ELSEVIER

PII: S0308-8146(97)00159-3

Food Chemistry, Vol. 63, No. 2, pp. 265–274, 1998

0308-8146/98 $19.00+0.00

Salt-soluble seed globulins of dicotyledonous and monocotyledonous plants II. Structural characterization

Massimo F. Marcone,* Yukio Kakuda & Rickey Y. Yada

Department of Food Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

(Received 20 February 1997; accepted 28 June 1997)

Structural characterization of 21 seed globulins from monocotyledonous and dicotyledonous plants revealed that they generally possessed low levels of α-helix and high levels of β-sheet secondary structure fractions. This finding suggested that the interior conformation of these globulins was very similar. In contrast to internal conformations, tertiary conformations indicated that very distinct surface properties existed between these two globulin classes. It now appears that surface properties are the most variable physico-chemical properties measured between globulins. Calorimetric analysis revealed that both classes of globulins possessed temperatures of denaturation (Td) which were in the temperature range of 83.8 to 107.8°C. Although dicotyledonous seed globulins had more pronounced thermal transitions than their monocotyledonous counterparts, most endothermic transitions occurred as co-operative events indicating that the various domains present in these globulins were held together by interdependent structural domains. These interdependent domains rendered the globulins stable to high temperatures and in connection with previous data (Marcone and Yada, 1998), it was believed that both monocotyledonous and dicotyledonous seed globulins share similar structural domains.

INTRODUCTION

Information about the secondary and tertiary conformational structures of seed globulin storage proteins is still limited (Pernollet and Mossé, 1983). It would be understandable, that if storage proteins were only copolymerized amino acids devoted strictly to the nutrition of young emerging seedlings, a very high degree of mutability in primary structure (amino acid sequence) would be expected resulting in a lack of conservation in secondary and tertiary structure. Although a lack of conservation of secondary and tertiary structure would be expected, previously observed N-terminal sequences and predicted secondary structure homologies strongly suggest some conservation of polypeptide chains, in spite of their general micro-heterogeniety (Pernollet and Mossé, 1983). One reason suggested for the above conservation is that storage proteins must maintain their folding patterns during evolution in order to fit the particular endoproteases which hydrolyse seed proteins in the course of germination (Fukushima, 1968; Pernollet and Mossé, 1983). Another reason suggested is that storage protein structure is mainly adapted for the efficient and maximal packing of storage proteins within protein bodies (Pernollet and Mossé, 1983). Maximal packing of storage proteins within protein bodies can be achieved in two levels, i.e. compacting of proteins is increased by the specific folding of polypeptide chain in favour of a maximal packing of amino acids within the protein molecule, and secondly, in the association of these polypeptide chains in order to form quaternary structures (Pernollet and Mossé, 1983).

Fukushima (1968) found that the major internal structures of both 7S and 11S seed globulins of soybeans possessed little α-helical but high β-sheet secondary structures. Using various alcoholic denaturants it was demonstrated that the internal structures of soybean 11S and 7S globulins were stabilized by hydrophobic and hydrogen bonds and were fairly compact. These results were later confirmed by Koshiyama (1971).

*To whom correspondence should be addressed.

Jacks *et al.* (1973) showed that four seed globulins from curcurbit, hemp, peanut and soybean, which comprise the principal storage proteins of dicotyledonous plants, had similar physico-chemical characteristics. Circular dichroic analysis indicated that storage proteins were consistently low in α-helical and high in β-sheet secondary structures. In a later study, Prakash and co-workers (1980) showed that the α-globulin of *Sesamum indicum* contained relatively low levels of α-helix and concluded that all seed storage protein, in general, contained low α-helix.

No attempts have yet been made to determine the relationship between the secondary and tertiary structures of globulins from a large set of genetically diverse dicotyledonous and monocotyledonous plants. The present study was conducted in order to determine the commonalities among these proteins.

MATERIALS AND METHODS

Materials

Non-heat treated milled seeds of *Amaranthus hypochondriacus* K343 marketed under the trade name Ambake Lot No. 1010 were purchased from American Amaranth Inc. (Bricelyn, MI.). Pea (*Pisum sativum* L.), peanut (*Arachis hypogaea* L.), wheat (*Triticum aestivum*), buckwheat (*Fagopyrum esculentum*), barley (*Hordeum vulgare*), corn (*Zea mays*), rye (*Secale cereale*), oat (*Avena sativa*), alfalfa (*Medicago sativa*), caraway (*Carum carvi*), celery (*Apium graveolens*), cumin(*Cuminum cyminum*), dill (*Anethum graveolens*), fennel (*Foeniculum vulgare*), flax (*Linum usitatissimum*), mustard (*Brassica alba*) and pine nut (*Pinus edulis*) were obtained from the 1993 or 1994 growing season (from the University of Guelph, Department of Crop Science). The seeds were ground with a mechanical grinder and material passing through a 60 mesh screen was used for further study. The pumpkin globulin (*Cucurbita pepo*) and the hemp globulin (*Cannabis sativa*) were obtained twice crystallized from Sigma Chemical Co. (St. Louis, MO).

All seed meal materials were mixed and quartered in order to ensure representative composite samples. The samples were stored in sealed polycarbonate containers at room temperature throughout the study. Chromotagraphic materials, i.e. a Separon Hema-Bio 1000 DEAE anion exchange column and Concanavalin A-Sepharose 4B affinity chromatography media were both obtained from Fisher Scientific (Toronto, ON), while Sephacryl S-300 Superfine gel permeation media Mono-Q 5/5 anion exchange column and high molecular weight calibration proteins were products of Pharmacia LKB (Montreal, PQ).

All other reagents and chemicals were of AR or HPLC grade from Sigma Chemical Co. (St. Louis, MO), Aldrich (Milwaukee, WI) or Fisher Scientific (Toronto, ON).

Determination of protein concentration

Protein was measured using the Bio-Rad protein dye-binding assay (modified Bradford) with bovine serum albumin(BSA) as the standard (Bio-Rad Laboratories, 1989). Analyses were performed in triplicate. In addition, a spectrophotometric method was used based on the difference in absorbance of proteins at 235 and 280 nm (Whitaker and Granum, 1980).

Isolation and purification of a variety of salt-soluble seed storage globulins from dicotyledonous and monocotyledonous plants

(i) Sample preparation

Seed meals were prepared as described in the previous paper by Marcone and Yada (1998).

(ii) Protein extraction

The salt-soluble protein from each grain was extracted from the defatted meal as described in the previous paper by Marcone and Yada (1998).

(iii) Gel filtration and anion exchange chromatographies

Gel filtration and anion-exchange chromatographies were performed as described in the previous paper by Marcone and Yada (1998).

Spectroscopic cryoaggregation profiles

Protein solutions of 0.375 mg ml^{-1} in the buffer described above were equilibrated at 23, 4, 2°C for 15 min and their turbidities measured at 600 nm. A Shimadzu UV-260 dual-beam recording spectrophotometer (Tekscience, Mississauga, ON) equipped with variable temperature control was used for the measurements. Analyses were performed in duplicate.

