECM proteins. This possibly occurred by the GAGS binding to the ECM proteins and, in so doing, masking a preferred ligand for *C. albicans* adherence.

2. INTRODUCTION

The adherence of *Candida* species to extracellular matrix (including the basement membrane) may be important in the pathogenesis of all candidal diseases [1]. The extracellular matrix proteins of particular interest include laminin, type I collagen, and fibronectin. The adherence of *Candida albicans* to fibronectin, a plasma and extracellular matrix protein, is believed to be important in the pathogenesis of such specific entities as disseminated candidiasis [2], candidal endocarditis [3], and mucosal candidiasis [4].

The ECM proteins laminin, and types I and IV collagen possess one heparin-binding site whereas fibronectin possesses two heparin-binding domains [5]. *C. albicans* has been shown to avidly adhere to all of these proteins. Indeed, it has been reported that *C. albicans* possesses ~8000 receptors for fibronectin [2] and laminin [6] with $K_d$ of $\sim 10^{-7}$ M and $10^{-8}$ M, respectively. In this report, we show that the glycosaminoglycans (GAGS) heparin, heparan sulfate, and dextran...
sulfate all inhibit the ability of *C. albicans* yeast cells to adhere individual ECM proteins possibly by masking preferred ligands for yeast cell adherence. Furthermore, heparin and heparan sulfate also inhibit yeast cell adherence to the complex target of subendothelial ECM.

3. MATERIALS AND METHODS

3.1. *Candida albicans*

Isolates were maintained on Sabouraud dextrose agar slants and transferred monthly. Fungi were grown in Sabouraud dextrose broth with agitation at 26°C for 20 h yielding the yeast cell phase. Cells were washed in Earle’s balanced salt solution containing calcium and magnesium by centrifugation [7].

3.2. Adherence targets

Bovine corneal subendothelial ECM was purchased from Accurate Laboratories (Westburg, NY) in 24-well tissue culture trays. Bovine skin type I collagen was obtained from Collagen Corporation (Palo Alto, CA) and murine laminin and type IV collagen were obtained from Collaborative Research Inc. (Bedford, MA). Human plasma fibronectin was isolated as previously described [2]. The ECM proteins were immobilized in 24-well trays as previously described [8]. Porcine intestinal heparin, bovine lung heparan sulfate and dextran sulfate were obtained from Sigma (St. Louis, MO).

3.3. Adherence assay

Yeast cells were diluted to $1.25 \times 10^3$/ml (estimated by hemacytometer counting) with or without GAGS and 200 µl placed in each well for 30 min at 37°C without agitation. The percent of yeast cells adhering to each ECM protein of the total of yeast cells added to each well was then determined by the colony-forming unit technique as previously described [7]. The data were not normalized, thus the percentage adherence for controls when performed weeks apart were different.

4. RESULTS

Heparin at 0.1 mg/ml or greater concentration significantly reduced *C. albicans* yeast cell adherence to immobilized fibronectin, laminin, and type IV collagen (Fig. 1). This inhibition occurred in a dose-dependent manner. The inhibition of adherence by the GAG appeared to be due to the binding of the GAG to the immobilized ECM protein. For example, pretreatment of immobilized fibronectin, or yeast cells, with heparin demonstrated that the pretreatment of the ECM protein reduced adherence, whereas pretreatment of yeast cells with heparin did not affect adherence (Table 1).

The ability of other GAGS to affect the adherence process was then investigated. Heparin, heparan sulfate and dextran sulfate all reduced yeast cell adherence to type I collagen in a dose-dependent manner. At the highest concentration of each GAG (1 mg/ml), the percent inhibition of yeast cell adherence to type I collagen was 39% for heparin, 70% for heparan sulfate, and 68% for dextran sulfate (Fig. 2). Furthermore, heparin and heparan sulfate caused a reduction in yeast cell adherence to the more complex target,

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Adherence of <em>Candida albicans</em> yeast cells to immobilized fibronectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>$n$</td>
</tr>
<tr>
<td>(1) Yeast cells suspended in buffer</td>
<td>4</td>
</tr>
<tr>
<td>(2) Yeast cells suspended in buffer plus heparin</td>
<td>6</td>
</tr>
<tr>
<td>(3) Yeast cells pretreated with heparin</td>
<td>6</td>
</tr>
<tr>
<td>(4) Yeast cells added to fibronectin that had been pretreated with heparin</td>
<td>6</td>
</tr>
</tbody>
</table>

Yeasts were suspended in buffer (1), (3), (4) or buffer plus 1 mg/ml heparin (2). Immobilized fibronectin (4) or yeast cells (3) were treated for 2 h with 1 mg/ml heparin and then washed thoroughly before adherence assay performed.
Fig. 1. Inhibition by heparin of *Candida albicans* adherence to four ECM proteins. Each well was pretreated with 20 μg protein in 200 μl of buffer, washed and then yeasts added with heparin. Filled circles, fibronectin; open circles, laminin; filled triangles, type IV collagen; and open triangles, type I collagen. The means representing four replicates are plotted + 2 S.E.M. (space bars).

