Safety of GM Food Crops: Protein Allergenicity Assessment

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General Considerations - Proteins

Proteins and the human diet

- Macronutrients: protein, fat, carbohydrate, fiber
- Exposure is relatively “High” - Essential component of the human diet
  - 100 g/day consumed
- Most dietary proteins are not absorbed intact but are digested and exposure is not to the fully functional protein
- Protein consumption is not inherently associated with adverse effects
- ***Only a small number of proteins are known to be allergenic***

Safety – A Weight of Evidence Approach

Interpretation of study results are taken into consideration using a holistic approach – all studies are used to perform a risk assessment.

Risk = hazard *multiplied by* exposure

- If no adverse findings: the protein has ***"reasonable certainty of no harm"***
- When an uncertain finding for any one characterization is observed:
  - Could indicate that the protein represents a hazard
    - (E.g. – shares some characteristic with known allergens)
  - ***Further testing*** would be indicated and necessary to demonstrate safety or potential allergy

Allergy Health Risks – Biotech Proteins

- Has the protein been **unintentionally transferred** from a known allergen source and is the protein itself a known allergen?

- Has the transformation **process increased the normal expression of endogenous allergens** in such a way to increase the risk to allergic patients?

- Will the novel protein expressed in the biotech product become an allergen once exposed to workers/consumers? *i.e.*, **is there risk of a *de novo* allergen**?

Case Study; Identifying the Transfer of an Allergen into a GM Crop

- Mid - 1990s, Soybean product was **genetically modified** with the 2S albumin (now **known as allergen, Ber e1**) to enhance the nutritional content.

  - soybean are deficient in methionine, so sulfur-rich proteins from nuts make a good additive.

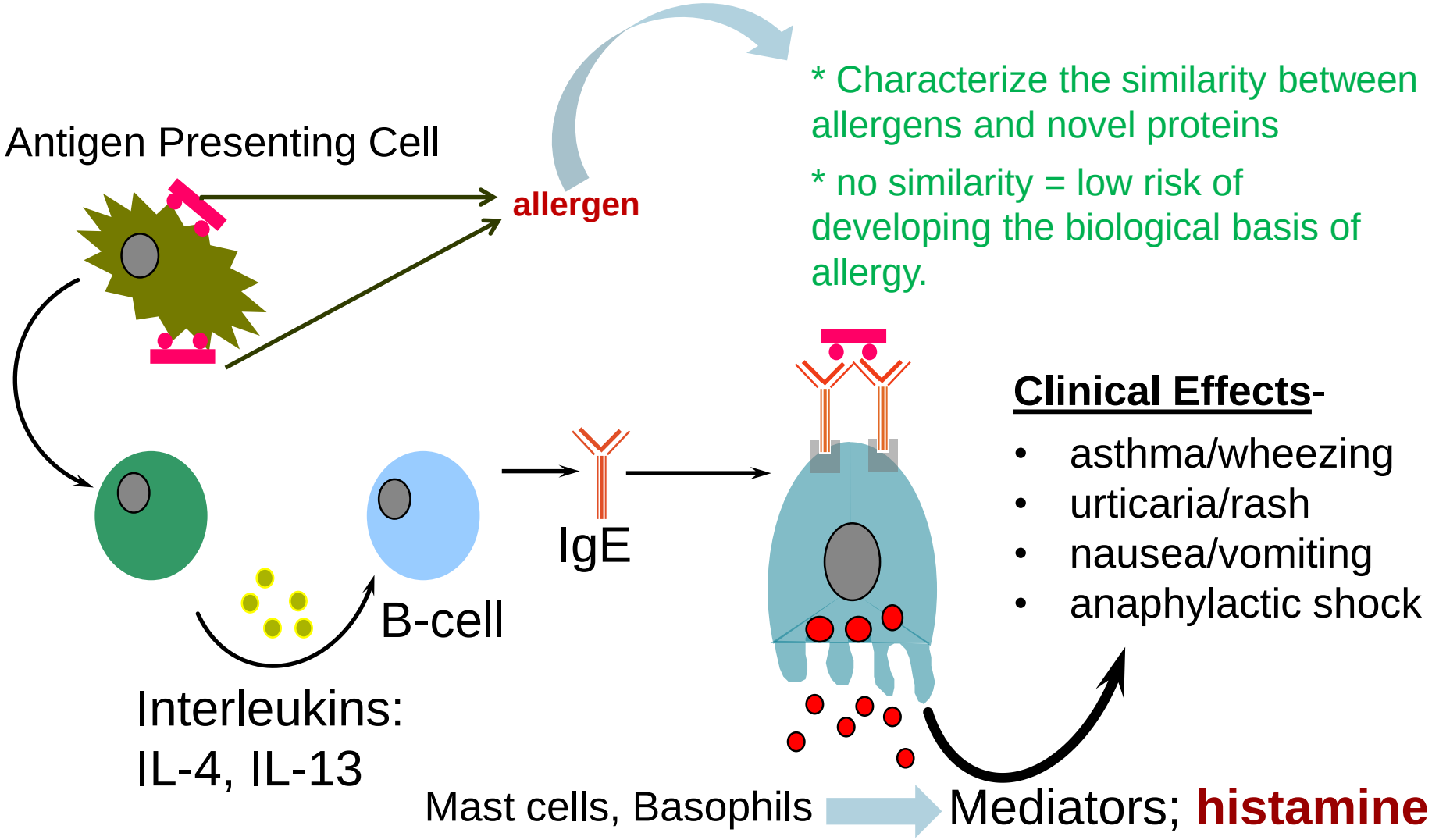
- Company **proactively studied** the risk of allergic reactivity to the GM soybean containing the Ber e1 gene and protein using

