Protein safety: Allergenicity

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Assessment of allergenicity potential of GM crop proteins

Concerns →

-Transfer of a major allergen/cross reactive protein into a food crop
-Transfer a pepsin-stable, abundant protein,
-Increase endogenous allergens

Codex strategy-weight of evidence →

Source of gene
Sequence homology to known allergens
Specific Serum IgE binding
Stability to pepsin in SGF in vitro (& heat stability)
Abundance in food

Existing knowledge →

Food allergy-IgE mediated most important
Eight foods account for ~ 90% of food allergies & require labels: Peanuts, eggs, milk, fish, crustacea, tree nuts, wheat, soybeans. EU: celery (root); mustard & sesame seeds

Occurrence of food allergy in the US and Europe: 2-4% of adults ,4-8% of young children

No single test is predictive of allergy

Allergenicity assessment of GE plants/foods for premarket approval in India: RCGM, DBT, ICMR, GOI

Protocols for Food and Feed Safety Assessment of GE crops	Guidelines for the Safety Assessment of Foods Derived from Genetically Engineered Plants
I. Acute Oral Safety Limit Study In Rats and Mice	7.2 Assessment of Possible Allergenicity
II. Sub-chronic Feeding Study In Rodents	7.2.1 Assessment Strategy
III. Protein Thermal Stability	7.2.2 Source of the Protein
IV. Pepsin Digestibility Assay	7.2.3 Amino Acid Sequence Homology
V. Livestock Feeding Study	7.2.4 Pepsin Resistance
	7.2.5 Specific Serum Screening
	7.2.6 Other Considerations

Protein Allergenicity concerns with GM crops

- Potential to elicit allergic reaction in individuals sensitive to introduced proteins.
  - Transfer an existing allergen into another crop
  - Transfer a highly identical, potentially cross-reactive protein
- Potential to sensitize susceptible individuals.
  - Transfer a pepsin-stable, abundant protein
- Potential to increase endogenous allergens due to insert

Critical risks of food allergy

- Incidence of food allergy in India: uncertain
- EU and US: 6-8% in children, 2-4% in adults.
- Predominant foods: The Big Eight proteins (US), or 14 (EU)
- Important food allergens: tropomyosin (crustaceans and mollusks), parvalbumin (fish), 2S albumins, 7S vicillins and 11S legumins (legumes, tree nuts and other seeds).
- Primary risks for allergic subjects: Accidental ingestion of primary allergens or nearly identical proteins

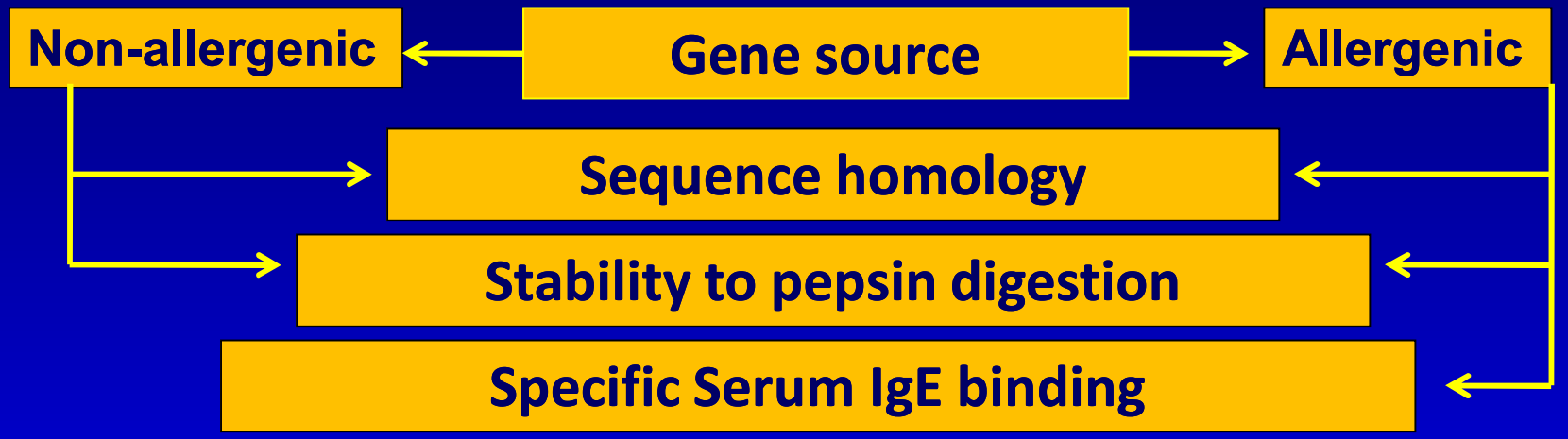
Assessment of allergenicity potential of GM crops- the rationale

Primary focus: ***To prevent avoidable increase in risk of allergy while transferring novel gene/proteins into food crops.***

