feature of each line. Work is in progress to ascertain whether these differences are linked to alternative pathways of maturation of the precursor molecules.

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Pattern of Zinc-65 Incorporation into Soybean Seeds by Root Absorption, Stem Injection, and Foliar Application[1]

Alam Khan and Connie M. Weaver*

The pattern of [65]Zn incorporation into soybean seeds of plants grown hydroponically and intrinsically labeled with [65]Zn by root absorption, stem injection, and foliar application was studied. Stem injection resulted in the greatest (64.5% of dose) accumulation of [65]Zn while incorporation of [65]Zn through root absorption was the least (23.4%) and through foliar application was intermediate (37.5%). Regardless of the labeling techniques, approximately 40–45% of the seed [65]Zn was associated with the subcellular organelles. The pattern of zinc incorporation did not change appreciably as a result of the labeling technique. The major portion of the soluble zinc was not associated with the major proteins (11S and 7S) of soybeans but either was free or was associated with very low molecular weight amino acids, peptides, or their complexes with phytic acid. Zinc in soybean seems to be ionically bound, and this association is affected by the pH of the extracting buffer.

The bioavailability and chemical association of zinc in soybeans can be studied with radiotracers. Incorporation of [65]Zn into soybeans can be accomplished either by extrinsic labeling where the radionuclide is physically mixed with soy or by intrinsic labeling where [65]Zn is incorporated into the plant system biologically (Weaver, 1985). If extrinsic labeling of [65]Zn exchanges completely with the endogenous zinc, then extrinsic labeling would be an easy approach as it is less costly and easier than intrinsic labeling (Evans and Johnson, 1977). However, individual foods and processing conditions should be checked before

assuming that extrinsic labels exchange with endogenous zinc (Weaver, 1984, 1985).

Intrinsic labeling is assumed to incorporate [65]Zn into the plant in the same pattern as zinc is incorporated in the general field conditions. Although intrinsic labeling could be done through soil media, it is not practical because of the low efficiency of incorporation of an applied dose (Weaver, 1984, 1985). Labeling through hydroponic culture (root absorption) is more practical and efficient in comparison to soil (Weaver, 1985). However, labeling through hydroponic culture (root absorption) is less efficient in terms of incorporation and more specialized, laborious, and time-consuming than stem injection and foliar application (Weaver, 1984, 1985; Schmitt and Weaver, 1984; Janghorbani et al., 1983; Starks and Johnson, 1985; Zeind, 1976). Stem injection usually incorporates a higher percentage of the applied dose into the seeds than root ab-

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sorption. Incorporation through foliar application is expected to be less than stem injection becuase of the major (leaf cuticle) and minor (stomata) barriers to the penetration of nutrients (Currier and Dybing, 1959; Teubner et al., 1957; Van Overbeek, 1956; Biddulp et al., 1956). However, incorporation through stem injection and foliar application may result in deposition of nonphysiological levels of minerals or in a different form than the natural form (Weaver, 1985). This study was initiated to answer the question of whether ^{65}Zn is incorporated in the same pattern of distribution when applied by stem injection or foliar application as when it is absorbed through roots.

MATERIALS AND METHODS

Labeling Soybeans with ^{65}Zn. Soybeans (*Glycine max* L. Merr Century) were germinated in Perlite. After 3 weeks, seedlings for root absorption were transplanted to a nutrient film technique (NFT) system (Weaver, 1985) located inside a greenhouse where 0.5 strength modified Hoagland and Arnon nutrient solution was circulating. For stem injection and foliar application, the seedlings were transplanted to 16-L aerated plastic pots, having full-strength modified Hoagland and Arnon (1950) nutrient solution. These pots were set outside the greenhouse. Four plants were set per pot, and in total there were five pots for stem injection and four pots for foliar application. The nutrient solution of the NFT system and pots were monitored for pH, conductivity, and level of nitrate. The pH values of the two systems were maintained in the range 6.1–6.3.