  1. serum IgE reactivity and

  2. reactivity with skin prick testing (Nordlee, et al., 1996).

- **Product Development was stopped**

Cellular Basis for the Formation of Type I, IgE Mediated Allergic Responses



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Allergy Health Risks - Biotech Proteins

Higher

Risk Level

Low

Allergy Risk Characterization

Gene from allergenic source?

Is sequence similar to known allergens/toxins
Is pepsin digestibility rapid?

Glycosylated?

Exposure likely after heat/processing?

Is Abundance low?

Does protein have a history of safe exposure?

Evidence Supports Allergy Safety

Allergy and Biotechnology: Safety Guidance

Evolving allergy strategy to manage health risks

- CODEX
  - Intergovernmental body (>180 member states)
  - Implements joint FAO/WHO Food standards programs
  - Protects health of consumers and facilitates trade by setting international safety standards
  - **No single test can predict protein allergenicity**

Allergy and Biotechnology: Codex Guidance

<u>If introduced protein from a non-allergenic source</u>

- assess amino acid sequence similarity to known allergens (bioinformatics) (*Thomas et al., 2006, Mol. Nutr. Food Res., 50:591-670*).
- assess in vitro pepsin resistance by using a standardized protocol (*Thomas et al., 2004, Regul. Toxicol. Pharmacol., 39:87-98*)

<u>If introduced protein from an allergenic source</u>

- assess amino acid sequence similarity to known allergens (bioinformatics)
- assess in vitro pepsin resistance
- assess specific IgE binding, when applicable

Allergy and Biotechnology : Codex Guidance

Adaptive allergy strategy to manage health risks

- CODEX recommended allergy assessment
  - ***Other considerations***
    - ✓Exposure level of the introduced protein
    - ✓As science and technology evolves other methods may be considered

❖**Targeted serum screens**

❖**Animal models**

❖**T-cell epitopes, structural motifs associated with allergens**

Allergy and Biotechnology: Allergy Safety

Adaptive allergy strategy to manage health risks

- Science of allergy is still evolving
- Current assessment process utilizes best science available
- Current assessment process is successful at preventing the introduction of known or cross-reactive allergens into the food supply
- Harmonization of the allergy assessment process is underway
- **Safety process utilizes a "weight of evidence" approach;**
  - the goal is to add to this approach with scientifically justified methods, when appropriate.

Bioinformatics - Standardized Approaches for Allergy Risk

- Focused on one *primary* question:
  - Is the query (novel) protein similar to an existing allergen?
  - Can also use the results to identify source organism of a trait protein.
- Screening based on knowing the sequences of hundreds of food and respiratory protein allergens
- Identifying significant similarity and possible homology with known allergens means that a novel protein may cross-react with an existing allergen.
- However, bioinformatics is not intended to predict whether a protein will *"become"* an allergen.
- Bioinformatics is rarely an answer in and of itself.