Circular dichroism spectroscopy (near-UV) (CD)

Circular dichroism measurements were carried out in the near-UV (240–320 nm) at 20°C under constant nitrogen purge using a Jasco J-600 spectropolarimeter (Japan Spectroscopic Co. Ltd., Tokyo) with cell path-length of 1.0 cm. Globulin concentrations of approximately 1.0 mg ml^{-1} in a 32.5/2.6 mM K_2HPO_4/KH_2PO_4, buffer pH 7.5, with NaCl added to a final ionic strength of 0.5 were used in the determinations. Analyses were performed in triplicate.

Circular dichroism spectroscopy (far-UV) (CD)

Circular dichroism measurement were performed in the far-UV (190–250 nm) on 0.1 mg ml^{-1} globulin/buffer solutions (32.5/2.6 mM K_2HPO_4/KH_2PO_4), pH 7.5, using a Jasco J-600 Spectropolarimeter (Japan Spectroscopic Co. Ltd., Tokyo Japan) with a cell path-length of 0.1 cm. Secondary structure was determined using the

Jasco Protein Secondary Structure Estimation Program (Japan Spectroscopic Co). Secondary structure fractions were determined using the Jasco SSE program, which is based on the algorithm of Chang *et al.* (1978) and the data base of Hennessey and Johnson (1981). Analyses were performed in triplicate.

Micro differential scanning calorimetry (DSC)

DSC measurements were performed on a MC-2D Instrument (Upscan Ultrasensitive Differential Scanning Calorimeter) with twin 1.2 ml total-fill tantalum cells. Data were collected using the Origin DSCITC Data Collection Software V.1.1. and analyzed using the Origin Version 2.9 DSC Program (Micro Cal, Inc., Northampton, MA, USA) at a heating rate of 1.37°C min^{-1} from 30°C to 118°C (under 20 psi pressure). Globulins were diluted and dialyzed against 32.5/2.6 mM K_2HPO_4/KH_2PO_4 buffer, pH 7.5, containing 0.4 M NaCl to a concentration of 0.05%. The identical buffer served as the reference. Analyses were performed in duplicate.

Modulated differential scanning calorimetry (DSC)

Modulated DSC® measurements were performed on a DuPont Differential Calorimeter Model 2910 (DuPont de Menours and Co., Wilmington, Delaware) equipped with a TA Instruments DSC Cell (New Castle, Delaware, USA) and a Liquid Nitrogen Cooling Accessory (TA Instruments).

Thermograms were obtained employing a heating rate of 5°C per min (on 10% protein solution in the above stated buffer) from 30 and 130°C and performed in duplicate. High purity indium and gallium were used to calibrate the system and data evaluated using General V4 IC DuPont 2000 analysis software.

RESULTS AND DISCUSSION

Structural characterization

Secondary structural conformations of globulins

Quantitative estimation of the relative amounts of α-helical, β-sheet, β-turn and random coil secondary structure fractions for the purified dicotyledonous and monocotyledonous 11S seed globulins examined in this study are tabulated in Table 1. Examination of the relative quantities of each of these secondary structure fractions revealed that all the globulins typically possessed low levels of α-helical and very high β-sheet secondary structure fractions with the exception of the mustard globulin. In addition to these general observations, striking similarities were also noted in the amounts of β-turn fraction found in each of the studied globulins. The amounts of random coil in each globulin were also found to be in the range of 23.7–32.0% with the exception of soybean globulin (7.1%). The present findings support those by Jacks *et al.* (1973) where it was reported that 4 oil seed globulins (curbit, hemp,

Table 1. Circular dichroic secondary structure from various purified seed storage globulins

Globulin Source	Fraction[a]			
	α-helix	β-sheet	β-turn	Random
Pea *(Pisum sativum L.)*	8.9 (±0.4)	49.6 (±1.1)	17.6(±0.8)	24.0 (±0.9)
Peanut *(Arachis hypogaea L.)*	8.1 (±0.3)	50.8 (±0.3)	16.6 (±0.4)	24.5 (±0.5)
Soybean[b] *(Glycine max. Merr.)*	15.8 (±0.4)	56.0 (±2.0)	21.2 (±0.3)	7.1 (±1.1)
Corn *(Zea mays)*	10.3 (±0.4)	44.9 (±1.6)	17.1 (±0.6)	27.6 (±1.9)
Barley *(Hordeum vulgare)*	17.4 (±0.4)	34.7 (±1.1)	15.7 (±0.5)	32.0 (±1.0)
Rye *(Secale cereale)*	8.2 (±0.3)	49.7 (±0.8)	14.3 (±0.6)	27.7 (±0.7)
Oat *(Avena sativa)*	12.7 (±0.3)	45.0 (±1.2)	12.7 (±0.6)	29.6 (±0.4)
Wheat[c] *(Triticum aestivum)*	8.0 (±0.0)	53.4 (±0.4)	13.8 (±0.2)	24.8 (±0.2)
Amaranth[d] *(Amaranthus hypochondriacus)*	7.8 (±1.3)	57.6 (±1.1)	17.6 (±0.5)	16.9 (±0.3)
Buckwheat *(Fagopyrum esculentum)*	16.8 (±0.6)	32.0 (±1.8)	22.0 (±2.3)	29.3 (±1.2)
Hemp *(Cannabis sativa)*	11.4 (±0.4)	45.7 (±1.5)	16.4 (±0.1)	26.4 (±1.0)
Pumpkin *(Cucurbita pepo)*	6.0 (±0.4)	53.7 (±0.9)	16.6 (±1.1)	23.7 (±0.6)
Alfalfa *(Medicago sativa)*	5.1 (±0.5)	55.2 (±1.0)	14.2 (±0.9)	25.5 (±0.4)
Caraway *(Carum carvi)*	0.5 (±0.1)	82.7 (±0.1)	10.5 (±0.5)	6.3 (±0.5)
Celery *(Apium graveolens)*	4.6 (±0.3)	68.0 (±2.0)	12.1 (±1.3)	15.3 (±0.5)
Cumin *(Cuminum cyminum)*	7.2 (±0.1)	58.2 (±0.4)	18.6 (±1.6)	16.0 (±1.8)
Dill *(Anethum graveolens)*	1.0 (±0.6)	68.4 (±2.3)	12.7 (±1.6)	18.0 (±0.9)
Fennel *(Foeniculum vulgare)*	3.9 (±0.6)	73.5 (±1.5)	10.8 (±2.0)	11.8 (±1.1)
Flax *(Linum usitatissimum)*	4.0 (±0.2)	62.8 (±0.9)	16.2 (±0.2)	17.0 (±0.9)
Mustard[e] *(Brassica alba)*	35.6 (±0.7)	11.7 (±1.7)	24.0 (±3.1)	28.7 (±2.1)
Pine nut *(Pinus edulis)*	0.3 (±0.2)	68.1 (±1.3)	9.9 (±1.7)	21.6 (±0.4)
Mean values	9.2	53.4	15.7	21.6

[a]Results are the mean values (± standard deviation) of three replications.
[b]Data from Marcone *et al.*, 1994a.
[c]Data from Marcone and Yada, 1995a,b.
[d]Data from Marcone and Yada, 1991.
[e]Data from Marcone *et al.*, 1997b.

peanut and soybean) had low levels of α-helix, and high β-sheet secondary structure fractions. Unfortunately, in their study they did not differentiate between β-turn and random coil.