At flowering of plants labeled by root absorption, 1.5 μCi of ^{65}Zn L^{-1} week^{-1} was added to the nutrient solution of the NFT system containing 0.025 ppm Zn, and this dose was repeated for 7 weeks (682.5 μCi total). Before addition of ^{65}Zn, a zinc-free nutrient solution was circulated in the NFT system for 24 h. Plants grown in pots were used for stem injection and foliar application. At the flowering stage, a dose of 15.3 μCi in 225 μL per plant was injected into the stem of the plants. One-third of the total dose was injected in each of three places above the third node of the stem of each plant. Sterile hypodermic needles (26 gauge) equipped with a hypodermic needle luerlock hub were used for injection. Solution was taken up in the needle and the adjoining hub. Care was taken not to bring the liquid up into the syringe, and also in air space in the top of the needle was avoided. A double puncture was made consecutively in the same spot. The first was to remove a plug of plant tissue to avoid plugging the second needle used for ^{65}Zn infusion. The syringe was removed, and a small piece of tape was put over the hub to guard against evaporation. After the solution was infused into the plant, the hubs were refilled with distilled water to ensure that residual traces of ^{65}Zn were also infused. The needles were removed after 36 h. For foliar application, a dose of 15.3 μCi/plant in a total volume of 225 μL was placed as droplets on the top younger leaves of the plant at the same stage of growth (flowering) as that of stem injection and the first dosing of root-labeled plants. The leaves were kept straight by tying their respective branch to three opposite branches for complete absorption of the droplets. They were also shaded to prevent rapid evaporation of the ^{65}Zn solution prior to absorption. Mature beans were harvested, weighed, assayed for radioactivity, and then stored at 25 °C prior to analysis.

Processing of Soybeans. Soybean seeds from each labeling technique were pooled, dehulled, and ground in a Wiley mill (mesh size 1 mm) as described by Levine et al. (1982). The different flours were then ground to fine flours on a Wig L bug amalgamator (Crescent Dental Manufacturing Co., Chicago, IL). Portions of these flours were saved for the subcellular distribution study while the remaining flours were defatted with hexane in a Soxhlet apparatus for 24 h. These defatted flours were further processed into an isolated soy protein (Moreira et al., 1979; Cataldo et al., 1978).

Efficiency of ^{65}Zn Incorporation. The efficiency of ^{65}Zn incorporation into soybean seeds by the different labeling techniques was calculated as follows: % efficiency = (total activity in seeds at harvest × 100)/(total applied activity corrected for decay to harvest).

Subcellular Distribution of ^{65}Zn. The method of Huffman and Allaway (1973) was used to separate the different organelles

Defatted Soy Flour

```
                    Extract twice for 3 hrs. with
                    0.035M potassium phosphate
                    buffer pH 7.7, containing 0.4M
                    Sodium chloride, 0.02% sodium
                    azide and 0.01M mercaptoethanol,
                    flour buffer ratio 1:8
                         |
           Centrifuge at 5000 x g
                15 min.
                         |
          _____
         |                           |
   Soluble fraction           Insoluble fraction
   (⁶⁵Zn activity)            (⁶⁵Zn activity)
         |                           |
  pH adjusted to 4.5 and      Dialysis against same
  centrifuged at 20,000 x g       buffer pH 7.7
      for 10 min                     |
         |                  _____
         |                 |                   |
         |            Dialyzable          Undialyzable
         |           (⁶⁵Zn activity)     (⁶⁵Zn activity)
         |                 |
   _____
  |                                  |
Supernatant of soy whey      Precipitate or soy curd
  (⁶⁵Zn activity)              (⁶⁵Zn activity)
         |                           |
   _____            _____
  |              |          |                |
10% TCA treatment       Dialysis against DIH₂O
centrifuge at 5,000 x g        pH 4.5
  for 20 min                     |
         |              _____
         |             |                   |
         |        Dialyzable          Undialyzable
         |       (⁶⁵Zn activity)     (⁶⁵Zn activity)
   _____
  |                              |
Precipitate                 Supernatant
(⁶⁵Zn activity)             (⁶⁵Zn activity)
                                 |
                        Dialysis against DIH₂O
                               pH 1.7
                      _____
                     |                    |
                 Dialyzable          Undialyzable
                (⁶⁵Zn activity)     (⁶⁵Zn activity)
```
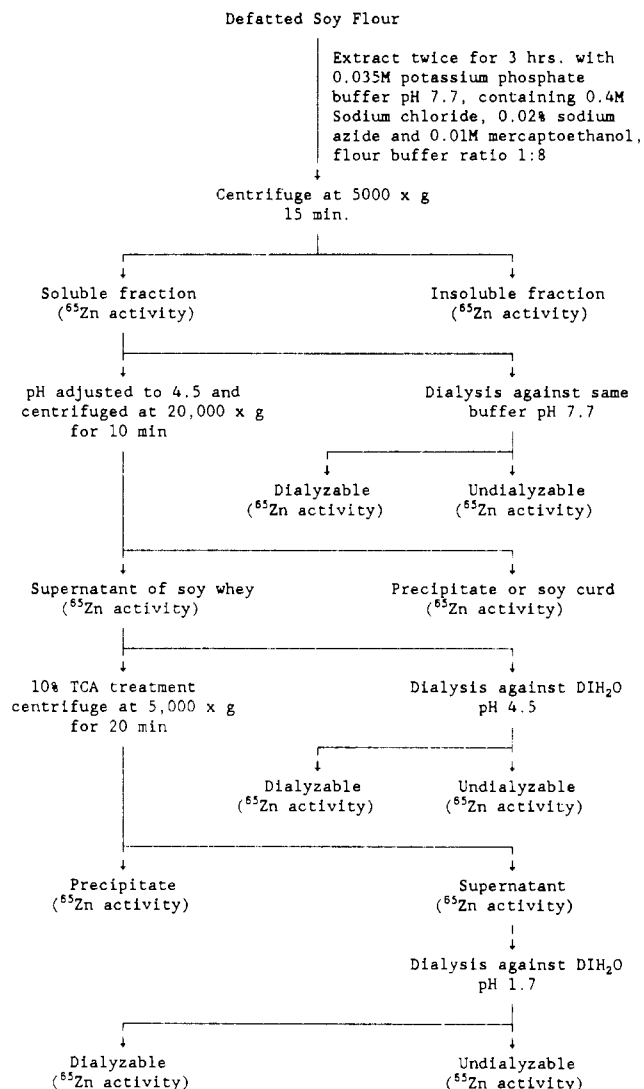
Figure 1. Schematic for determining ^{65}Zn association with isolated soy protein and soy whey.