Bioinformatics - Principles

- Protein structure is determined by amino acid sequence
- Similar amino acid linear sequences have similar structure/function
  - Similar sequence and structure infers a common ancestor and related function across species (Taxonomic relatedness)
- Allergen homologues (highly similar proteins) from two species are often allergens, but not always
- Non-homologous proteins do not cross-react

Bioinformatics -*comparing one-to-many-* Is there a match?

GM Protein Sequence

…A R Y Q P E R S T R…

Qualified Database of Allergen Sequences

…A R Y Q P E R S T R…

…S S E Q Q T M G F T A…

…M T Y Y Q S D V E K E T…

…F Y V E Q D S E D V V Y…

GM sequence is compared in a one-to-many alignment process

Sequences may not match at all or match so little that the process does not report them

Database sequences that match the *Query* are judged on the significance of the match

Homologous match!

…A R Y Q P E R S T R…

…A R Y Q P E R S T R…

Guidance for Determining Significant Similarity

1. 35% shared identity between an novel biotech/GMO protein and a known allergen

➢ PLUS

2. Minimum of 80 amino acids in overlap length

❖ Why (2) criteria?

- Because being short is not a feature of shared homology between cross reactive allergens

- Short amino acid matches
  - In the past there has been a requirement for exact matching 8 or amino acid matches –
  - Although there is an expectation for this

TYQRTSQV	Novel protein
TYQRTSQV	**known allergen**

There is a lowered requirement (EU) for this given the lack of biological evidence backing this “in silico” approach.

Bioinformatics – Where does it play a role in safety?

- Bioinformatics plays a continual role throughout a novel protein's development life cycle; this is because -
  - Bioinformatic reviews can be updated frequently
  - New allergens and toxins are discovered which creates an update to the databases used for bioinformatics
- Helps to inform other aspects of the protein safety assessment
  - HOSU: similarity with proteins with safe records
  - Mode-of-action

Critical to any kind of bioinformatics – a well understood database

- Industry consortium database at University of Nebraska
  - Leaders in the field, Rick Goodman and Steve Taylor host a peer-reviewed allergen sequence database

food allergy research & resource program
farrp

University of Nebraska Lincoln

- What is it?
  - A database of 1,706 whole or partial protein sequences with a minimum of at least one publication supporting IgE binding or other clinical evidence and the sequence itself as a relevant human allergen.

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Simulated Gastric Fluid: protein stability to pepsin enzyme proteolysis

- Characterizes the rate at which a protein is degraded:
  - **Interpretation**: if a novel protein is “stable”, *i.e*., as stable as some allergens, then risk of exposure and therefore, the potential for allergy is greater.
- **Method standardization** has been key for screening novel food proteins (Thomas et al, 2004 – An ILSI/PATC publication); regulators are able to compare results from one company to the next for similar proteins.
- The characterization of simulated gastrointestinal stability for novel proteins has expanded to include an additional characterization.
  - **Simulated Intestinal Fluid assay** (pancreatic enzyme mix) can be included in assessments of novel proteins.

Glycosylation

- Is not consistently associated with clinically relevant allergenic potential
  - Mari A, et al., Evaluation by double-blind placebo-controlled oral challenge of the clinical relevance of IgE antibodies against plant glycans. *Allergy 63: 891-896.*
- **Industry**: characterization is by a **standardized in vitro kit** to measure presence of carbohydrate moieties on plant-extracted novel proteins.
- Does not have the priority status of SGF and bioinformatics, but is unlikely to be removed from registrations (and expectations).
  - *Does provide a conservative approach and does add to the weight of evidence for novel protein not undergoing unintended changes, in planta*.

Concluding an Assessment of Allergy Safety

Evidence Does Support Allergy Safety

Allergy Risk

Higher

Risk Level

Gene from allergenic source?

Is sequence similar to known allergens?

Is pepsin digestibility similar to allergens?

Glycosylated?

