Conformation of Bowman–Birk Inhibitor

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Bowman–Birk inhibitor, a major trypsin and chymotrypsin inhibitor from soybean, has 71 amino acids with 7 disulfide bonds. Conformation of Bowman–Birk inhibitor in native state, after heating, and after disulfide bonds were broken by sodium metabisulfite was determined by circular dichroism. The native Bowman–Birk inhibitor has 61% β-sheet, 38% unordered form, 1% β-turn, and no α-helical structure. There was no significant change in conformation after Bowman–Birk inhibitor was heated at 80 °C for 1 h in phosphate buffer. There was a decrease in β-sheet and an increase in β-turn structure after Bowman–Birk inhibitor was heated at 80 °C for 1 h in sodium metabisulfite–phosphate buffer. Although the change in conformation after disulfide bonds of Bowman–Birk inhibitor were broken was statistically significant ($P < 0.05$), the magnitude of the change was not large. The data support Bowman–Birk inhibitor's having a stable conformation even after disulfide bonds are broken.

Keywords: *Bowman–Birk inhibitor; soybean; circular dichroism; conformation change*

INTRODUCTION

Bowman–Birk inhibitor, a trypsin and chymotrypsin inhibitor from soybean, is a protein molecule consisting of 71 amino acids with 7 disulfide bonds. The other major type of soybean protease inhibitor is Kunitz inhibitor, which inhibits trypsin. Feeding soybeans containing active trypsin inhibitors to animals depresses growth in comparison with feeding of inhibitor-free soybeans. Heat treatments were used to inactivate trypsin inhibitors from soybean (Hackler et al., 1965; Borchers et al., 1972; Collins and Beaty, 1980; Naim et al., 1982; Chae et al., 1984; DiPietro and Liener, 1989). Chemical inactivation of soybean trypsin inhibitor was studied by Hogle and Liener (1973), Friedman and Gumbmann (1986), Sessa and Ghantous (1987), Sessa et al. (1988, 1990), and Sessa and Nelsen (1991). Sodium metabisulfite is an excellent inactivator of soybean trypsin inhibitors (Sessa and Ghantous, 1987; Sessa et al., 1988) and will not significantly alter the amino acid composition of soy flour (Sessa and Nelsen, 1991). Sulfiting agents will cleave disulfide bonds to *S*-sulfo cysteine derivatives, which leads to loss of inhibitory activity and increased protein digestibility and nutritive value (Friedman and Gumbmann, 1986). Inactivation of Bowman–Birk inhibitor can occur when four of the seven disulfides are cleaved (Hogle and Liener, 1973).

The conformation of Bowman–Birk inhibitor has been reported by a number of investigators. Steiner and Frattali (1969) concluded from circular dichroism study that Bowman–Birk inhibitor had low α-helical content. Ikeda et al. (1968) found that Bowman–Birk inhibitor was nonhelical from circular dichroism and optical rotatory dispersion study. Kay (1976) reported the circular dichroism spectrum of Bowman–Birk inhibitor without any structural calculation such as α-helix. Bewley and Birk (1978) found that Bowman–Birk inhibitor did not contain appreciable amounts of α-helix from its circular dichroism spectrum. Werner and Wemmer (1991, 1992) used two-dimensional proton nuclear magnetic resonance spectroscopy to determine the three-dimensional structure of Bowman–Birk inhibitor and found that residues Q11–T15 form an antiparallel β-sheet with residues Q21–S25 in the tryptic inhibitory domain and an analogous region of antiparallel β-sheet between residues S38–A42 and Q48–V52 in the chymotrypic inhibitory domain. No quantitative conformation of Bowman–Birk inhibitor by circular dichroism and no conformation of heated and sodium metabisulfite-treated Bowman–Birk inhibitor were reported previously. In an effort to better understand the impact of sodium metabisulfite on the secondary structure of Bowman–Birk inhibitor, the objective of this research was to define via circular dichroism measurements the conformational changes that occur in the native state of this inhibitor when heated in the presence and absence of sodium metabisulfite.