α-Helical and β-sheet secondary structure fractions have a tendency of being deeply buried within the polypeptide chain (Hopp and Woods, 1982, 1986). The observed similarity in the amounts of α-helix and β-sheet secondary structures shared by all dicotyledonous as well as monocotyledonous seed globulins may, therefore, indicate that the interior conformation are very similar or highly conserved. Interestingly, the molten globule theory (already found to exist in globulins) (Marcone *et al.*, 1997*a*) suggests a model for protein folding in which α-helices at least, are formed early in folding and are retained throughout the folding process (Baldwin, 1991). This view was supported by Radford *et al.* (1984) where it was demonstrated that α-helical domains in protein molecules become substantially folded before the formation of stable β-sheet domains. In this present study, the possibility, therefore, exists that these conserved α-helical structures may also play an important role in the folding of oligomeric seed protein globulins.

Tertiary structural conformation of seed globulins

It is well recognized that near-UV CD spectra reflects the tertiary structure of a protein (Strickland, 1974) brought about by the interaction and orientation of the aromatic rings of tyrosine, tryptophan, and phenylalanine with other amino acid moieties in the protein. In the present study, identification of the fine structure of the individual aromatic amino acid groups for all the tested dicotyledonous and monocotyledonous seed globulins was somewhat difficult due to the low intensities of the CD spectral scans (Figs 1–3). Tentative identification of the fine structure of phenylalanine was made at 258.0 and 264.0 nm, while the 0–0 cm^{-1} ^1L_b of tryptophan was assigned to 290.2 nm and its 0 + 850 cm^{-1} ^1L_b band at 286.4 nm for these globulins. Tyrosine showed its characteristic 0 + 800 cm^{-1} band around 275.0 nm.

Each globulin was shown to possess its own characteristic near-UV CD fingerprint with the amplitude or intensity of the CD signal being the major distinguishing feature. This observation indicated that substantial differences existed in the arrangement/proximity and exposure of aromatic amino acids on the globulin surfaces. All globulins except those isolated from rye and barley showed a substantial tryptophan fine structure pattern, whereas the tyrosine fine structure was the least visible in all globulins with the exception of barley, hemp and pumpkin. Unlike tyrosine, phenylalanine fine structure pattern was highly visible in all studied globulins except for buckwheat. The pea globulin showed the highest phenylalanine aromatic fine structure of all the globulins. It is interesting to note that the nature of tertiary structure/conformation of globulins has been previously shown to be closely associated with the

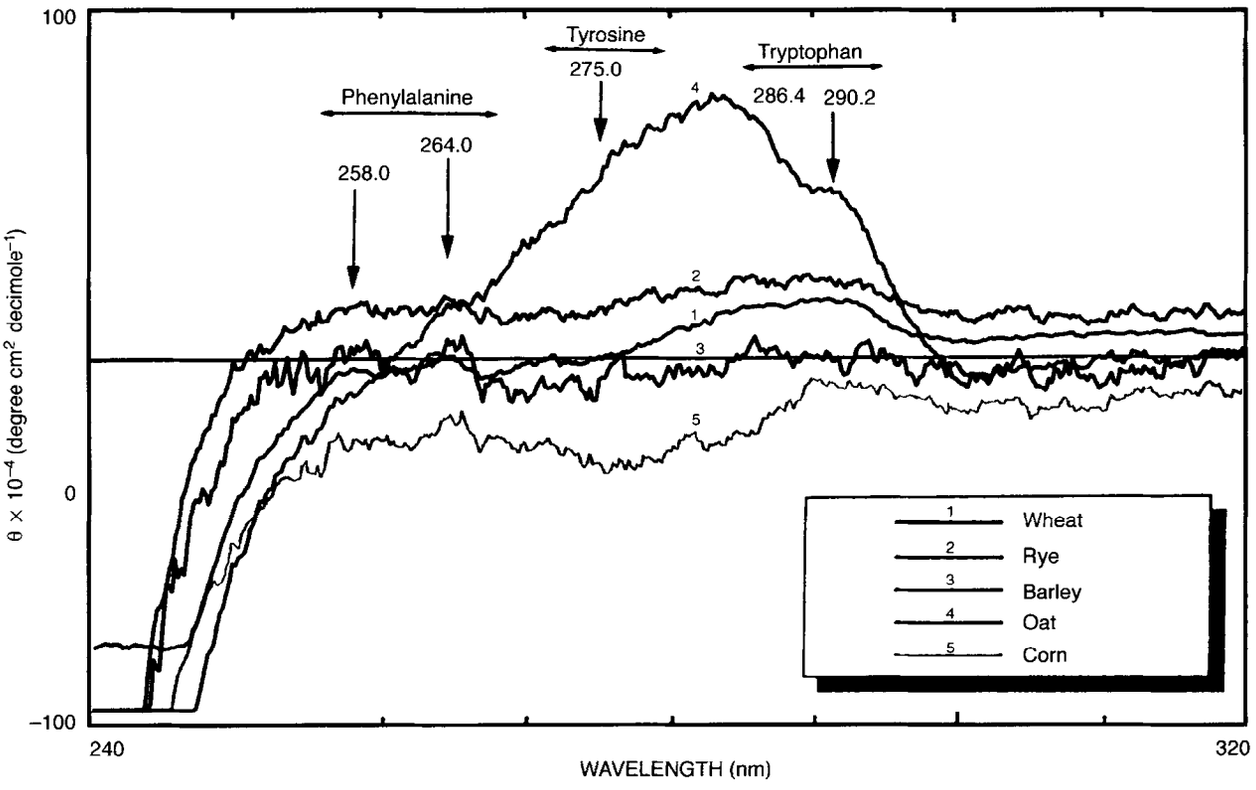

Fig. 1. Near-UV CD spectral (320–240 nm) scans of the purified cereal seed storage globulins (monocotyledonous) (1.0 mg ml^{-1}) i.e. wheat, corn, barley, rye, and oat. Average of six scans.