Exposure likely after heat/processing? → More applicable to toxicity assessment

Is Abundance low? → Low abundant proteins = lower exposure

Does protein have a history of safe exposure? → Taking on more prominence.

If a protein is derived from a commonly consumed safe food then it should still be safe

Low

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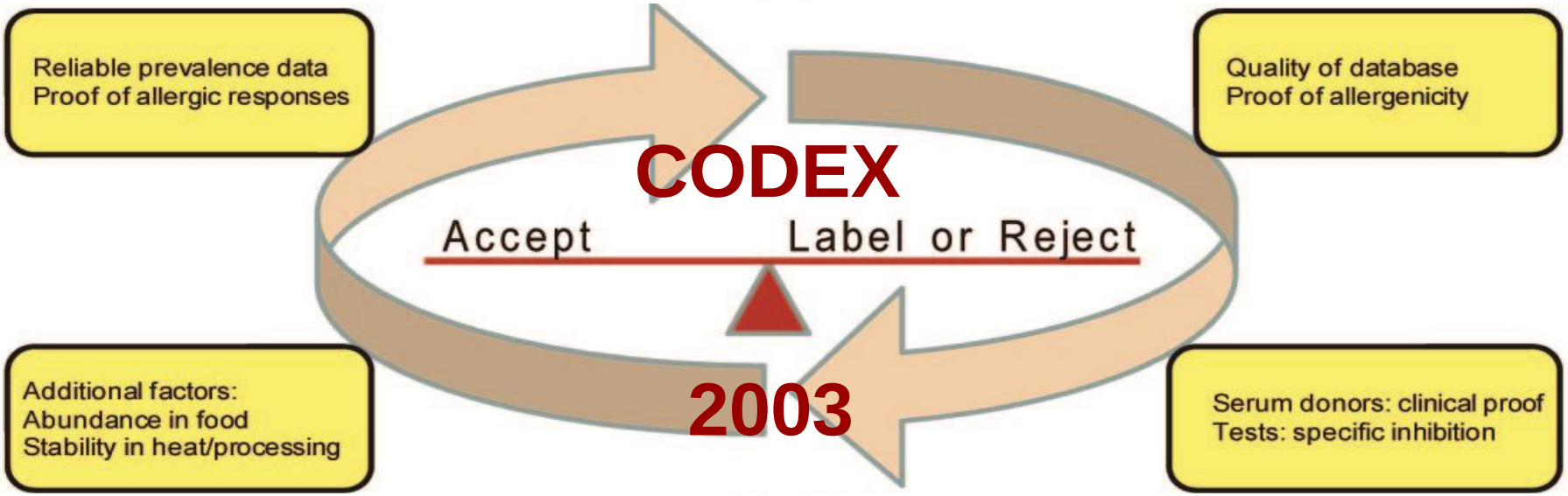
Weighing results from tests with imperfect correlations (Codex 2003)



from R.Goodman (Nature Biotech 2008)

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What's the Missing Piece?

The current allergy assessment process is useful and robust for novel protein allergy assessments

- ***However, We are still missing a true… Model***

- New methods are encouraged if they:
  - Are scientifically justified
  - Lend value to the allergy safety assessment process
- In vitro cell assays, animal models, and proteomics are evolving techniques that require validation in allergy assessments.

Areas of Advancement: Allergy Assessment Methods

How can we measure allergy potential of novel proteins and how can we measure and better characterize known allergens?

- *Bioinformatics*: *to address novel protein similarity to known allergens*
- Serum screening, when appropriate; *measure cross-reactivity*
- Animal Models; *measure novel proteins or known allergens*
- Proteomics; *characterize and measure concentrations of known allergens*
- *New approaches to determining epitopes*: *in-depth characterization of known allergens with potential application to novel proteins*
- *Cell-based in vitro assays*; *assess known allergens - develop test platform for predictive allergy of novel proteins*

Questions for the Industry…

- What is the risk that transformation issues, such as “moving” a known allergen into a GMO crop, can happen and proceed past the development phase?

- What has improved in the last 15 years to reduce allergy risk with GM food crops?

  ???? What does the allergy safety assessment process actually have for “process” and allergy science that it did not have back in 1995?