- **Ensure** ⟶ **Risks not > than risks from non-transgenic varieties.**
- **Evaluate** ⟶ **Possibility - introduced protein is an allergen (source and sequence)**
- **Evaluate** ⟶ **Possibility of cross reactivity (source and sequence)**
- **Evaluate** ⟶ **Characteristics of protein compared to known food allergens (stability and abundance)**

No single test is predictive of food allergy for humans

Codex Weight of evidence approach



Other considerations-
(Gaps in knowledge/Future R&D)

- Abundance and Effect of heat processing
- Targeted serum screen
- Animal testing/ model
- Assess T-cell epitopes, structural motifs

Role of bioinformatics in allergenicity assessment

Purpose and application

- To identify proteins known to be allergens or similar to allergens that could induce allergic cross reactions. Not a stand alone test.
  - ↓
  - Helps by identify transgenic proteins requiring specific serum IgE testing.
    - ↓
    - Helps by identify specific allergic populations likely at risk and who could be serum donors.

- Need simple, straightforward protocol – UNDER REVIEW

Bioinformatics protocol requires:

- Selection of allergen specific database
- Selection of search strategy and criteria for cross reactivity
- GM protein sequence
- Positive control sequence

Criteria for cross reactivity Based on Historical Data from a Variety of Sources

- Proteins sharing > 70% identity over their lengths are highly likely to be cross reactive
- Proteins sharing < 50% identity over their lengths are unlikely to share cross-reactivity

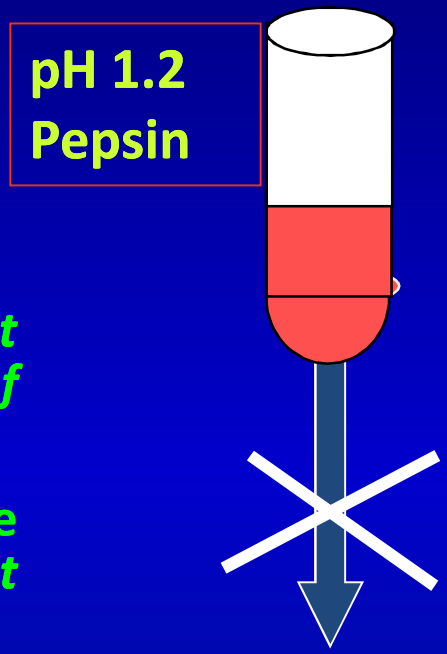
(Rob Aalberse, 2000, J Allergy Clin Immunol 106:228)

FAO/WHO 2001 and subsequently CODEX (2003) chose a criteria of >35% identity over any alignment of 80 OR MORE amino acids as a very conservative mark of potential significance

Amino Acid Sequence Comparison Strategy:

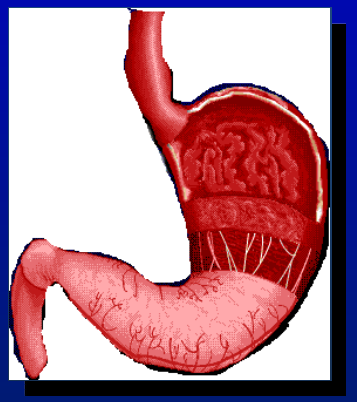
1. **Overall FASTA** vs. AllergenOnline (>50% identity or E score < 1 e -7 = structural similarity and modest to significant chance of cross reactivity
2. **FASTA scanning 80 aa window** (79 aa overlap), (>35% identity = some possibility of cross-reactivity
3. **Scanning 6 or 8 aa identity** NO PROVEN VALIDITY, unlikely to indicate cross-reactivity – no point in doing
4. **If matches in steps 1 or 2:** Do serum IgE tests if possible (Evaluate the evidence of allergenicity for the matched "allergen" first.)