of the cell. One gram of dehulled finely ground flour labeled by each technique was homogenized (Brinkman Instrument, Westbury, NY) in 12 mL of 10 mM potassium phosphate buffer, pH 7.0, containing 0.5 M sucrose, 2 mM cysteine, 2 mM magnesium chloride, 2 mM calcium chloride, and 1% bovine serum albumin. Homogenates were centrifuged at 1500g for 2 min to pellet the nuclei and debris. The supernatants were again centrifuged at 15000g for 15 min to separate the mitochondria. For separation of the microsomes, the supernatant was recentrifuged at 100000g for 60 min. The activity of the ^{65}Zn in the pellets and the supernatants was determined on a Gamma 4000 Beckman counter. Zinc distribution in the supernatants was further investigated by dialysis and trichloroacetic acid (TCA) precipitation. Of the supernatant, half was dialyzed against deionized water (pH 7.0) at 4 °C for 24 h while the remaining half was treated with 10% TCA for 24 h at 4 °C or 5 min at room temperature. The dialyzed and undialyzed fractions and the TCA precipitate and supernatant were assayed for ^{65}Zn activity.

Association of ^{65}Zn with Soy Proteins. Association of ^{65}Zn with isolated soy protein and soy whey prepared by a modified procedure based on the Moreira et al. (1979) and Cataldo et al. (1978) methods as shown in Figure 1 was determined.

Individual soybean proteins were separated by isoelectric pH precipitation in order to determine the association of zinc with each protein fraction. The separation of soybean proteins into 11S, 7S, and 2S fractions was done according to the method of Thanh and Shibasaki (Thanh and Shibasaki, 1976; Thanh et al., 1975). The procedure for determining ^{65}Zn association with these proteins is given in Figure 2. The different protein fractions were redissolved in 0.03 M Tris buffer (pH 8.0) and divided into two

Table I. Subcellular Distribution (%) of ^{65}Zn in Soybean Seeds Labeled by Different Labeling Techniques[a,b]

method	debris, nuclei	mito-chondria	micro-some	super-natant
root abs	25.9 ± 1.2[a]	9.1 ± 0.7[a]	5.1 ± 0.1[a]	59.9 ± 0.4[a]
stem inj	27.2 ± 0.3[b]	11.7 ± 0.4[b]	6.4 ± 0.5[b]	54.8 ± 0.5[b]
foliar appl	23.2 ± 0.4[c]	12.9 ± 0.37[b]	6.7 ± 0.6[b]	57.3 ± 0.7[c]

[a] Mean ± SD, n = 4. [b] Means followed by different letters in columns are significantly different at $p < 0.05$ as determined by an analysis of variance and Student–Newman–Keuls test.

portions. Half of each fraction was dialyzed against 0.03 M Tris buffer at the isoelectric pH of each protein. The dialyzed and undialyzed portions were assayed for radioactivity. The purity of each protein fraction was verified by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (7.5–20% linear gradient acrylamide) using the Laemmli (1970) system. Concentration of ^{65}Zn was determined using a Gamma 8000 Beckman counter. Dialysis tubing of 6–8K molecular weight cutoff was used.