  - We have Codex Alimentarius; And a strong consensus in the Agricultural Biotech and Allergy Science communities to improve on the knowledge base of allergen characterization.
    - If we know the mechanisms of how allergens interact with the immune system we can work towards a predictive platform for novel proteins

Thank you for your attention!

Slides for Breakout Discussion

Bioinformatics - Alignment Scoring. Communicating the interpretation of the Results

>>gi|444 – QUERY SEQ - (160 aa) initn: 44 init1: 44 opt: 116 Z-score: 155.0 bits: 35.3. **E-Score = 0.001,** Smith-Waterman score: 119; **27.95% identity** (56.989% similar) in **93 aa overlap**

My search (query) sequence – **Question: Has it significantly aligned with a known allergen?**

```
    30        40        50        60        70        80
betv1  NYPHHHQKQVQKVSLTRGMADVPEHVELSHTHVVGPSQCFSVVVQDVEAPVSTVWSILSR
                                   : :. : . :...: .... .  .
gi|444                      MGVFTYADESTSVIPPPRLFKALVLEADTLIPKI------
                                     10        20        30

            90       100       110       120       130       140
betv1  FEHPQAYKHFVKSCHVVIGDGREVGSVREVRVVSGLPAAFSLERLEIMDDDRHVISFSVV