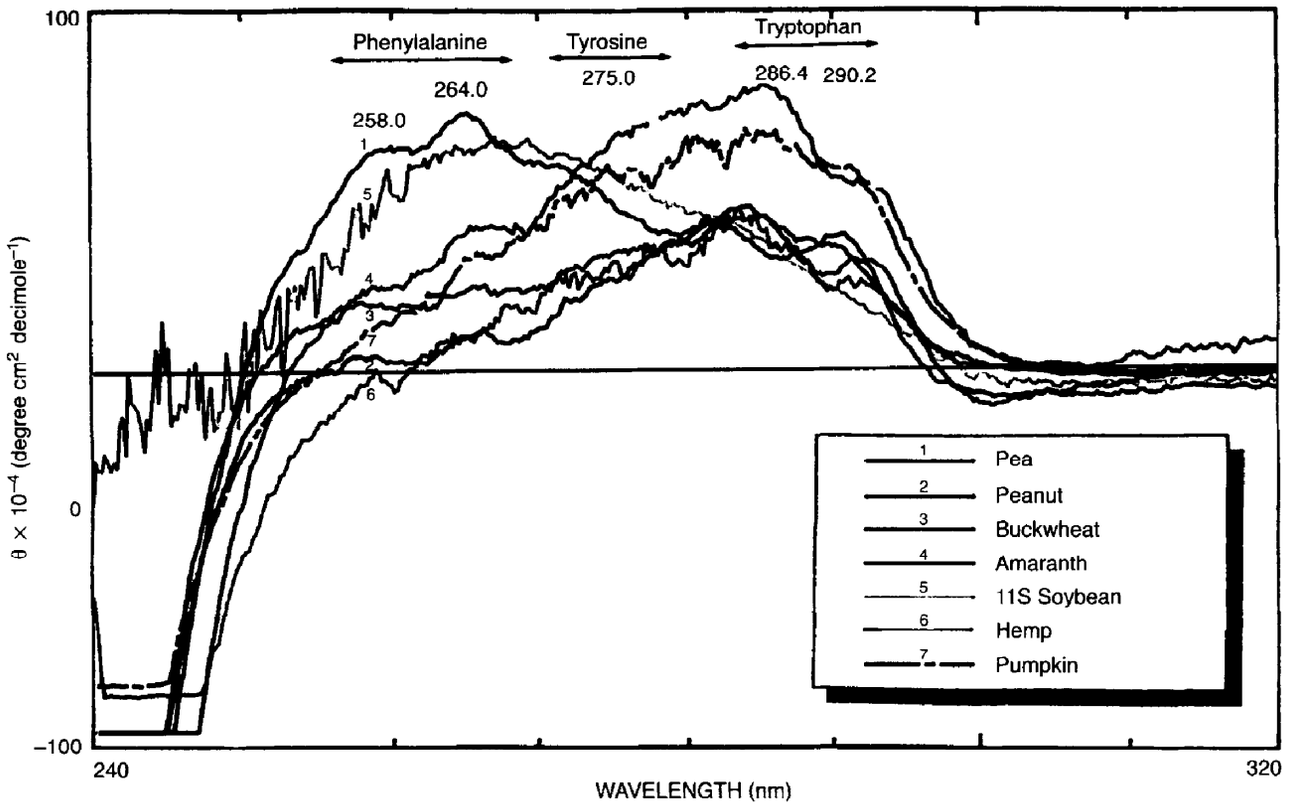

Fig. 2. Near-UV CD spectral (320–240 nm) scans of the purified non-cereal seed storage globulins (dicotyledonous) (1.0 mg ml^{-1}) i.e. pea, peanut, buckwheat, amaranth, soybean, hemp and pumpkin. Average of six scans.

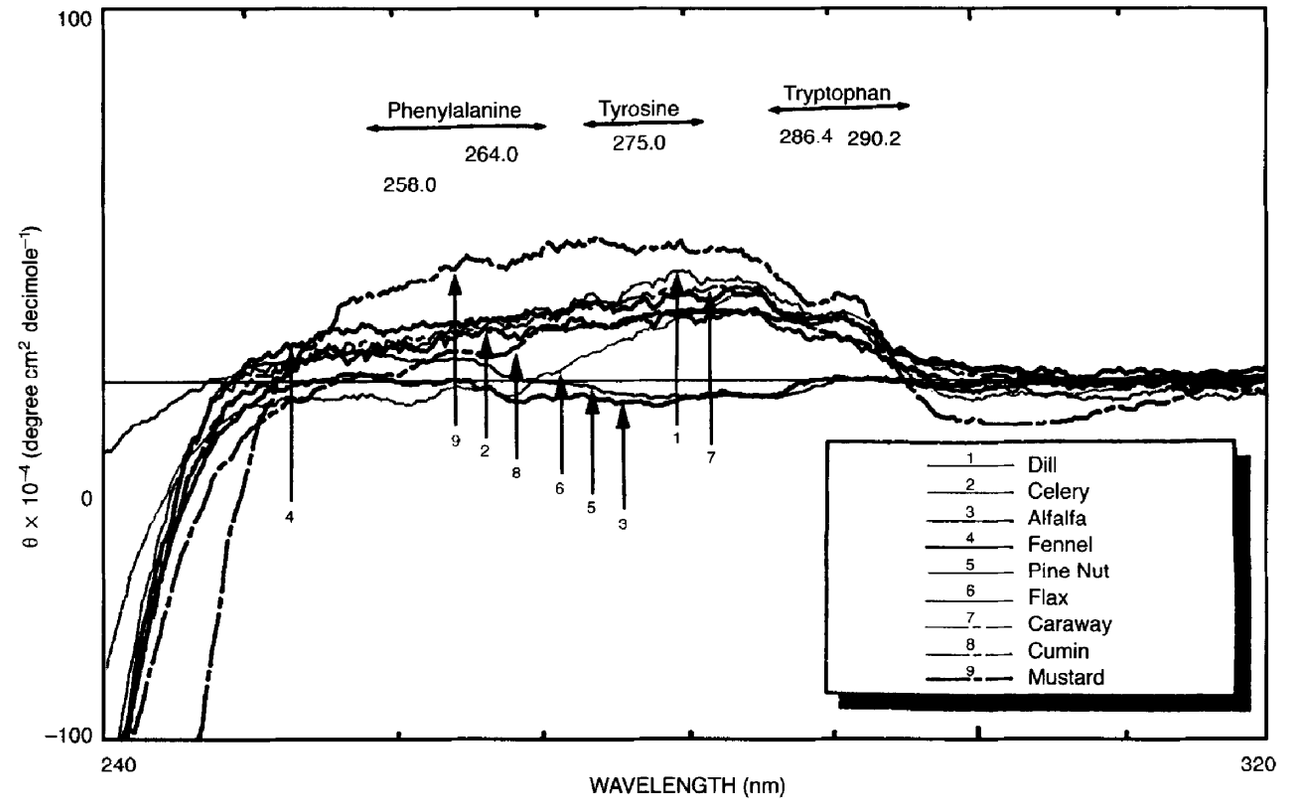

Fig. 3. Near-UV CD spectral (320–240 nm) scans of the purified seed storage globulins, (1.0 mg ml^{-1}) i.e. alfalfa, caraway, celery, cumin, dill, fennel, flax, mustard and pine nut. Average of six scans.

extent to which globulins cryoaggregate when temperatures of the solutions are reduced (Marcone and Yada, 1995). In that study, ultraviolet (UV) and circular dichroism (near and far-UV) spectral analyses revealed that the cryoprecipitable globulins were more susceptible to surface conformational changes than the more rigid globulins with decreasing temperature. It was believed that globulins that were more flexible at their surfaces would allow for subtle changes in protein conformation. This change in conformation would permit the surface aromatic groups to interact with other hydrophobic groups on neighbouring protein molecules in a co-operative manner, and therefore, initiate and enhance the aggregation process. When the various monocotyledonous and dicotyledonous seeds were evaluated, all globulins were shown to cryoprecipitate (Table 2) to varying degrees consistent with the diversity shown in their tertiary structures.

