Sucrose Gradient Analysis of Proteins

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[Abstract] The sedimentation rate of a protein in a linear sucrose gradient can be used to determine its S value when compared to proteins of known S values which are run in a parallel gradient. This can be used to estimate an approximate molecular weight of the protein and test the potential interaction of two proteins if they peak in the same gradient range.

Materials and Reagents

1. Sucrose (Sigma-Aldrich, catalog number: S0389)
2. Gelatin (Sigma-Aldrich, catalog number: G9136)
3. Aldolase (Bio-Rad Laboratories)
4. Thyroglobulin (Bio-Rad Laboratories)
5. Sucrose solutions (see Recipes)

Equipment

1. Centrifuges (Beckman Falcon, TLS-55)
2. Gradient mixer (Sigma-Aldrich)
3. Stirring bar

Procedure

1. Make 10% and 40% sucrose solutions in the same buffer used for the protein sample.
2. Coat the tubes with 1% gelatin.
3. Fill the tube with 1% gelatin solution.
4. Pour the gelatin out and wash several times with ddH_2O.
5. Make a 10-40% linear sucrose gradient.
   Note: There are two chambers in a gradient mixer, a reservoir and a mixing chamber, with an interconnecting valve. A second valve regulates the output flow from the mixing chamber. A magnetic stirring bar can be placed in the mixing chamber to maintain a

constant gradient. All of the mixers have a flat base to enable them to be placed on a magnetic stirrer.

6. Put the gradient mixer on top of a stir plate so that the gradient maker is level. Put a small stir bar in mixing chamber.
7. With all valves closed, fill each chamber with 1/2 the total amount of desired gradient [*i.e.* 1.1 ml for a 2.2 ml gradient (TLS-55)]. Use 40% sucrose in mixing chamber and 10% sucrose in reservoir chamber.
8. Turn on the stir plate to a setting so it is mixing gently.
9. Place the output tube in the centrifuge tube so that it is just touching the side near the top.
10. Open both valves at the same time. The 40% sucrose should start filling the centrifuge tube and the 10% sucrose should start mixing with the 40% sucrose.
11. Close both valves when the gradient nears the top of the centrifuge tube. Be sure to leave enough room to layer the sample on top of the gradient.
12. Put the gradients and rotor in the cold room for between 2-48 h. Make sure the tubes are balanced and then layer the sample on top. The samples should be of a small volume (*e.g.* 100 µl for a 2.2 ml gradient).
13. Spin at 55,000 rpm for 2.5 h.
14. Collect fractions to analyze by western blotting. Aldolase (158 kDa, 7S) and thyroglobulin (690 kDa, 19S) were used as size markers and analyzed in parallel. Ideally the protein of interest should be in the middle of the gradient.

Recipes

1. 10% sucrose solutions
   10 mg sucrose in 100 ml ddH_2O
2. 40% sucrose solutions
   40 mg sucrose in 100 ml ddH_2O

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