MATERIALS AND METHODS

Whole soybeans (Century variety) were cracked, dehulled, flaked, and defatted by hexane at 30 °C (Sessa et al., 1990). Crude Bowman–Birk inhibitor was prepared from the defatted soy flour by alcohol extraction and acetone preparation as described by Frattali (1969). Crude Bowman–Birk inhibitor was purified by an anhydrotrypsin affinity column (Pusztai et al., 1988) by using methods described by Sessa et al. (1990). Native Bowman–Birk inhibitor was dissolved in 0.008 M sodium phosphate buffer, pH 6.5. Some Bowman–Birk inhibitor solution was heated at 80 °C for 1 h (heated), and another Bowman–Birk inhibitor solution in 0.6 mM sodium metabisulfite ($Na_2S_2O_5$) plus 0.008 M sodium phosphate, pH 6.5, was heated at 80 °C for 1 h (metabisulfite treated). Sodium metabisulfite treatment was stopped by cooling in ice water, and salt was removed by dialysis against distilled water for 2 days at 4 °C (Sessa and Nelsen, 1991).

A Jasco (Easton, MD) J-600 spectropolarimeter was used to measure circular dichroism (CD) of Bowman–Birk inhibitor from 240 to 190 nm. The Path lengths of cells used were 0.0053 and 0.0056 cm. Nine scans of protein solution at 24 °C were made followed by nine scans of solvent. The same protein solution was scanned nine times followed by nine solvent scans on a different day to check for reproducibility. The spectropolarimeter was calibrated with ammonium camphorsulfonate solution. The protein solution varied from 1.8 to 2.0 mg/mL. CD data were expressed as the mean residue ellipticity, $[\theta]$, in deg cm²/dmol: $[\theta] = M\theta°/10lc$, where M is

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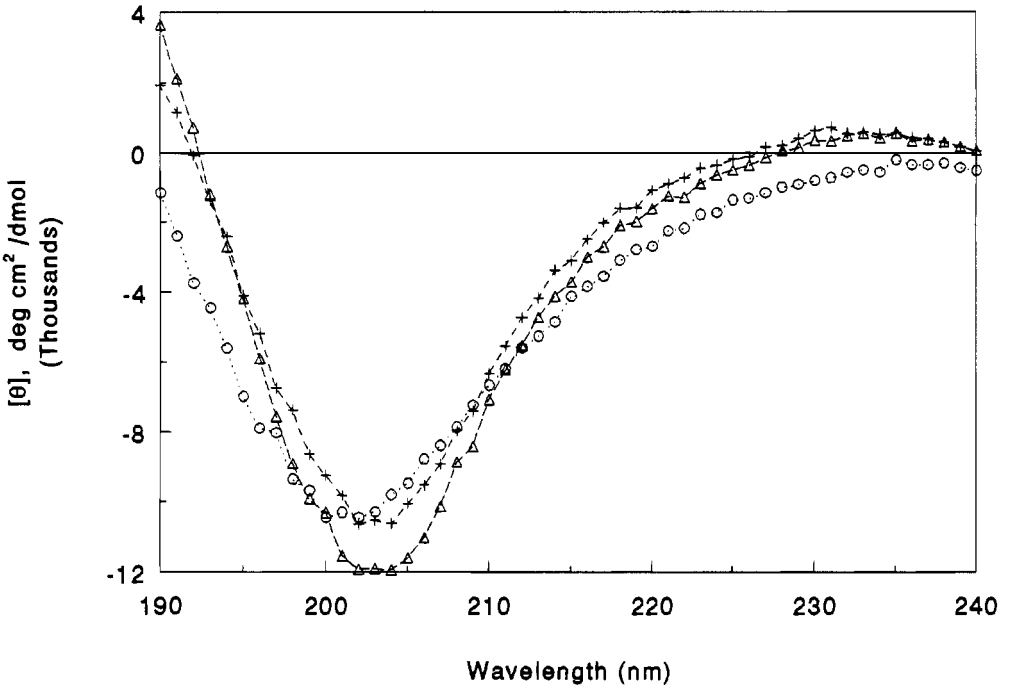

Figure 1. Circular dichroism spectra of Bowman–Birk inhibitor: +, native protein in 0.008 M sodium phosphate buffer, pH 6.5; △, protein in sodium phosphate buffer, pH 6.5, heated at 80 °C for 1 h and then cooled; ○, protein in 0.6 mM sodium metabisulfite + 0.008 M sodium phosphate, pH 6.5, heated at 80 °C for 1 h and cooled (excess salt removed by dialysis). Protein concentrations were 1.8–2.0 mg/mL, 24 °C.