Thermal analysis

In calorimetric analyses like those performed in the present study, the overall effect of heating on globulin denaturation was to disrupt bonds (Stanley and Yada, 1994) involved in the formation and maintenance of the higher orders of protein structure, i.e. secondary, tertiary and quaternary levels of organization. The temperature and the extent of these conformational changes are determined by the thermal stability of the protein (Stanley and Yada, 1994). The thermal stability of a particular globulin results from a large number of stabilizing structural factors including amino acid composition, compact packing/protein–protein contacts, binding of metals and other prosthetic groups, as well as intramolecular interactions and linkages (Stanley and Yada, 1994). Micro-differential scanning calorimetric analysis of the dicotyledonous and monocotyledonous purified globulins revealed distinctive thermograms (Figs 4 and 5) which possessed high temperatures of denaturations T_D in the range of 83.8 to 107.8°C as compared with 7S globulins which have denaturation temperatures of approximately 67°C (German *et al.*, 1982; Babajimopoulis *et al.*, 1983; Damodaran, 1988). Figure 6 clearly illustrates that the globulins denatured in a very narrow temperature region.

Table 2. Effect of storage temperature on solubility of purified globulins as measured by turbidity

Globulin source	Turbidity[a] (A_{600})		
	23°C	4°C	2°C
Pea *(Pisum sativum L.)*	0.017	0.057	0.077
Peanut *(Arachis hypogaea L.)*	0.018	0.063	0.085
Soybean *(Glycine max. Merr.)*	0.010	0.050	0.075
Corn *(Zea mays)*	0.019	0.049	0.069
Barley *(Hordeum vulgare)*	0.017	0.054	0.080
Rye *(Secale cereale)*	0.016	0.060	0.088
Oat *(Avena sativa)*	0.015	0.061	0.081
Wheat *(Triticum aestivum)*	0.030	0.070	0.090
Amaranth *(Amaranthus hypochondriacus)*	0.025	0.068	0.092
Buckwheat *(Fagopyrum esculentum)*	0.009	0.041	0.070
Hemp *(Cannabis sativa)*	0.018	0.050	0.075
Pumpkin *(Cucurbita pepo)*	0.021	0.059	0.083
Alfalfa *(Medicago sativa)*	0.011	0.050	0.081
Caraway *(Carum carvi)*	0.023	0.064	0.096
Celery *(Apium graveolens)*	0.030	0.075	0.085
Cumin *(Cuminum cyminum)*	0.018	0.068	0.090
Dill *(Anethum graveolens)*	0.033	0.080	0.095
Fennel *(Foeniculum vulgare)*	0.025	0.070	0.090
Flax *(Linum usitatissimum)*	0.021	0.068	0.088
Pine Nut *(Pinus edulis)*	0.011	0.040	0.063
Mustard *(Brassica alba)*	0.031	0.078	0.091

[a]Analysis was performed in duplicate.

It was generally noted that all dicotyledonous seed globulin examined in this study had more pronounced thermal transitions than their monocotyledonous counterparts except wheat globulin. This might well indicate that different internal forces stabilize these two types of globulins. In addition to micro-calorimetric analysis, the proteins were analyzed using Modulated DSC® which confirmed the T_D results obtained by Micro-DSC (data not shown). The only exception was the oat globulin which revealed an additional transition at 112.4 ± 0.5°C which was not detected using Micro-DSC. The use of modulated DSC in this study facilated the examination of the thermal properties over a wider temperature range (25–>160°C) than is possible with micro-calorimetric analysis. Except for wheat, peanut, buckwheat and hemp globulins, all endothermic transitions occurred as cooperative events, i.e. thermal denaturation (lose of secondary and tertiary conformation) did not occur in a series of discrete stages but occurred as one major endothermic transition. This finding would indicate that the various structural and conformational domains present in the globulins were held together by many interdependent bond forces. For dicotyledonous seed globulins this would be understandable since the majority of these globulins were shown to have interchain disulfide linkages which link some of the constituent subunits together (Marcone and Yada, 1997).

It is currently thought that the majority of proteins are constructed in a modular fashion with structural domains acting as components (Stanley and Yada, 1994). It becomes important to consider protein domains when discussing thermal denaturation and in understanding the present endothermic results obtained for globulins, since according to the accepted domain theory, structural disruption appears to proceed in several discrete stages, each of which corresponds to an 'all-or-none' breakdown of these co-operative blocks (Stanley and Yada, 1994).

In light of the observed similarities in the globulins overall molecular weights, amino acid composition, subunit composition (i.e. acidic/basic) and the forces holding them together (Marcone and Yada, 1998), and secondary structures fractions, it is postulated that similar structural domains exist resulting in observed

