PARTIAL GLOMALIN PURIFICATION BY PRECIPITATION AND DIALYSIS

(Wright *et al.*,1998)

Introduction

Precipitation and dialysis are used to partially purify glomalin prior to chemical or molecular analysis or lyophilyzation (freeze-drying) and analysis or storage. The Bradford total protein concentration (see Bradford total protein methodology) should be at least 5 mg or the freeze-dried amount will be rather small and difficult to measure accurately. Several different chemicals may be used to precipitate and reconstitute glomalin which may be dialyzed against a dilute buffer solution (such as borate) or water. Like other proteins, glomalin will precipitate in a high ionic (salt) solution such as NH_4SO_4. Glomalin is insoluble in acid solution and will precipitate in HCl at pH 2.0 to 2.5. In addition, glomalin may be reconstituted in many alkaline solutions for dialysis. We use the precipitation in HCl, reconstitution in NaOH, and dialysis against water procedure most often.

Materials

Precipitation solutions*: (1) 20% TCA (Trichloroacetic acid), (2) 1 N HCl, or (3) 4 M NH_4SO_4
Reconstitution solutions*: (1) 100mM sodium borate, pH 9.0, (2) 0.1 M NaOH, or (30 any alkaline salt solution
Dialysis solutions*: (1) 10 mM sodium borate, pH 8.0, or (2) deionized (Milli-Q) water (dH_2O)

Dialysis tubing (≤12,000 Daltons) and clamps
Alkaline solution proof labels (optional)
Rubber bands (optional)
Dialysis jar, large beaker or bucket (5 to 10 L container)
Thin foam or plastic circle that fits in top of dialysis jar, beaker, or bucket or weights to keep samples submerged in dialysis solution
Centrifuge and centrifuge tubes
Ice bucket and ice
Pasteur pipettes and bulbs
Freeze drying apparatus

***Note: Several different chemicals may be used in this procedure depending on experimental needs. There is a possibility that some of the TCA or NH_4SO_4 will adhere to glomalin during these procedures. If you are measuring carbon and nitrogen concentrations in glomalin, this may produce inaccurate results. In this case, it is recommended that you use HCl, NaOH or sodium borate to eliminate problems.**

Methods

1) Place samples in centrifuge tubes.
2) Balance tubes and centrifuge at 10,000 rpm* for 10 min.
3) Collect supernatant.
4) In new centrifuge tubes, precipitate glomalin by mixing samples 1:1 with ice cold 20% TCA or with 4 M NH_4SO_4 or titrating samples to pH 2.0 to 2.5 with 1 N HCl.

5) Incubate in ice for 1 hr.
6) Balance tubes and centrifuge at 10,000 rpm* for 10 min.
7) Remove supernatant.
8) Reconstitute pellet at the bottom of the tube with 100mM sodium borate at pH 9.0 or 0.1 M NaOH in 1-mL increments.
9) Use a Pasteur pipette to mix solution trying to minimize volume. Mix slowly to prevent excessive bubbling
10) Make sure that all of the precipitate has been reconstituted.
11) Fill dialysis jar, beaker or bucket with 10mM borate at pH 8.0 or dH_2O.
12) Prepare dialysis tubing: Cut a strip of dialysis tubing (1.5X the amount needed to hold the reconstituted sample). Soak the piece of tubing in a large volume of dH_2O to remove glycerine, clamp or tie bottom, and rub other end between fingers to open.
13) Transfer sample into bags using Pasteur pipette, clamp or tie the top (making sure that the sample is labeled), and place bags in dialysis solution. Cover with a thin piece of foam or plastic to keep bags submerged in solution, and incubate for at least 6 hr while stirring continuously.
14) Change dialysis solution at least five times (more if a large volume is being dialyzed) at 6 to 12 h intervals.
15) Remove bags from solution and transfer contents into centrifuge tubes.
16) Centrifuge at 10,000 rpm* for 10 min.
17) Collect supernatant and freeze at $-20^{o}C$ until completely frozen. Place in freeze-drying apparatus until dry.

* Centrifugation may be conducted at any speed from 3000-10000 xg. Higher speeds or longer duration will assist in pelleting the sample.