RESULTS

The relative efficiency of incorporation of ^{65}Zn into seeds was stem injection (64.5%) > foliar application (37.8%) > root absorption (23.4%). The zinc concentrations of the seeds were 38.5 ± 0.4, 23.3 ± 0.6, and 25.6 ± 0.6 ppm for root absorption, stem injection, and foliarly applied ^{65}Zn, respectively. The distribution of ^{65}Zn among the different organelles and supernatant of seeds for each method of labeling is given in Table I. About 23–27% ^{65}Zn stayed with the debris and nuclei, 9–13% with the mitochondria, 5–7% with microsomes, and 55–60% in the supernatant. Although the differences in incorporation of ^{65}Zn into the different organelles by the different labeling techniques were significantly different at $p < 0.05$, they were very small. The percent ^{65}Zn remaining in the supernatants of seeds labeled by the different labeling techniques was also significantly different at $p < 0.05$. The dialyzable and undialyzable ^{65}Zn in the 100000g supernatant and ^{65}Zn in the TCA precipitate and supernatant are given in Table II. For all the seeds labeled by different techniques, 43–48% of the supernatant ^{65}Zn was undialyzable. Differences due to labeling technique were small but significant at $p < 0.05$. Approximately 80% of the ^{65}Zn in the supernatant did not precipitate with TCA and remained in the TCA supernatants irrespective of the labeling technique. Again differences among the labeling tech-
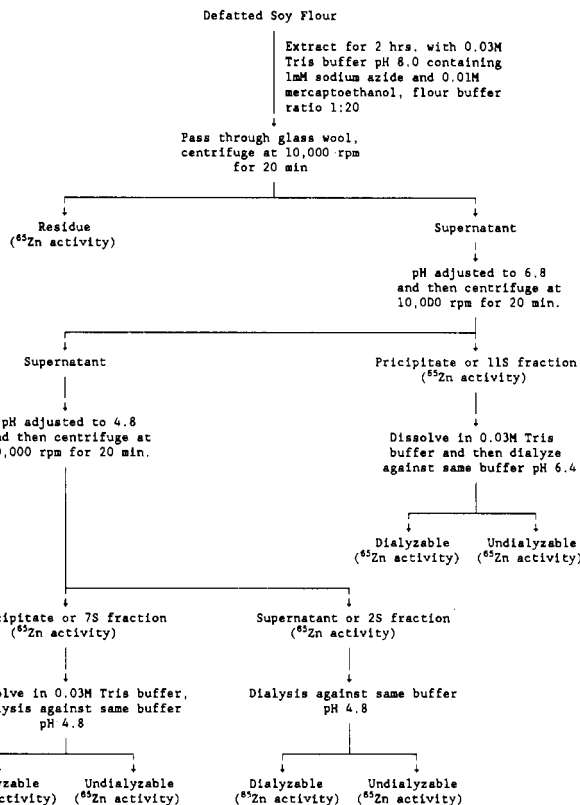

Figure 2. Procedure for determining ^{65}Zn association with individual soybean proteins.

niques in concentration of ^{65}Zn in the TCA supernatant were small but significant at $p < 0.05$.

The distribution of ^{65}Zn in soybean proteins based on solubility, molecular weight, and isoelectric precipitation is given in Table III. Approximately 69–74% ^{65}Zn of the total flour was obtained in the soluble fraction while the remaining ^{65}Zn of the flour did not dissolve and remained in the residue. On the basis of distribution of ^{65}Zn between soluble and insoluble fractions, plants labeled by root absorption were significantly different from those labeled by stem injection and foliar application. When the soluble fraction was dialyzed, about 33–36% of the ^{65}Zn of the soluble fraction was found to be undialyzable. There was no significant difference ($p > 0.05$) either in the undia-