Table 1. Conformation of Bowman–Birk Inhibitor[a]

	% β-sheet	% β-turn	% unordered
native	61A[b]	1B	38A
heated	58AB	1B	41A
metabisulfite treated	53B	5A	42A

[a] There was no α-helix structure in Bowman–Birk inhibitor. [b] Values in each column followed by the same letter are not significantly different. Means were compared by least significant difference tests at $P < 0.05$.

mean residue weight (=110.7), $\theta°$ is the measured ellipticity in degrees, l is the path length in the sample solution in cm, and c is the concentration of sample in g/cm^3. Percentages of α-helix, β-sheet, β-turns, and unordered form in each protein were calculated from the reference spectra from 15 proteins of known structure by CD estimate computer program from Yang et al. (1986).

The concentration of Bowman–Birk inhibitor was calculated from the nitrogen content of 16.0% from amino acid sequence. Nitrogen was determined by micro-Kjeldahl in triplicate (AACC, 1983). Trypsin inhibitory activity was measured according to the method of Hamerstrand et al. (1981).

Statistical analysis was carried out by general linear model procedure.

RESULTS AND DISCUSSION

The circular dichroism spectra of native, heated, and sodium metabisulfite-treated Bowman–Birk inhibitor are shown in Figure 1. All spectra had minima around 202–204 nm. The spectra were the average CD measurements of 18 scans of protein solution minus the solvent blanks for each curve.

Native Structure. The conformation of native Bowman–Birk inhibitor is shown in Table 1, where 61% β-sheet, 38% unordered form, and no α-helix were found. Our result agrees with those reported by Steiner and Frattali (1969), Ikeda et al. (1968), and Bewley and Birk (1978), who found little or no α-helix. Werner and Wemmer (1991, 1992) found 37% β-structure, 25% turn, and 20% unordered form by two-dimensional proton nuclear magnetic resonance spectroscopy. Although Werner and Wemmer's finding did not define all 100% of structure, they also found β sheet accounted for the highest percentage of Bowman–Birk inhibitor structure and that no α-helix was located.

Heated Structure. When Bowman–Birk inhibitor was heated at 80 °C for 1 h and cooled to room temperature, there was no significant difference in conformation compared with the native structure (Table 1) and 96% of the original trypsin inhibitory activity remained. Our results indicated that either Bowman–Birk inhibitor conformation was stable to heating at 80 °C for 1 h or the change is reversible when the protein is cooled to room temperature. Birk (1961) found that purified Bowman–Birk inhibitor retained its antiproteolytic activity after heating in an aqueous solution at 100 °C for 10 min. DiPietro and Liener (1989) reported that Bowman–Birk inhibitor solution heated to 100 °C lost inhibitory activity very slowly and that remaining trypsin inhibitory activity approximately paralleled chymotrypsin inhibitory activity during heating. Our finding that no conformation change was observed when Bowman–Birk inhibitor solution was heated and cooled was consistent with the heat stability of this protein reported by Birk (1961) and DiPietro and Liener (1989). For comparison, arachin (principal storage protein of the peanut) showed increased content of unordered structure when heated above 140 °C from circular dichroism measurement (Jacks et al., 1975). On the other hand, arachin maintained the native secondary structure of the multimeric form after irreversible dissociation by acidic hexane into subunits (Jacks et al., 1983).

Metabisulfite-Treated Structure. After Bowman–Birk inhibitor in sodium metabisulfite solution was heated and cooled, the conformation was lower in β-sheet but higher in turn structure compared with the native protein (Table 1), and 16% of the original trypsin inhibitory activity remained. The increase in unordered form from native to metabisulfite-treated Bowman–

Birk inhibitor was not significant. When the conformations of the heated and metabisulfite-treated protein were compared, the former had lower turn structure (Table 1). Although there were statistical differences in conformation when disulfide bonds of Bowman–Birk inhibitor were broken by heating in sodium metabisulfite solution compared with the native protein, the differences are relatively small in magnitude and indicate the native Bowman–Birk inhibitor has very stable conformation. Though the conformation of Bowman–Birk inhibitor is quite stable, disulfide bonds are critical to the antitryptic activity of this inhibitor since it retained only 16% of antitryptic activity with the metabisulfite treatment versus 96% without metabisulfite.

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