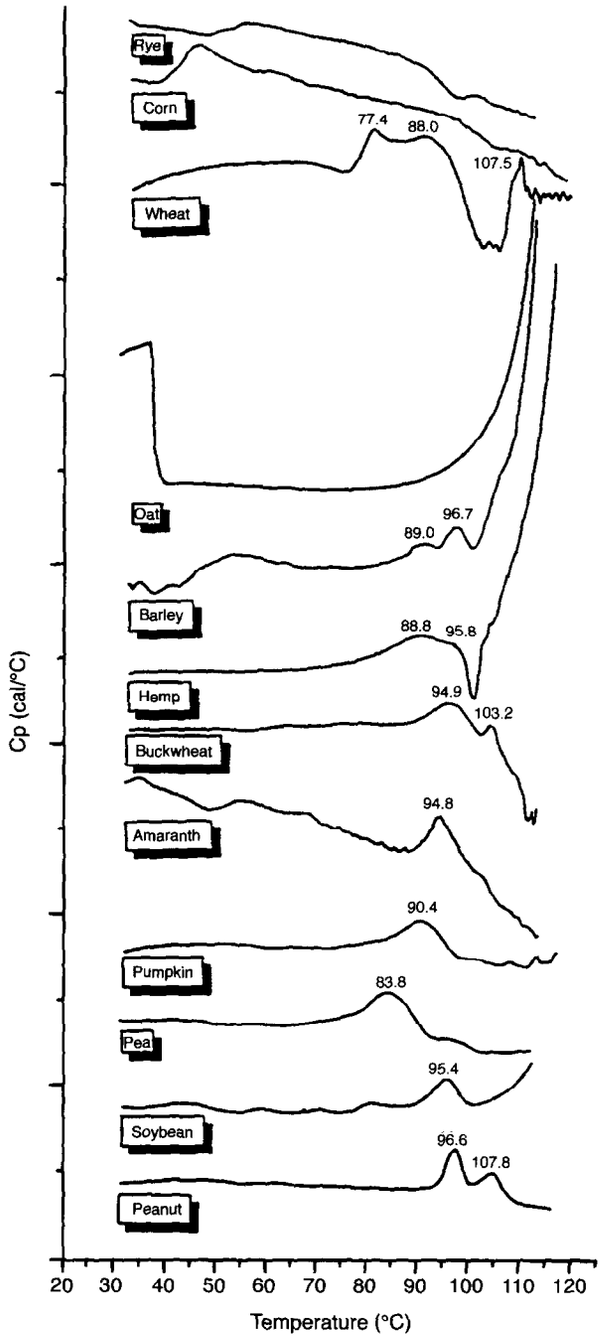

Fig. 4. Micro-differential scanning calorimetry thermograms of various purified dicotyledonous and monocotyledonous seed storage globulins run between the temperatures of 30 to 118°C at a ramp rate of 1.37°C per min. Analyses performed in duplicate.

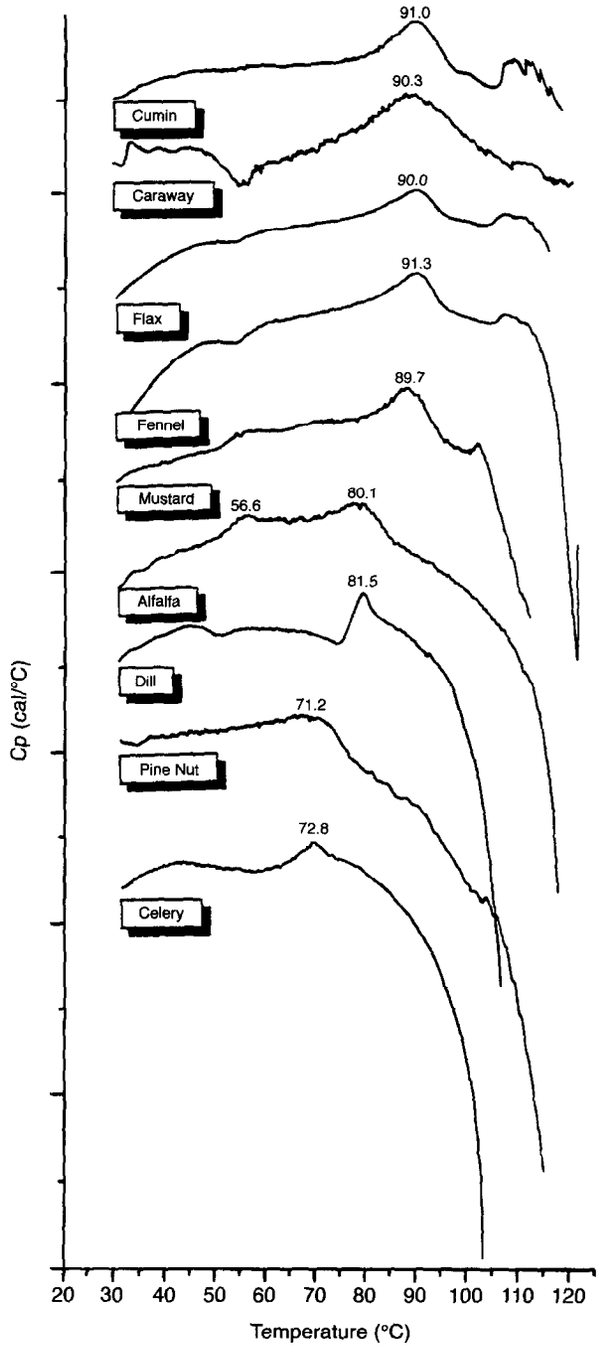

Fig. 5. Micro-differential scanning calorimetry thermograms of various purified dicotyledonous seed storage globulins, i.e. alfalfa, caraway, celery, cumin, dill, fennel, flax, mustard and pine nut run between the temperatures of 30 to 118°C at a ramp rate of 1.37°C per min. Analyses performed in duplicate.

similarity in denaturation temperatures (Stanley and Yada, 1994). Even in seemingly different proteins have been shown to share similar structural domain (Privalov, 1989).

Surface diversity as a function of protein evolution

If dicotyledonous and monocotyledonous globulins share a common ancestral origin (Marcone and Yada, 1997), differences in surface properties between these two classes as noted in this present study would seem confusing. One possible reason for the observed differences among globulins is that they may have undergone mutations during their normal course of evolution and divergence from one another.

Go and Miyazawa (1980) conducted a systematic study in an effort to learn more about the mutability of