          ::.    ::: ..: :::  ::.....    :   ..  .:.. .: :  : :.:.:
gi|444 --APQS----VKSAEIVEGDGG-VGTIKKISFGEGSHYSYVKHRIDGLDKDNFVYSYSLV
                 40        50         60        70        80

           150       160       170       180       190       200
betv1  GGDHRLMNYKSVTTVHESEEDSDGKKRTRVVESYVVDVPAGNDKEETCSFADTIVRCNLQ
        ::
gi|444 EGDALSDKVEKISYEIKLVASADGGSIIKSTSNYHTTGDVEIKEEDVKAGKEKATGLFKL
        90       100       110       120       130       140
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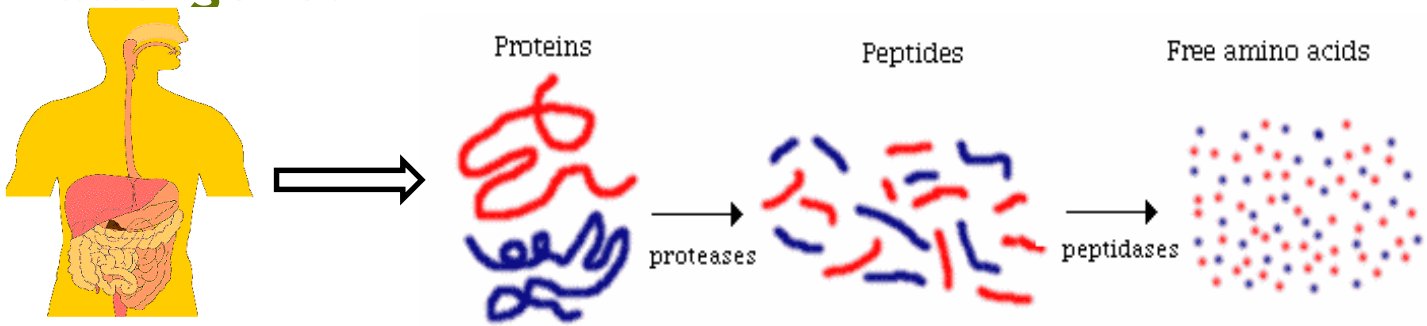
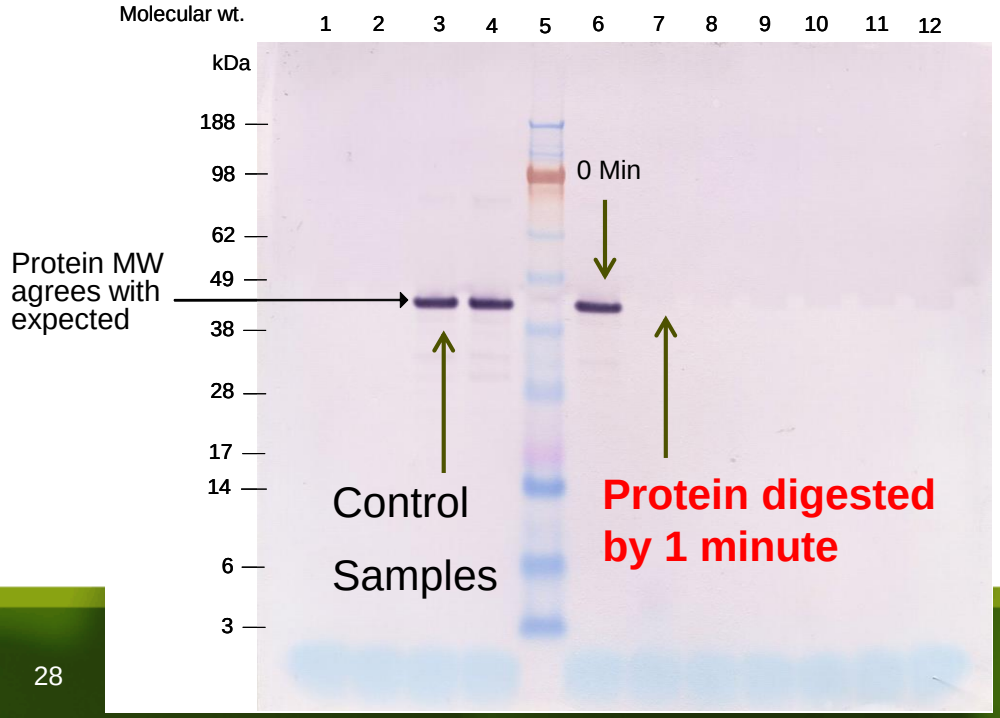
Pepsin Digestibility - Does the protein behave like known allergens?



Some known allergens are stable to pepsin digestion

In vitro digestibility study

Is the protein stable in ***simulated gastric fluid*** (SGF) or ***simulated intestinal fluid*** (SIF)?



Quickly digested proteins indicate low likelihood of gastric exposure to immune system

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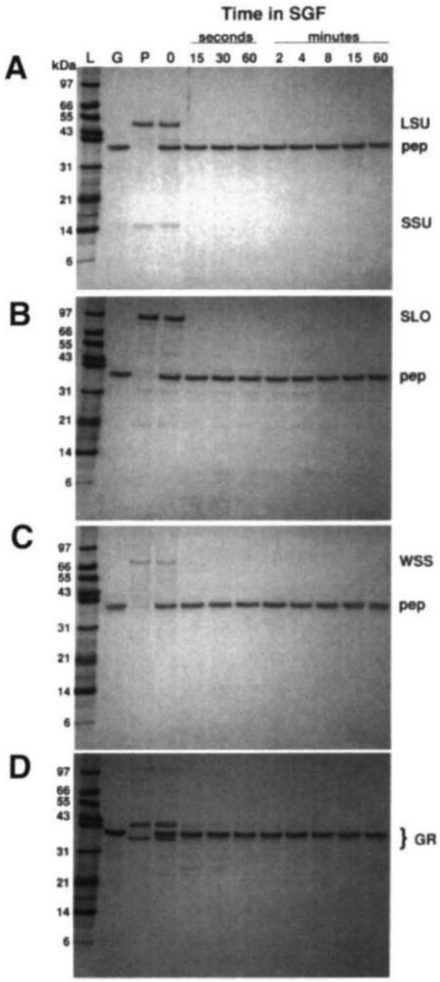
Comparators for SGF Breakout Discussion

Expectation for Novel GM Crop Proteins is ~ 2 – 5 min

Astwood, Leach and Fuchs, 1996

Table 1. Summary of allergen and protein stability in SGF.