Protein Stability to Pepsin Digestion & allergenicity potential

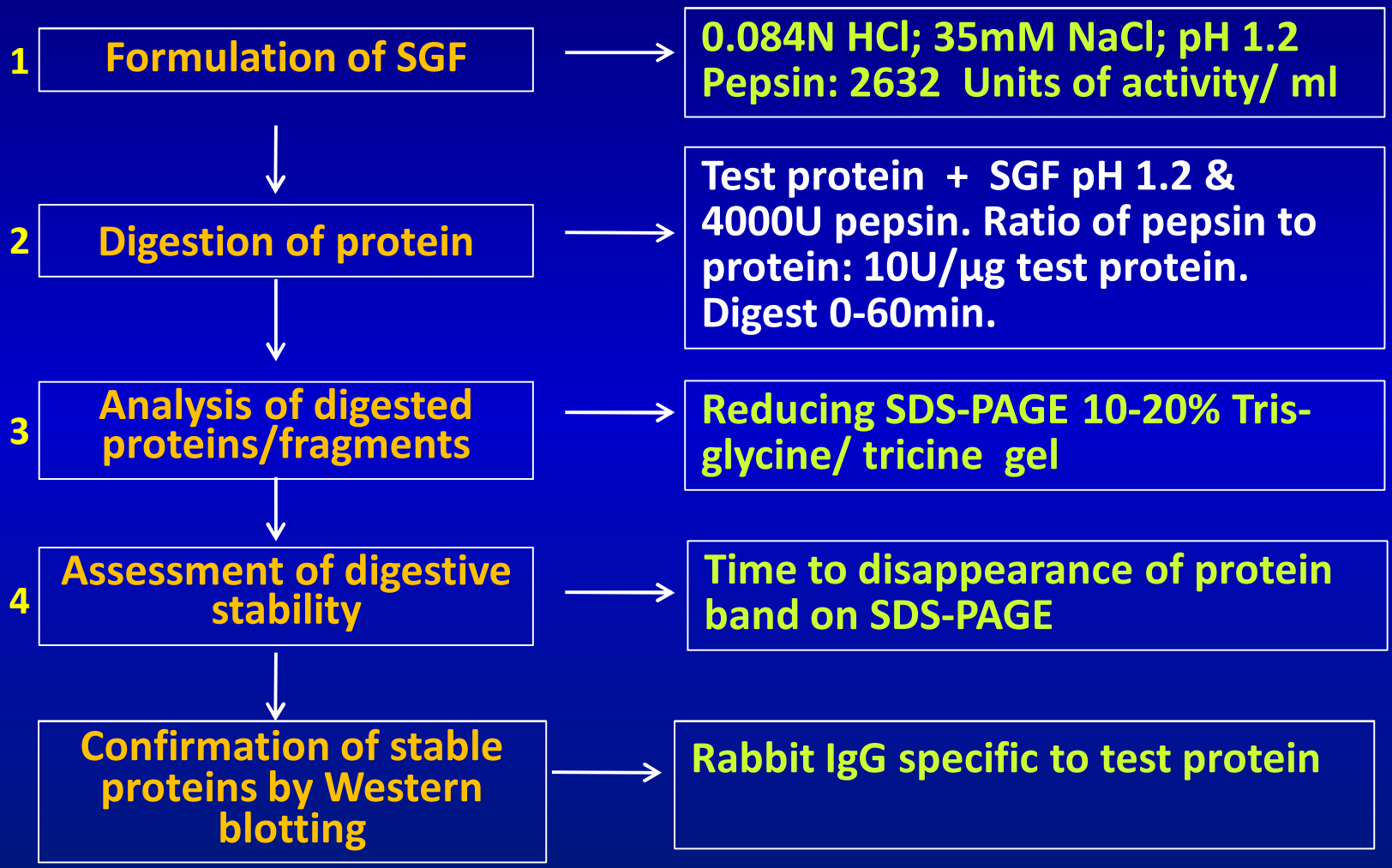


Rationale:

- Stability relative to known major food allergens
- Resistance of a protein to digestion- *retain sufficient structural integrity and increased probability of stimulating allergic response.*
- Provides a simple in vitro correlative assay to evaluate protein digestibility. *(assay not meant to predict digestibility of a given protein).*
- Used in conjunction with other evidence (Codex 2003 weight of evidence) to help predict whether a dietary protein may become a food allergen.



Pepsin digestibility assay- The Basic protocol



Improving sensitivity and reproducibility of the assay- Determining the limit of detection

Ofori-Anti, et al proposed objective detection limits for the pepsin digestion assay.
Regulatory Toxicology and Pharmacology 52 (2008) 94–103

Establishing objective detection limits for the pepsin digestion assay used in the assessment of genetically modified foods

A.O. Ofori-Anti, H. Ariyarathna, L. Chen [1], H.L. Lee [2], S.N. Pramod, R.E. Goodman *

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- ***Test capability of detection of SDS-PAGE and Imaging system for test protein over an experimental range (10-100%) of undigested protein mass in SGF***
- ***Detection of 10% undigested protein mass***
- ***Generate Standard curve using conc. and pixel densitometric values.***
- ***Determine Coefficient correlation and least conc. of protein that can be detected on the gel determined.***

Criteria for evaluating digestibility

Shortest time-digested sample with a band intensity equal to, or less than the 10% undigested test protein in the well.

- **Stable: Proteins with >10% stainable full-length protein band remaining at 60 minutes.**
- **Intermediate stability: Proteins reduced to < 10% stainable band at 5-30 minutes.**
- **Rapidly digested/labile: Proteins reduced to < 10% stainable band by 2 minutes.**
- ***Analyse fragments above3,000 da generated as intermediate products of digestion would be noted as stable (or partially stable) intermediate proteolytic fragments in addition to the test protein.***

Astwood et al, 1996 Nature Biotechnology 14:1269-1273
Goodman and Hefle 2005 Expert Rev. Clin. Immunol.1(4):561-578
DBT, GOI 2008 Protocols for food &feed safety assessment of GE crops

Thermal Stability protocols in India (DBT/ICMR 2008)

Protocol suggests looking at effects of heating on:

- Enzymatic activity (e.g. if the GM protein is an enzyme like CP4 EPSPS, PAT)
- Insecticidal properties (e.g. if the GM protein is a Cry protein)