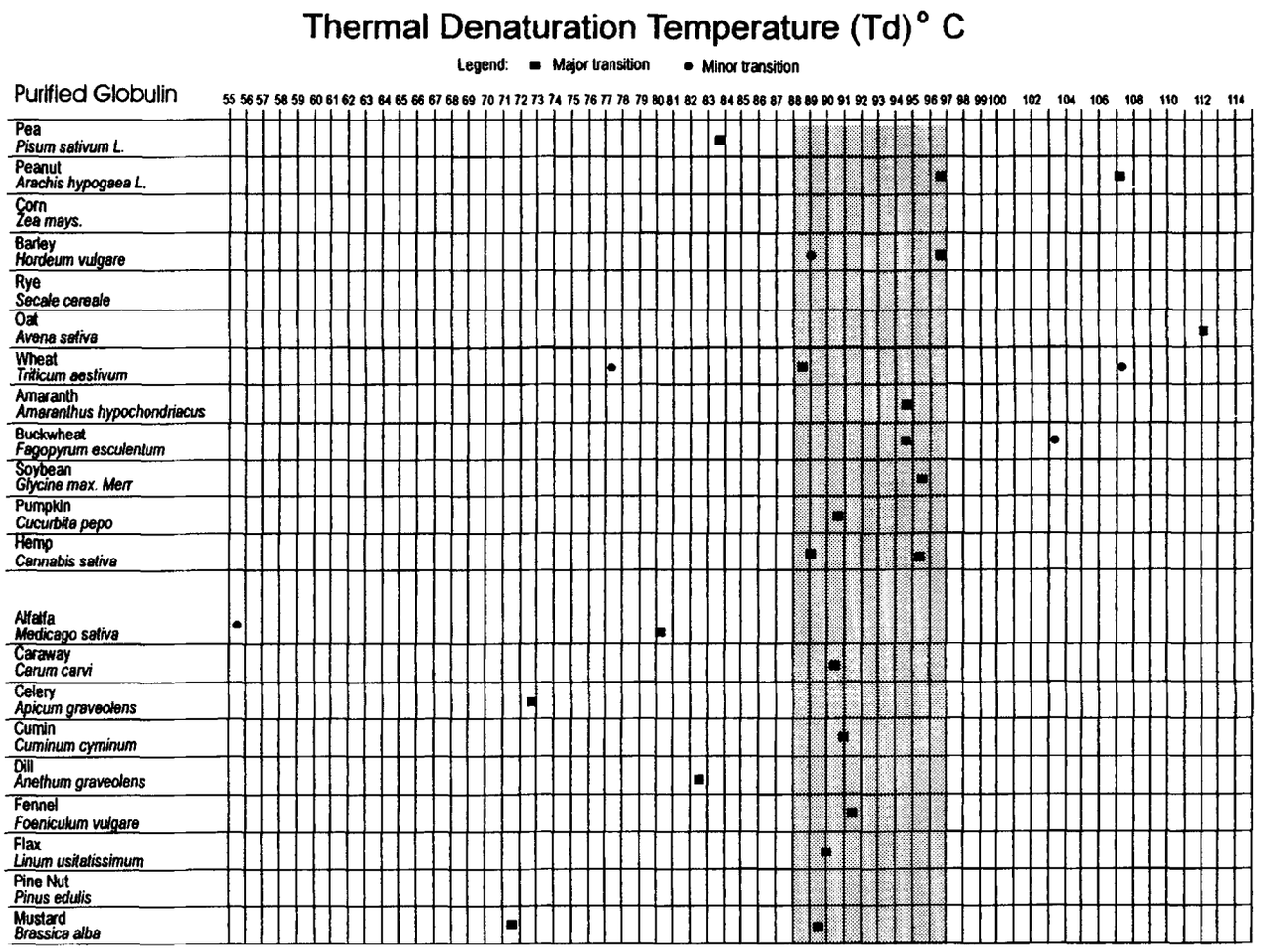

Fig. 6. Tabular representation of thermal denaturation temperatures for various purified globulin from various sources.

amino acids in evolving proteins especially in relation to their polarity and location within the three-dimensional structure of proteins. According to their findings, more frequent amino acid substitution on exterior protein sites was observed in eight sets of homologous protein families regardless of their biological function, and in the presence or absence of prosthetic groups. These observations would support data from the present study, indicating differences in the surface tertiary structure which revealed that the distribution, relative abundance and degree of mobility and type of polar/charged/aromatic amino acids found on the surface of evolutionary related globulins are not highly conserved (Marcone and Yada, 1992; Marcone and Yada, 1995).

According to Go and Miyazawa (1980), amino acid residues could be classified into three main groups depending upon their polarity: polar (Arg, Lys, His, Gln, Asn, Asp and Glu), weakly polar (Ala, Pro, Gly, Thr and Ser) and non-polar (Cys, Val, Met, Ile, Leu, Phe, Tyr and Trp). Inter-group replacement is such that weak polar residues are replaced more often by non-polar residues if they are situated in the interior and by polar residues if they are on the surface of the protein (Go and Miyazawa, 1980).

The work of Go and Miyazawa (1980) was modelled on the pioneering work on molecular evolution by Zuckerkandl and Pauling (1965). Pauling's group was one of the first to propose that exterior amino acids were more variable than those in the interior of proteins. Zuckerkandl (1976) further developed the concept of mutability by studying the direct effects that such mutuations (amino acid substitution) would have on the function and/or stability of the entire protein molecule. They generalized the above in terms of functional properties which they defined as any molecular property of a polypeptide chain that is discernable by natural selection. In the present study of globulin-type proteins, a functional property would refer to any property of the polypeptide (subunits in the case of globulins) that would contribute to a certain equilibrium state that expresses the adaptation of the polypeptide or subunits to its environment and of components of the polypeptide to the polypeptide itself (Zuckerkandl, 1976). According to Zuckerkandl's work, a polypeptide selected by nature will at all times exert a *general* as well as a *specific function*. A specific function is one that can be carried out by a certain molecular site with little or no leeway as to the residue and very little or none as to

the site, e.g. the active site of an enzyme. An example of a protein which acts as an enzyme and a storage protein which could be affected by an indiscriminate mutuation is the protein urease (Pernollet and Mossé, 1983). On the other hand, a general function can be carried out by several residues at a certain number of sites. General functions, in the case of globulins, would include for example, solubility, charge density, isoelectric point, pK and mean polarity of residues. According to Zuckerkandl (1976) the most variable sites were shown to be involved in general functions only. These sites are usually located on the surface of the globulin proteins. One single amino acid substitution could have a relatively modest effect (from negligible to significant) on the value of general function of a protein. In the case of specific functions, however, the effect of one single substitution may impair the function completely, therefore, only residues involved in specific functions are highly conserved. Sites in the interior of the protein molecule although carrying out general structural functions at the same time, may also exercise specific contact functions, and therefore, may be conserved.

It is interesting to note that similar levels of α-helical and β-sheet secondary structure functions (which have the propensity of being deeply buried in proteins) were found to exist in all dicotyledonous as well as monocotyledonous seed globulins examined. These results were interpreted as indicating that the interior conformation of the globulins were similar and highly conserved. This conservation of interior structure would be consistent with the general trend for mutations not to be favoured in the interior of these proteins. Such mutations could interfere with subunits' ability to fold at the point of biosynthesis and assemble into a quaternary structure which would then allow for their maximal packing within protein bodies for future utilization during seed germination.

SUMMARY

Structural characterization of 21 seed globulins from monocotyledonous and dicotyledonous plants revealed that they generally possess low levels of α-helical and high levels of β-sheet secondary structure fractions. This finding would indicate that the interior conformation of these globulins are very similar or highly conserved. The conservation of both α-helical and β-sheet structures in both globulin classes may indicate that they play a similar important role in the internal folding of these globulins.

In contrast to internal conformations, tertiary conformations indicated that distinct surface properties existed between these two globulin classes. Tertiary conformation was shown to be important in the mechanism leading up to cryoaggregation of globulins. It now appears that surface properties are the most variable physico-chemical properties measured between globulins.

Calorimetric analysis revealed that both classes of proteins possessed relatively high temperatures of denaturation (Td), as compared to 7S globulins, and were in a narrow temperature range, i.e. 83.8 to 107.8°C. Although dicotyledonous seed globulins had more pronounced thermal transitions than their monocotyledonous counterparts, most transitions occurred as co-operative events indicating that the various structural/conformational domains present in these globulins were held together by interdependent bond forces. These interdependent domains rendered the globulins stable to elevated temperatures and in connection with other data, it was believed that both monocotyledonous and dicotyledonous seed globulins shared similar structural domains.

On the basis of the structural and chemical data obtained in this study, it was concluded that the 11S storage globulin having several similar properties exists in many leguminous and non-leguminous dicotyledonous plants as well as monocotyledonous plants. This similarity among 11S storage globulins could be due either to convergent evolution in response to a common functional need, or to common ancestry. Observed surface differences between globulins were probably due to the propensity of a higher frequency of amino acid substitutions (mutabilities) to occur in the surface of the protein molecules.