Protein	Stability (min) Whole Protein	Fragments
Egg allergens		
Ovalbumin	60	—
Phosvitin	60	—
Ovomucoid	8	—
Conalbumin	0	15
Milk allergens		
ß-lactoglobulin	60	—
Casein	2	15
BSA	0.5	15
Soybean allergens		
ß-conglycinin (ß-subunit)	60	—
SKTI	60	—
Soy lectin	15	—
ß-conglycinin (α-subunit)	2	60
Gly m 1	0.5	8
Mustard allergens		
Sin a 1	60	—
Bra j IE	60	—
Peanut allergens		
Ara h2	60	—
Peanut lectin	8	—
Common plant proteins		
Glycolate reductase (spinach leaf)	0.25 (15 sec)	—
Rubisco LSU (spinach leaf)	0 (<15 sec)	—
Rubisco SSU (spinach leaf)	0 (<15 sec)	—
Lipoxygenase (soybean seed)	0 (<15 sec)	—
PEP carboxylase (corn kernel)	0 (<15 sec)	—
Sucrose synthetase (wheat kernel)	0 (<15 sec)	—
ß-amylase (barley kernel)	0 (<15 sec)	—
Acid phospatase (potato tuber)	0 (<15 sec)	—
Phosphofructokinase (potato tuber)	0 (<15 sec)	—



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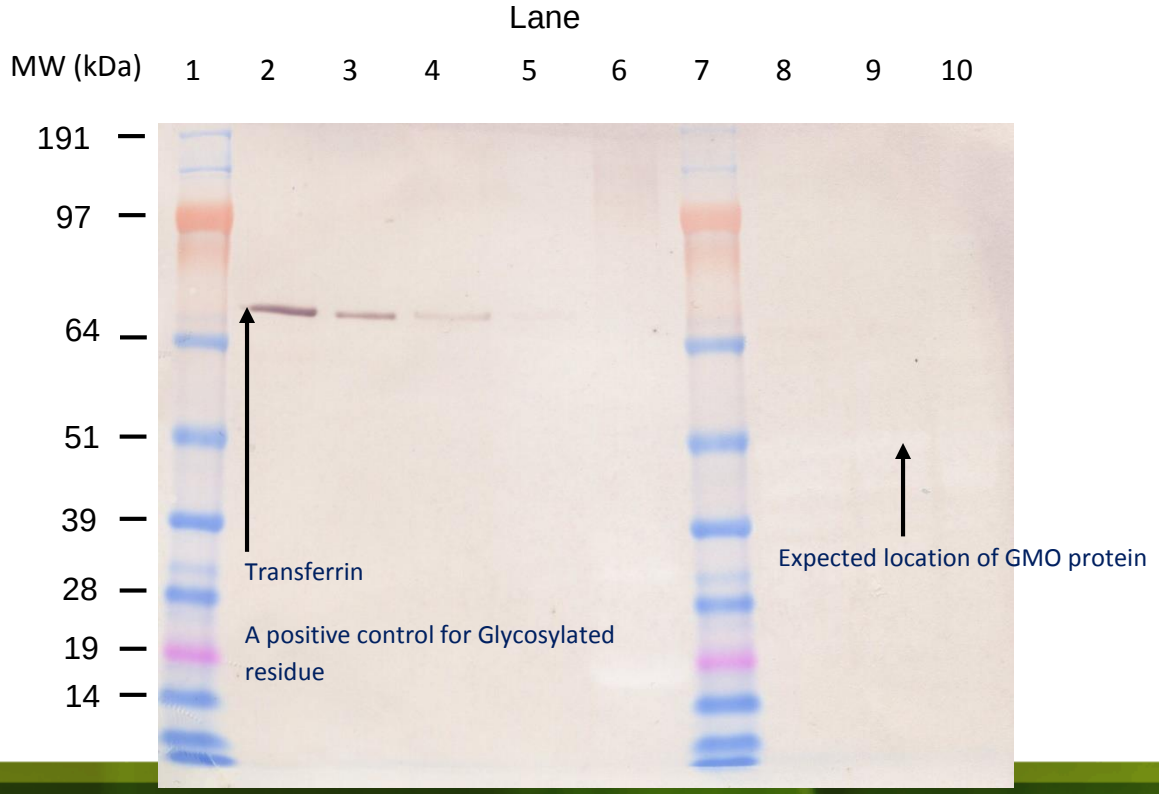
Glycosylation – Is the plant-produced GMO protein modified?

SDS-PAGE is used to separate proteins by molecular weight and reagents specific to glycosylated residues are used for detection.

Plant-expressed GMO proteins are expected to not have post-translational changes.



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