



Challenges in the Determination of Lectins

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COVANCE.



Topics

- Brief lectin background
- Methodology – the past
- Methodology – the present
- Methodology – the future?

Background

- Lectins are a type of proteins that bind to sugars
- Etymology – from Latin legere – to choose
- High affinity for a specific sugar
- Can bind with free sugars, polysaccharides, glycoproteins, or glycolipids
- Can occur in both plants and animals

History

- Early recognition of the toxicity of castor beans
- Agglutination of blood cells
- Ricin isolated in late 1800s
- 1960s - Agglutination of other types of cells
- 1970s - Lectins as powerful tools in the investigation of carbohydrates

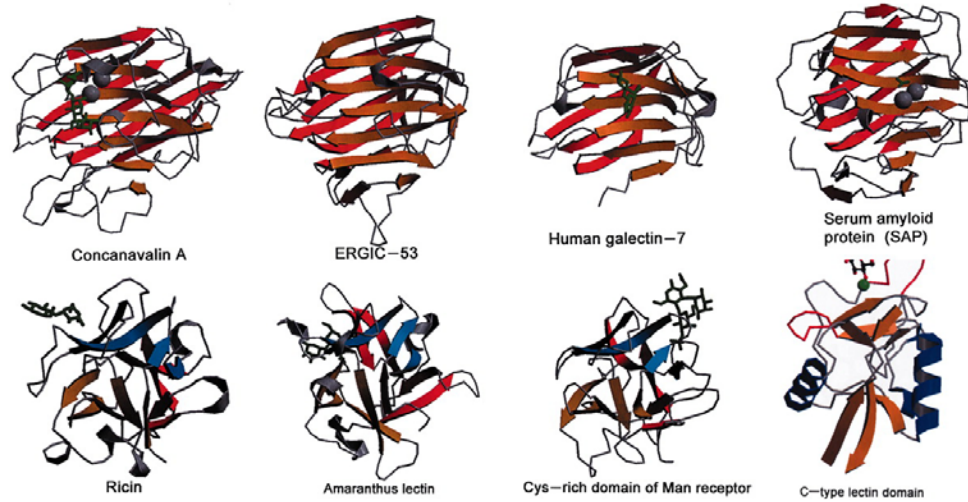


Functions

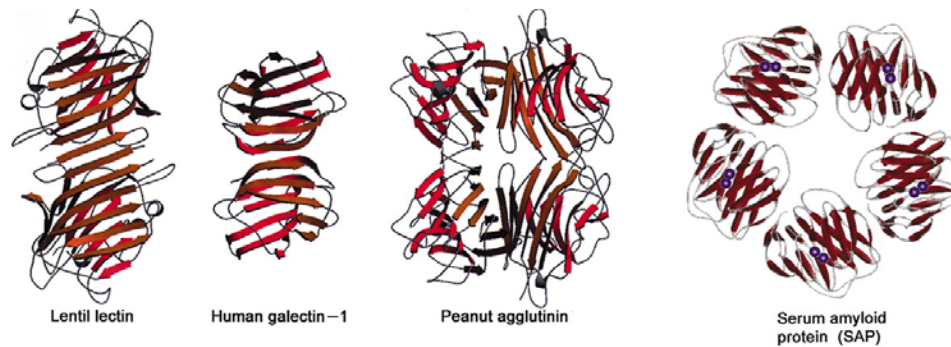
- Play a role in the defense mechanism of plants against attack from microorganisms, pests, insects.
- Fungal infections or wounding seems to increase lectin content.
- In legumes, recognition of nitrogen fixing bacteria and binding them to plant roots.
- Storage of protein, mitogenic stimulation and transport of carbohydrates.

Structures of different lectins represented as ribbon diagrams.