Fig. 2. Inhibition of *Candida albicans* adherence to type I collagen in the presence of various concentrations of glycosaminoglycans. Open triangles, heparin; open circles, heparan sulfate; and filled triangles, dextran sulfate. Each point represents the mean of four replicates with space bars equal to 2 S.E.M.

subendothelial extracellular matrix. For example, heparin caused a 32% inhibition of adherence of yeast cells to ECM, whereas heparan sulfate caused a 33% inhibition of yeast cell adherence to subendothelial ECM. By contrast, dextran sulfate had no effect on the adherence of yeast cells to subendothelial ECM (data not shown).

5. DISCUSSION

The adherence of *C. albicans* to subendothelial ECM is believed to be a virulence factor of this organism [1]. The ECM not only includes the amorphous interstitium but the electron dense limiting basement membranes as well. The major proteins contained in subendothelial basement membrane are type IV collagen and laminin whereas fibronectin and type I collagen predominate in the interstitium [9]. The ECM, including basement membranes, also contain proteoglycans which possess characteristic highly charged glycosaminoglycans (GAGS) attached to a protein moiety. Therefore, a yeast cell encountering subendothelial basement membrane is likely to encounter GAGS contained in proteoglycans in addition to the matrix glycoproteins such as laminin or type IV collagen.

In previous work, we have shown that *C. albicans* adheres avidly to immobilized laminin, types I and IV collagen, and fibronectin as well as subendothelial ECM [8,10]. Conversely, when GAGS were immobilized in tissue culture trays and yeast cells incubated with the immobilized GAGS, there was no increased adherence. On the contrary, there was actually decreased adherence of yeast cells to immobilized heparin, chondroitin sulfate, hyaluronic acid and keratan sulfate. The inhibition of adherence of yeast cells to immobilized GAGS was believed to be due to electrostatic interactions.

The inhibition of *C. albicans* adherence to ECM and ECM proteins by GAGS appears to be due primarily to the binding of the GAG to the matrix protein and in so doing perhaps covering or masking a potential ligand for *C. albicans*. This is suggested by the data in Table 1 showing that pretreatment of fibronectin with heparin but not pretreatment of yeast cells reduces yeast cell adherence significantly to the immobilized fibronectin. This is further supported by the data...
comparing the inhibitory activity of the GAGS with subendothelial ECM. Dextran sulfate is the most sulfated of the three GAGS and therefore is the most negatively charged in solution. If electrical charge were the only explanation of the inhibitory activity of the GAGS, dextran sulfate should have been the most active compound. However, dextran sulfate had little or no inhibitory activity with subendothelial ECM, suggesting that a specific interaction between heparin and heparan sulfate occurred with subendothelial ECM.

In conclusion, it is possible that the GAGS within the proteoglycans which are an integral part of the ECM may actually retard *C. albicans* adherence to ECM. This may occur by masking preferred ligands of *C. albicans* or by electrostatic interactions, or perhaps both. Furthermore, this report shows that GAGS in solution can affect the adherence process and it would be interesting to determine if such molecules would affect the outcome of metastatic candidiasis induced by intravenous inoculation of yeast cells. Preliminary work by us demonstrates that a 45 kDa fibronectin fragment containing the heparin binding domain (Telios Pharmaceuticals, Inc., San Diego, CA) binds as avidly to *C. albicans* yeast cells as does a 120 kDa fibronectin fragment containing the cell binding domain (Arg-Gly-Asp). Previous work by us has established that the fibronectin peptides containing the sequence Arg-Gly-Asp ameliorates the metastatic process of disseminated candidiasis [11]. It is possible that selective GAGS could do the same, perhaps by binding to exposed ECM glycoproteins such as fibronectin, laminin or type IV collagen. Inhibition of epithelial cell adhesion to these same ECM molecules with proteoglycans has been demonstrated, due probably to the masking of preferred ligands for the integrin receptors borne on the surface of epithelial cells [12]. In this regard it is important to note that we and others have proposed that *C. albicans* possess integrin-like molecules that may be responsible for yeast cell adherence to ECM and/or endothelium [2,13].

ACKNOWLEDGEMENTS

This work was supported by a grant from the Veterans Affairs Research Service.

REFERENCES