Oilseed Protein Solubility After Exposure to Hydrogen Peroxide

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Cucurbitin, a physicochemically typical oilseed storage protein from pumpkin and other cucurbits, was treated with 20% H_2O_2 for periods up to 36 h to determine the length of exposure needed to render it water-soluble. Maximum solubility was reached in 8 h from virtual insolubility and started decreasing after about 12 h. These results not only indicate that H_2O_2 was effective in rendering oilseed protein water-soluble and can be used to prepare several ratios of soluble-to-insoluble protein products, but also show the lengths of exposure needed to obtain the required solubilities.

KEY WORDS: Cucurbitaceae, cucurbitin, globulin, hydrogen peroxide, oilseed, protein, protein solubility.

Globulins, which comprise the bulk of proteins stored in oilseeds (1), are insoluble in water and in neutral salt solutions of low ionic strength (2). Their solubilities in water increase, however, after chemical modifications such as acylation, mild oxidation and partial hydrolysis (3–5). Regarding oxidatively induced solubilization, cucurbitin, which is the principal seed storage protein of Cucurbitaceae (cucurbits such as gourds, melons, pumpkins) as well as a physicochemically typical oilseed globulin (6,7), becomes soluble in salt-free and peroxide-free water after being exposed to H_2O_2 (8,9). However, the length of exposure required to render cucurbitin water-soluble has not been determined. In this communication we describe changes in the solubility of cucurbitin after several different periods of exposure to H_2O_2.

EXPERIMENTAL PROCEDURES

Cucurbitin was purchased from United States Biochemical Corp. (Cleveland, OH) and then recrystallized by the method of Vickery *et al.* (10). One-gram specimens were suspended in 20 mL of 20% H_2O_2 as described earlier (8), and 5 min to 36 h later, 4000 units of catalase (crystalline A grade from bovine liver; Calbiochem, La Jolla, CA) were added directly to each suspension to immediately end the exposure by destroying the peroxide. Peroxide destruction was estimated colorimetrically with $TiOSO_4$ (11) and corresponded to the cessation of O_2-bubble formation. The amount of protein that was subsequently soluble in distilled water at saturation (8) was determined spectrophotometrically (12, 13). Protein content was corrected for the presence of catalase. Experiments were conducted in triplicate.

RESULTS AND DISCUSSION

The effects of exposure time of cucurbitin to 20% H_2O_2 on protein solubility in water are shown in Figure 1. The greatest solubility at 13 mg/mL occurred after an 8-h (approximate) exposure and began decreasing about 4 h later. After 30 h of exposure (not shown), the solubility of cucurbitin in water decreased to only about one-third that at 8 h.

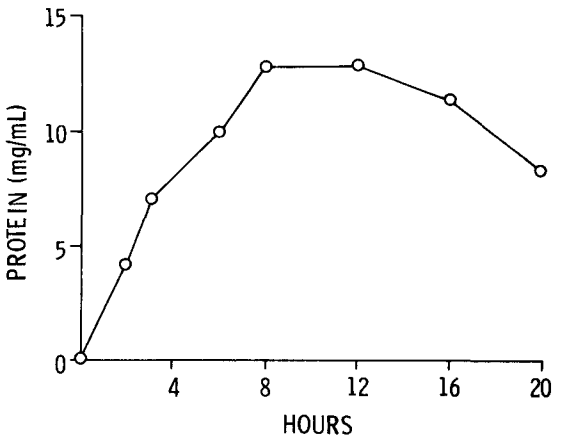

FIG. 1. Effect of the length of exposure of cucurbitin to 20% H_2O_2 on its solubility in water. Abscissa is the amount of soluble protein (mg/mL); ordinate represents reaction time (h). Symbol sizes of data points approximate SE.

In our earlier studies (8,9), cucurbitin was exposed to H_2O_2 for 2 h and then dialyzed against catalase for peroxide removal. Dialysis, however, produced additional time of actual contact to peroxide while it was being depleted by irreversible destructive diffusion. In the current study, according to the $TiOSO_4$ test (11) and to bubble formation, peroxide was virtually depleted immediately by the addition of catalase to each reaction mixture, permitting complete control of peroxide exposure. These results indicate that about 8 h of actual contact with 20% H_2O_2 is needed to substantially increase protein solubility (Fig. 1).

Because our method of protein determination was based one detection of peptide bonds, the decrease in soluble protein content after 12 h of exposure to peroxide (Fig. 1) was actually a decrease in intact peptide linkages. If our results were based on nitrogen solubility, then amino acids and short peptides released from the globulin by oxidatively induced protein scission (14) would be measured as protein but would not be indicative of the actual protein content. Based on peptide linkages, results presented here specifically indicate solubilities of polymeric peptides and protein.

The amount of protein that became soluble after peroxide exposure was dependent on the concentration of peroxide (9) and the length of exposure (Fig. 1). These relationships indicate that short exposures of protein to stronger concentrations of H_2O_2 than were used here should be equivalent to long exposure with weaker concentrations.

In conclusion, these and other results (8,9) show that oilseed storage proteins are rendered water-soluble by treatment with H_2O_2. Because both the concentration of H_2O_2 and the length of treatment affect the degree of solubility, any desired ratio of soluble-to-insoluble protein can be generated by varying the peroxide concentration

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and/or treatment time. The ability to control processing conditions to obtain proteins with specific solubility properties is highly desirable in developing new products from oilseeds.

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