Table II. Percent Distribution of ^{65}Zn in the 100000g Supernatants from Soybeans Labeled by Different Techniques[a,b]

	dialysis		TCA treatment			
			24 h		5 min	
method	dialyzable	undialyzable	precipitate	supernatant	precipitate	supernatant
root abs	37.1 ± 4.0[a]	48.2 ± 0.3[a]	17.1 ± 0.2[a]	81.7 ± 0.5[a]	12.6 ± 0.2[a]	77.6 ± 0.2[a]
stem inj	43.5 ± 1.2[b]	43.0 ± 0.5[b]	15.5 ± 0.3[b]	82.3 ± 0.3[a]	12.3 ± 0.1[a]	80.4 ± 0.4[b]
foliar appl	50.5 ± 1.3[c]	45.1 ± 0.2[c]	17.9 ± 0.4[c]	80.6 ± 0.8[b]	10.9 ± 0.3[b]	83.0 ± 0.5[c]

[a] Mean ± SD, n = 3. [b] Means followed by different letters in columns are significantly different at $p < 0.05$ as determined by an analysis of variance and Student–Newman–Keuls test.

Table III. Percent Solubility of ^{65}Zn and Distribution between Soy Whey and Isolated Soy Protein (Soy Curd) in Soybean Seeds Labeled by Different Labeling Techniques[a]

	solubility[b]		soluble fraction			
			dialysis[c]		isoelectric precipitation[b]	
					ppt or soy curd	supernatant or soy whey
technique	soluble	insoluble	dialyzable	undialyzable		
root abs	68.9 ± 1.5[a]	25.9 ± 5.2[a]	56.8 ± 5.7[a]	32.8 ± 3.7[a]	7.8 ± 4.1[a]	86.0 ± 3.2[a]
stem inj	73.1 ± 2.7[b]	24.1 ± 0.3[a]	60.6 ± 1.0[a]	35.9 ± 12.7[a]	5.1 ± 3.3[a]	82.9 ± 4.0[a]
foliar appl	73.9 ± 1.9[b]	22.8 ± 3.1[a]	58.9 ± 3.4[a]	34.8 ± 1.5[a]	6.7 ± 4.4[a]	83.0 ± 7.3[a]

[a] Means followed by different letters in columns are significantly different at $p < 0.05$ as determined by an analysis of variance and Student–Newman–Keuls test. [b] Mean ± SD, n = 6. [c] Mean ± SD, n = 3.

Table IV. Percent Dialyzable and TCA-Precipitable ^{65}Zn in the Supernatant (Soy Whey) of Soybean Seeds Labeled by Different Techniques[a,b]

technique	dialysis		TCA treatment		TCA supernatant	
	dialyzable	undialyzable	ppt[c]	supernatant	dialyzable[c]	undialyzable
root abs	76.8 ± 6.6	19.0 ± 1.9	1.9	98.1 ± 0.5	91.5	8.5 ± 2.7
stem inj	74.2 ± 6.7	15.6 ± 1.9	2.2	97.8 ± 2.2	95.7	4.3 ± 1.4
foliar appl	74.5 ± 9.3	16.8 ± 3.4	1.3	98.7 ± 1.4	91.0	8.9 ± 4.8

[a] Mean ± SD, $n = 5$. [b] There was no significant difference ($p < 0.05$) in ^{65}Zn distribution in soy whey based on dialysis and TCA precipitation due to labeling techniques as determined by an analysis of variance and Student–Newman–Keuls test. [c] Values for TCA ppt and TCA dialyzable are by substraction.

Table V. Association of ^{65}Zn with Protein Fractions of Soy Flour Labeled by Different Techniques[a,b]

	technique of labeling, % of ^{65}Zn act. in flour					
	root absorption		stem injection		foliar application	
protein fraction	before dialysis	after dialysis	before dialysis	after dialysis	before dialysis	after dialysis
sol fraction[c]	65.0 ± 6.5	26.1 ± 1.9	62.8 ± 1.6	33.6 ± 2.9	64.0 ± 0.4	31.0 ± 0.3
11S fraction	1.3 ± 0.1	1.0 ± 0	1.7 ± 0.1	1.3 ± 0.4	2.4 ± 0.9	1.5 ± 0.4
7S fraction	5.1 ± 1.1	4.3 ± 0.9	5.7 ± 1.2	3.9 ± 0.7	6.5 ± 1.4	5.2 ± 1.2
2S fraction	41.4 ± 4.6	12.5 ± 2.1	36.5 ± 1.8	14.5 ± 2.6	38.9 ± 0.5	15.9 ± 4.4