A



B



Sharon N , Lis H Glycobiology 2004;14:53R-62R

GLYCOBIOLOGY

Plant Lectins

- Many (but not all) lectins are toxic
- The most well studied lectins are found in legumes
- Stems and leaves of a plant may have different lectins than seeds
- Concentrations of lectins vary with the age and development stage of the plant

Example plant sources of lectins

Castor



Snowdrop



Wheat



Soy Lectin



- Soy was well known to poorly support growth of rats unless subjected to heat treatment. Thought to be due to trypsin inhibitor which had been isolated from raw soybeans.

Irv Liener's work

- Added trypsin inhibitor to heat treated soy and found that trypsin inhibitor only partially accounted to antinutrient activity.
- Purified soy lectin and published in 1952
- Developed assay using trypsinized rabbit red blood cells in 1955

With only minor modifications, this is still the assay being used today.

The Past - Liener agglutination method

- Trypsinized rabbit blood cells.
- Extraction
- Serial dilutions (none to 1:512).
- Add blood suspension
- 2.5 hours undisturbed in vertical position
- Read on photometer using special adapter.
- Measure against blood curve.
- Determine the dilution equivalent to settling of 50% of cells.
- Standardize results based on a known control soybean agglutinin sample.



Units for agglutination assays

1 hemagglutinating unit (H.U.) has been arbitrarily defined as that level of test solution which causes 50% of the standard cell suspension to sediment in 2.5 hr under the conditions described.

OECD Soy Guidance Document

From Section III – Antinutrients in Soybeans

B. Lectins

Lectins are proteins that bind to carbohydrate-containing molecules. Lectins in raw soybeans can inhibit growth and cause death in animals. It is expected that similar effects would occur in humans. **The ability of lectins to act as hemagglutinins that cause blood clotting is the basis for most quantitative analytical methods.**

Soybean lectin is sometimes referred to as soybean hemagglutinin. Lectin levels can vary from 37 to 323 Hemagglutinating Activity Units (HU)/ mg protein (Kakade et al, 1972).....

Method issues

- Obsolete equipment
- Vague description of spectrophotometer adapter
- Variability of assay due to blood preps



The Present

Recent modifications to Liener method

Modification made to the following:

- Concentration of blood cells
- Dilutions
- Calculations

Results are somewhat lower but in the same ballpark as the data referenced in OECD which used the traditional method.

Soy lectin agglutination assay issues

- Assay results are dependent on the exact conditions of the test which will vary from lab to lab
- Inaccuracy introduced in serial dilutions
- Accidental jostling of tubes during 2.5 hr agglutination period
- Variations in results from blood cell sources, preps

Problems with agglutination assays

- Lack of sensitivity
- Interferences
- Poor correlation with other methodology
- Poor correlation with other agglutination assays run under different conditions.

Data comparison agglutination vs ELISA

Determination of hemagglutinating activity and of biologically active SBA in commercially available foods

	Total protein in extracts mg/g of edible product	Hemagglutination assays mg/g of edible product	Quantitative ELISA mg/g of edible product
Soy sprouts	1.522	0.245 ± 0.210	0.30 ± 0.07
Soy hamburger	0.059	Undetected	2.5 x 10 ⁻³ ± 1 x 10 ⁻⁴
Soy milk	1.562	Undetected	4.7 x 10 ⁻³ ± 8 x 10 ⁻⁵
Seeds	84	8.64 ± 7.01	0.35 ± 0.03

From: Rizzi, C., Galeoto, L., Zoccatelli, G., Vincenzi, S., Chignola, R., and Peruffo, A. D. B. "Active soybean lectin in foods: quantitative determination by ELISA using immobilized asialofetuin", *Food Research International*, 36 (8):818 (2003)

Future for lectin assays

Stick with agglutination assay?

Or

Update to new methodology?

Steps to improve agglutination approach

1. Consider updated approaches
Coulter counter?
2. Publish updated method single lab validation
3. Collaborative study with other laboratories
4. Standardize results against a mutually agreed upon control substance
5. Publish reference data using new method
6. Consider “officializing method” via organization such as AOAC, AACC, etc

Other published methods

- RIA
- Functional lectin immuno assay
- Biosensor
- Affinity Chromatography
- ELISA
- Other?

Questions



References

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