BMOL2201/6201 Case Study – Proteases

2. Isolating a Storage Protein from Seeds of *Brassica nigra*

Focus concept

Purification of a novel seed storage protein allows for sequence analysis.

Prerequisites

- Protein purification techniques, particularly gel filtration.
- Protein sequencing using Edman degradation.

Background

Seedlings use seed storage proteins as an important nitrogen source during germination. The seed storage proteins are made as large precursors, then hydrolyzed to smaller products for the seedling's use during growth. In this case, the investigators discovered a new seed storage protein, which they named BN, in the oilseed *Brassica nigra*. These seeds are important nutritionally as a source of oil as well as protein. The storage protein described here was first purified and then characterized for its important biochemical properties. The storage protein turned out to be an inhibitor of serine protease enzymes. The authors hypothesized that the purpose of serine protease inhibitors like BN is to protect the plant from proteolytic enzymes of insects and microorganisms that would damage the plant.

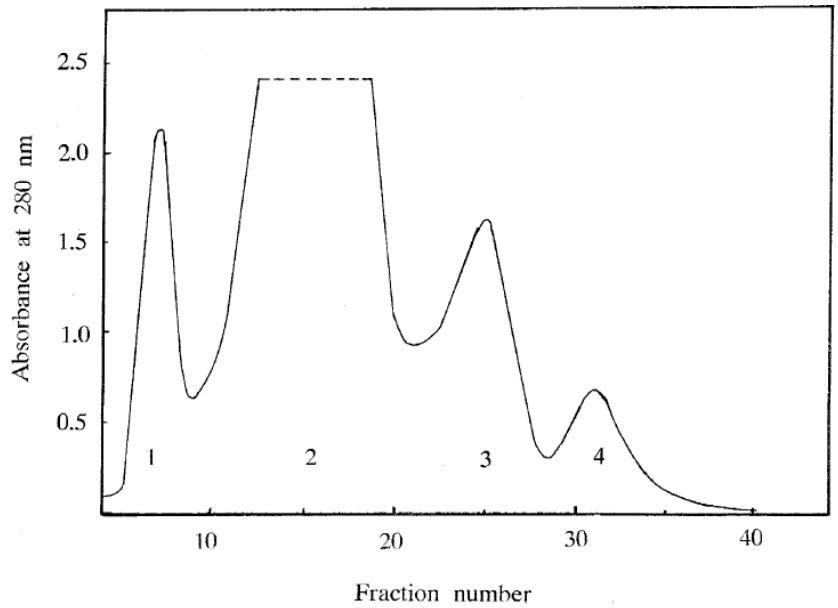
The protein BN likely belongs to a family of seed storage proteins called napins, which typically consist of two non-identical disulfide-bonded polypeptide chains. Napins make up as much as 20% of the protein content of seeds

Questions

In order to isolate the protein, seeds were ground and extracted with water. The proteins in the extract were precipitated with hydrochloric acid and the pellet isolated by centrifugation was discarded. The supernatant was heated to 70°C to remove heat-labile proteins, then lyophilized (freeze-dried). The lyophilized powder was dissolved in a small amount of ammonium acetate buffer at pH = 5 and the sample was loaded onto a Sephadex G-25 gel filtration column.

1. What is the property on which separation by a gel filtration column is based?

The elution profile showed four peaks in the figure below. Most of the BN protein eluted in the first peak.

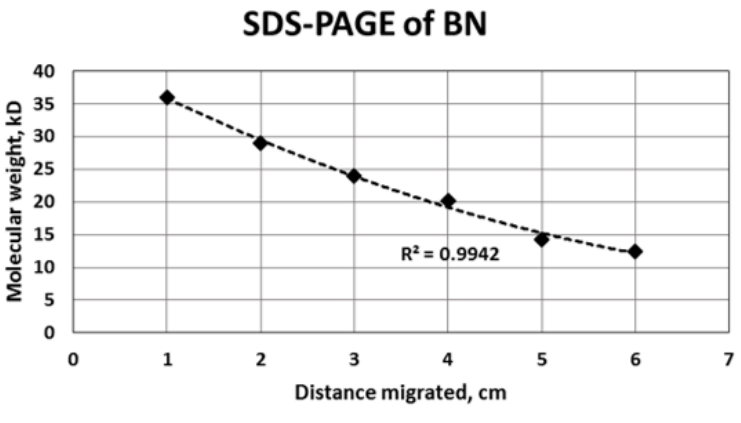


Compare the BN protein to other proteins found in the *B. nigra* seeds.

2. How is the BN protein different from the other proteins found in the *B. nigra* seeds from the elution profile shown?

Following gel filtration chromatography, the BN protein was further purified by dialysis using tubing with a 6000-8000 molecular weight cut-off. Analysis using SDS-PAGE (in the absence of β- mercaptoethanol) showed a single band. The results were tabulated and a simplified standard curve of molecular weight of the standards *vs.* migration distance in the gel is shown below (normal std. curve for SDS_PAGE is log (Mol. wt.) *vs.* distance):

Molecular weight, kD	Distance migrated, cm
36	0.3
29	1.4
24	2.6
20.1	3.5
14.2	5.4
12.5	6.1
BN protein	5.0



3. What is the molecular weight of the BN protein from the standard curve?

Next, the investigators attempted to sequence the protein using an Edman degradation procedure. However, this was initially unsuccessful because the amino terminus was blocked. Based on comparisons with other proteins in the same family as BN whose sequences are known, the investigators hypothesized that the amino terminal amino acid was *N*-acetyl serine.

4. **If *N*-acetyl serine is the amino terminal amino acid, why would sequencing using the Edman method be unsuccessful?**

5. **What are some other ways that the investigators could try and sequence the protein?**