[a] Mean ± SD, $n = 3$. [b] There was no significant difference ($p > 0.5$) in ^{65}Zn association with protein fractions before or after dialysis due to labeling techniques. [c] The amount of total ^{65}Zn solubilized in the initial extraction.

lyzable or in the dialyzable ^{65}Zn of the seeds labeled by different labeling techniques. On the basis of isoelectric precipitation, 83–86% ^{65}Zn of the soluble fraction remained in the supernatant (soy whey) while the remaining amount of ^{65}Zn of the soluble fraction associated with protein (isolate or soy curd) precipitated at the isoelectric pH (4.5). There was no significant difference in the distribution of ^{65}Zn in the supernatant (soy whey) due to labeling technique.

The distribution of ^{65}Zn in the supernatant (soy whey), based on dialysis and TCA precipitation, is given in Table IV. Less than 20% of the supernatant (soy whey) ^{65}Zn was undialyzable, and more than 75% ^{65}Zn of the supernatant was dialyzable regardless of the labeling technique. Neither the undialyzable nor dialyzable ^{65}Zn of the supernatant (soy whey) was significantly different among seeds labeled by different techniques at $p < 0.05$. Upon precipitation of the supernatant (soy whey) with TCA, about 98% of the ^{65}Zn of the supernatant remained in the supernatant after TCA precipitation. The remaining 2% ^{65}Zn of the supernatant (soy whey) associated with the TCA precipitate. Upon dialysis, the TCA supernatant gave a distribution of 4–7% undialyzable and 91–97% dialyzable ^{65}Zn. Distribution of ^{65}Zn based on TCA precipitation and dialysis of the TCA supernatant was statistically different among seeds labeled by the different techniques at $p < 0.05$.

The attachment of ^{65}Zn with different proteins of soybeans based on the isoelectric precipitation and dialysis of the separated protein fractions is presented in Table V. The protein fraction precipitated at pH 6.4 (11S) contained 1.2–2.4% of the total ^{65}Zn activity in the flour. When this fraction was redissolved and dialyzed, only 1.0–1.5% of the total ^{65}Zn activity of the flour remained within the dialysis tubing. Similarly, when the pH of the supernatant left after the pH 6.4 precipitation was adjusted to pH 4.8, about 5–7% of the total ^{65}Zn activity in the flour was precipitated with the 7S protein fraction. When the 7S fraction was redissolved and dialyzed, about 4–5% of the total Zn activity in the flour remained in the dialysis tubing. About 36–41% of the total ^{65}Zn activity in the flour remained in the supernatant. The protein in this fraction is referred as the soy whey protein or the 2S fraction (Thanh and Shibasaki, 1976; Thanh et al., 1975). After this fraction of the soybean protein was dialyzed, about 12–16% of the total Zn activity in the flour was

undialyzable. ^{65}Zn association with proteins of soybeans separated by isoelectric precipitation and dialysis was not significant ($p > 0.05$) among seeds due to labeling technique.

DISCUSSION

The percent incorporation of ^{65}Zn into seeds through root absorption was lower than via stem injection or foliar application. The selective absorption and transport mechanism of the root (Weaver, 1984), interference with ^{65}Zn uptake for other divalent ions, and competition for chelators (Schmitt and Weaver, 1981) justify the lower incorporation of ^{65}Zn through root absorption. Janghorbani et al. (1983) reported 21.3–27.6% incorporation of single dose of ^{65}Zn into soybean seeds grown in pot culture depending on the time of application of ^{65}Zn to soybeans. Weaver (1984) has reported 14–23% incorporation of the applied dose of a stable isotopes (^{70}Zn) into soybean seeds grown in noncirculating hydroponic system. A 28.3% incorporation of ^{65}Zn into soybeans grown in the same circulating hydroponic system as used in this study was reported (Schmitt and Weaver, 1984). The incorporation of 23.4% ^{65}Zn of the applied dose into soybeans in the present study is similar to these previously reported values whether given as a single dose or repeated application.