REFERENCES

American Association of Cereal Chemists (AACC) (1983) *Approved Methods of the American Association of Cereal Chemists*, 8th edn. Acc. Inc., St. Paul, MN.

Baldwin, R. L. (1991) Molten globules: specific or non-specific folding intermediates. *Chemstructures in Biochemistry and Molecular Biology* **2**, 379–389.

Babajimopoulos, M., Damodaran, S., Rizvi, S. S. H. and Kinsella, J. E. (1983) Effects of various anions on the rheological and gelling behaviour of soy proteins: thermodynamic observations. *Journal of Agriculture and Food Chemistry* **31**, 1270–1275.

Bio-Rad Laboratories Technical Bulletin. (1989) LIT 89-0931, 1089.

Chang, C. T., Wu, C. S.-S. C. and Yang, J. T. (1978) Circular dichroic analysis of protein conformation: inclusion of the β-turn. *Analytical Biochemistry* **91**, 13–31.

Damodaran, S. (1988) Refolding of thermally unfolded soy proteins during the cooling regime of the gelation process: effect on gelation. *Journal of Agriculture and Food Chemistry* **36**, 262–269.

Fukushima, D. (1968) Internals structure of 7S and 11S globulin molecules in soybean proteins. *Cereal Chemistry* **45**(3), 203–224.

German, B., Damodaran, S. and Kinsella, J. E. (1982) Thermal dissociation and association behaviour of soy proteins. *Journal of Agriculture and Food Chemistry* **30**, 807–811.

Go, M. and Miyazawa, S. (1980) Relationships between mutability, polarity, and exteriority of amino acid residues in protein evolution. *International Journal of Peptide and Protein Research* **15**, 211–224.

Hennessey, J. P. Jr. and Johnson, W. C. Jr. (1981) Information content in the circular dichroism of proteins. *Biochemistry* **20**, 1085–1094.

Hopp, T. P. and Woods, K. R. (1981) Prediction of protein antigenic determinants from amino acid sequences. *Proceedings of the National Academy of Sciences, USA* **78**(6), 3824–3828.

Hopp, T. P. (1986) Protein surface analysis: methods for identifying antigenic determinants and other interaction sites. *Journal of Immunological Methods* **88**, 1–18.

Jacks, T. J., Barker, R. H. and Weingang, O. E.Jr. (1973) Conformation of oilseed storage proteins globulins determined by circular dichroism. *International Journal of Peptide and Protein Research* **5**, 289–291.

Koshiyama, I. (1971) Some aspects of subunit structure of a 7S protein in soybean globulins. *Journal of Agricultural and Food Chemistry* **35**(3), 385–392.

Marcone, M. F. and Yada, R. Y. (1991) Isolation, purification and characterization of the oligomeric seed globulin from *Amaranthus hypochondriacus*. *Journal of Agricultural and Food Chemistry* **55**(9), 2281–2289.

Marcone, M. F. and Yada, R. Y. (1992) Study of the charge profile and covalent subunit association of oligomeric seed globulin from *Amaranthus hypochondriacus*. *Journal of Agricultural and Food Chemistry* **40**(3), 385–389.

Marcone, M. F., Bondi, M. C. and Yada, R. Y. (1994*a*) Isolation of the soybean 11S globulin by isoelectric precipitation and Sephacryl S-300 gel filtration chromatography: a new purification technique. *Bioscience, Biotechnology and Biochemistry* **58**(2), 413–415.

Marcone, M. F., Beniac, D. R., Harauz, G. and Yada, R. Y. (1994*b*) Quarternary structure and the model of oligomeric seed globulin from *Amaranthus hypocondriacus K343*. *Journal of Agriculture and Food Chemistry*, **42**, 2675-2678.

Marcone, M. F. and Yada, R. Y. (1995*a*) Isolation, purification and characterization of the seed storage globulin and its polymerized form from *Triticum aestivum*. *Journal of Food Biochemistry*, **18**, 123-163.

Marcone, M. F. and Yada, R. Y. (1995*b*) A proposed mechanism for the cryoaggregation of the seed storage globulin and its polymerized form from the *Triticum aestivum*. *Journal of Food Biochemistry*, **18**, 147-163.

Marcone, M. F. and Yada, R. Y. (1998) Salt-soluble seed globulins of various dicotyledonous and monocotyledonous plants. I. Isolation/purification and characterization *Food Chemistry* (in press).

Marcone, M. F., Yada, R. Y. and Kakuda, Y. (1997a) Evidence for a molten globule state in an oligomeric plant protein *Food Chemistry* (in press).

Marcone, M. F., Yada, R. Y., Aroonkamonsri, W. and Kakuda, Y. (1997b) Physico-chemical properties of the purified isoforms of the '12S' seed globulin from mustard seed (*Brassica alba*). *Bioscience, Biotechnology and Biochemistry*, **61** (1), 65-74.

Pernollet, J.-C. and Mossé, J. (1983) Structure and location of legume and cereal seed storage proteins. In *Seed Proteins*, eds. J. Daussant, J. Mossé and J. Vaughan. Academic Press, New York, pp. 155-191.

Prakash, V., Nandi, P. K. and Jirgensons, B. (1980) Effect of sodium dodecyl sulfate, acid, alkali and guanidine hydrochloride on the circular dichroism of α-globulin of *Sesamum indicum* L. *International Journal of Peptide and Protein Research* **15**, 305–313.

Privalov, P. L. (1989) Thermodynamic problems of Protein Structure. *Annual Reviews in Biophysical Chemistry* **18**, 47–69.

Radford, S. E., Dobson, C. M. and Evans, P. A. (1992) The folding of hen lysozyme involves partially structured intermidates and multiple pathways. *Nature* **358**, 302–307.

Stanley, D. W. and Yada, R. Y. (1994) Physical consequences of thermal reactions in food protein systems. In *Physical Chemistry of Foods*, eds. H. Schwartzberg and R. Hartel. Marcel Dekker, Inc. New York, pp. 669-733.

Strickland, E. H. (1974) Aromatic contributions to circular dichroism spectra of proteins. *Critical Reviews in Biochemistry* **11**, 113–175.

Whitaker, J. R. and Granum, P. E. (1980) An absolute method for protein determination based on the difference in absorbance at 235 nm and 280 nm. *Analytical Biochemistry* **109**, 156–159.

Zuckerkandl, E. (1976) Evolutionary processes and evolutionary noise at the molecular level. I. Functional density in proteins. *Journal of Molecular Evolution* **7**, 167–183.

Zuckerkandl, E. and Pauling, L. (1965) Evolutionary divergence and convergence in proteins. In *Evolving Genes and Proteins*, eds. V. Bryson and H. J. Vogel. Academic Press, New York, p. 97.