

Native BAD-1 Binding to Heparin-agarose

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[Abstract] BAD-1 is an adhesin created by the dimorphic fungus *Blastomyces dermatitidis*, the causative agent of blastomycosis. We have determined that it has an affinity for heparin, which may explain its impact on virulence and human immune function as a number of cells related to immune function have heparin like moieties on their surfaces. This assay allows a quantification of binding between soluble BAD-1 and immobilized heparin.

Materials and Reagents

1. Heparin-agarose resin (Sigma-Aldrich, catalog number: H6508) (prior to use it is washed 3x in five volumes of tricine buffer to eliminate free heparin)
2. 10 µg of BAD-1 [purified according to the method of Brandhorst *et al.* (2005)]
3. 25 microliters of soluble medical-grade sodium heparin for injection (50 mg/ml) (Elkins-Sinn Inc)
4. Tricine buffer (Sigma-Aldrich, catalog number: T0377) (see Recipes)

Equipment

1. Accuspin micro17 microcentrifuge (Thermo Fisher Scientific)
2. Nanodrop ND1000 spectrophotometer

Procedure

1. 100 µl of 0.1 mg/ml BAD-1 in tricine buffer was incubated with agarose heparin resin (5 µl bed volume) for 30 min at 25 °C with occasional agitation.
2. Resin beads were pelleted by centrifugation in an Accuspin microfuge at 7,000 x g for 1 min.
3. Samples of the BAD-1 containing supernatant were analyzed for optical density at 280 nm in a Nanodrop ND1000 spectrophotometer. This reading was compared to a control solution of BAD-1 to which tricine buffer was added in place of heparin-agarose beads.

The discrepancy in absorbance is assumed to be linear with respect to BAD-1 adhering to the heparin resin bed.

4. Binding inhibition studies were done by repeating steps 1-3 in the presence of soluble medical grade heparin in tricine buffer. Heparin was added to the tricine binding buffer at various concentrations (0.01, 0.1 and 1 mg/ml - significant inhibition was noted at 0.1 mg/ml heparin and above.). Measurements of baseline absorbance were corrected to account for absorbance of added heparin inhibitor.

Recipes

1. Tricine buffer
20 mM tricine (pH 7)
50 mM NaCl

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References

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