Stem injection incorporated the highest amount of the applied ^{65}Zn. This was expected as ^{65}Zn applied through stem injection bypasses the barrier of selective absorption, ion interaction, competition for chelators, and pH variation around the root and leaf barriers (Weaver, 1984, 1985; Schmitt and Weaver, 1984; Currier and Dybing, 1959; Teubner et al., 1957; Van Overbeek, 1956; Biddulph et al., 1956, Bukovac and Wittwer, 1957). Incorporation of ^{65}Zn into soybean seeds through foliar application was less than through stem injection because of leaf barriers to the penetration of ^{65}Zn and the intermediate mobility of ^{65}Zn from leaves to the storage areas (Currier and Dybing, 1959; Teubner et al., 1957; Van Overbeek, 1956; Biddulph et al., 1956). There are no other available data on incorporation of ^{65}Zn into soybean seeds by stem injection or foliar application techniques. However, Starks and Johnson (1985) reported higher incorporation of ^{65}Zn into wheat grain by stem injection than through root absorption. Neither stem injection nor foliar application of ^{65}Zn resulted in nonphysiological levels of zinc. In fact, the seeds labeled by root absorption had the highest zinc concentration in spite

65Zn Incorporation in Soybean Seeds

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of a lower concentration of zinc in the nutrient solution. The circulating NFT system used to label via root absorption probably favors uptake of zinc by the roots over pot culture, which was used to label by stem injection and foliar application. All seeds were within the normal range of 29–87 ppm for Zn content of soybeans grown in the United States (Wolnik et al., 1983). The different growing conditions also did not produce differences in proteins as evaluated by SDS-PAGE gel electrophoresis.

Subcellular distribution of ^{65}Zn in soybean seeds was similar but statistically different among the different labeling techniques. The small differences observed between labeling by root absorption and the other techniques could have been due to the environmental differences imposed by growing plants inside vs outside the greenhouse. However, seeds labeled by root absorption were not consistently different from seeds labeled by stem injection and foliar application, and the differences are likely too small to be biologically important. The high proportion of ^{65}Zn in the soluble fraction is similar to findings for pea seeds (Welch et al., 1974), spinach (Welch et al., 1977), and lettuce (Walker and Welch, 1987). However, more ^{65}Zn was associated with the debris and nuclei after differential centrifugation in soybeans than the 1% for spinach (Welch et al., 1977).

The similar distribution of ^{65}Zn in the supernatants from several extracting procedures and the similar results for dialysis and TCA precipitation for all the three labeling techniques suggest a similar pattern of incorporation of ^{65}Zn into soybean seeds. More than half of the seed ^{65}Zn was in the soluble form, which could be expected to be bioavailable. However, if this ^{65}Zn is complexed with protein and phytic acid, it may be unavailable. This possibility is evaluated by dialyzing the supernatants from the various extraction procedures. It was noted that much of the supernatant ^{65}Zn did not dialyze, indicating that this portion of ^{65}Zn was attached to proteins or complexes having a molecular weight >6–8K. However, the remaining ^{65}Zn of the supernatants (generally the major portion) was dialyzable and hence could be free or bound to substances having a molecular weight <6–8K. The low molecular weight substances could be proteins, peptides, amino acids, or complexes of these with phytic acid. It is difficult to differentiate between free zinc and zinc bound to low molecular weight substances. The supernatants from the subcellular distribution experiment and the soy whey were treated with 10% TCA to determine how much ^{65}Zn was associated with TCA-precipitable proteins. Approximately 80% or more of the ^{65}Zn from the supernatants remained soluble after TCA precipitation. Either the undialyzable substances of the supernatant are not TCA precipitable or TCA detached the ^{65}Zn from the undialyzable substances. If neither of the above assumptions were true, then the undialyzable ^{65}Zn and ^{65}Zn in the TCA precipitates should be almost equal, which was not the case (Tables II and IV). TCA precipitated only about half of the soy whey protein as determined by the method of Lowry (1951), and TCA is probably sufficiently strong to detach ionically bound ^{65}Zn. Although low pH could dissociate zinc, zinc solubility was little affected over the range pH 3.5–12.0 (unpublished data).

Very little of the ^{65}Zn was associated with the protein precipitated at pH 4.5 (Table III). The precipitated protein mainly contains 7S and 11S proteins of soybean seeds (Thanh and Shibasaki, 1976; Thanh et al., 1975). Since the 7S and 11S fractions constitute over 70% of the total soy proteins, the major portion of ^{65}Zn does not associate with the major proteins of soy. The amount of ^{65}Zn in the

isolate (soy curd) and the undialyzable ^{65}Zn of the supernatant (soy whey) was almost equal to the undialyzable ^{65}Zn of the soluble fraction. This confirms that at least two-thirds of the ^{65}Zn of the soluble fraction exists in complexes with molecular weights <6–8K (Tables III and IV) and that pH adjustment during preparation of the soy curd did not affect this distribution.

To provide another line of evidence that a nominal amount of zinc is associated with the major soy proteins, 11S, 7S, and 2S protein fractions of soybeans were separated by isoelectric precipitation as described by Thanh and Shibasaki, (Thanh and Shibasaki, 1976; Than et al., 1975). Each individual protein fraction before and after dialysis (against Tris buffer at isoelectric pH of each protein) was assayed for ^{65}Zn activity. As shown in Table V, less than 10% of the total ^{65}Zn activity in the flour before and less than 7% after dialysis were associated with 7S and 11S proteins. About 40% of the total ^{65}Zn activity in the flour before dialysis and less than 20% of the total ^{65}Zn activity after dialysis were with the 2S protein fraction. This fraction includes all those proteins and peptides that did not precipitate at pH 4.8. Data in Table V support that the major portion of ^{65}Zn is associated with low molecular weight compounds. No statistical differences were found in the pattern of incorporation of ^{65}Zn into soybean seeds by the different labeling techniques.

This work reveals that the method of labeling does not greatly influence the pattern of incorporation of zinc into soybeans. Likewise, Starks and Johnson (1985) observed that the pattern of incorporation of ^{65}Zn into wheat grain remained the same whether it was incorporated by root absorption, stem injection, or foliar application. The major portion of zinc in soybeans does not associate with the major proteins of soybeans. Further investigations on the chemical form of zinc complex in soybeans are currently under way in our laboratory.

Registry No. Zn, 7440-66-6.

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Changes Induced in β-Lactoglobulin B following Interactions with Linoleic Acid 13-Hydroperoxide

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β-Lactoglobulin B (βLG B) undergoes a number of deteriorative changes when exposed to linoleic acid 13-hydroperoxide. In the first stage, the lipid hydroperoxide caused destruction of tryptophan·and disulfide cross-linking of βLG B. Concurrently though more slowly, the hydroperoxide itself or some secondary product (SP) reacted with free amino groups of βLG B to generate fluorescent compounds and promoted polymerization. The presence of other degradation compounds, such as short-chain aldehydes, reacted with exposed amino groups of βLG B to form additional but different fluorescent and nonfluorescent compounds. However, the development of the principal fluorescence was closely related to the formation of stable dimers of βLG B. The blockage of the amino groups in βLG B by reductive alkylation prevented the development of fluorescence and the formation of stable dimers, suggesting that the presence of free amino groups is necessary for these types of reactions.

Lipid oxidation is one of the major causes of food spoilage and is undesirable not only from an acceptibility and economic point of view but also because oxidative reactions can decrease the nutritional quality of food and generate oxidation products that are potentially toxic (Matsuo, 1962; Morton, 1977; Nawar, 1985; Richardson, 1984). Among the negative effects produced by peroxidizing lipids in foods, their chemical interactions with proteins have received considerable attention (Desai and Tappel, 1963;, Gardner, 1983; Karel et al., 1975; Pokorny et al., 1988). This is an important deteriorative mechanism in the processing and storage of foods causing loss in flavor, color, functional properties, and nutritive value and, also, causing changes in biological tissues and is a basic pathological process in vivo (Funes et al., 1982; Kanner and Karel, 1976; Kanner et al., 1987; Tappel, 1973).

Exposure of proteins to peroxidizing lipids or their secondary breakdown products can produce changes in proteins, including loss of enzyme activity, polymerization,

insolubilization, scission, and formation of lipid–protein complexes, some of which are fluorescent (Chio and Tappel, 1969; Funes et al., 1982; Gardner, 1979; Pokorny and Janicek, 1975). These changes occur through two basic mechanisms, namely via protein–amino condensation reactions involving lipid peroxidation breakdown products and via reactions of proteins with lipid oxidation products (lipid free radicals, hydroperoxides, and volatile secondary products), resulting in the formation of protein-centered free radicals (Karel, 1977).

Most studies of interaction between peroxidizing lipids and proteins have been carried out between a model lipid, mostly linoleic acid or its hydroperoxides, and model proteins, usually lysozyme, egg albumin, or bovine serum albumin (BSA), and almost all have studied the changes produced in the protein.

The objective of this research was to determine the kinetics and products of the interaction between linoleic acid 13-hydroperoxide (13-LOOH) and β-lactoglobulin B (βLG B).

βLG B is a well-characterized protein that has not been used in these types of studies. However, it should be useful because its composition and secondary and tertiary

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