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Complete ^{1}H and partial ^{13}C resonance assignments at 37 and 22 °C for brazzein, an intensely sweet protein*

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Abbreviations: COSY, correlated spectroscopy; NOESY, nuclear Overhauser effect spectroscopy; TOCSY, total correlation spectroscopy; HSQC, heteronuclear single quantum coherence; DSS, 2,2-dimethyl-2-silapentane-5-sulfonate, sodium salt

Biological context

Although proteins and other macromolecules are generally devoid of taste, several proteins have been isolated that elicit intense sweet taste responses. One of them, brazzein, tastes 2000 times sweeter than sucrose on a weight basis and is exceptionally thermostable (Ming and Hellekant, 1994). This 54-residue, single chain polypeptide is isolated from the fruit of *Pentadiplandra brazzeana*, a climbing vine that grows in Gabon, Zaire, and Cameroon. Virtually complete proton resonance assignments have been determined at 37 °C at pH 3.7 and 5.2, and at 22 °C at pH 5.2. Carbon resonance assignments have been determined for most of the C^{α} and C^{β} carbons at 37 °C and pH 5.2. These assignments have enabled us to conduct structural studies, which are currently underway.

Methods and results

Brazzein was extracted from the pulp surrounding the seed of the wild fruit as described previously (Ming and Hellekant, 1994). The concentration of brazzein in each 400 μl sample ranged between 0.8 and 8 mM. Samples at pH 5.2 were buffered with 50 mM succinic-d_4 acid in 90% H_2O/10% D_2O or 99.9% D_2O. Samples at pH 3.7 were prepared by dissolving brazzein without buffer in 90% H_2O/10% D_2O. Each sample contained 0.1 mM DSS as the internal chemical shift reference and 1 mM sodium azide to inhibit bacterial growth. All samples were studied in 5 mm Wilmad (Buena, NJ, U.S.A.) NMR sample tubes.

NMR spectroscopy was carried out on Bruker AM600 (600 MHz), DMX600 (600 MHz), and DMX750 (750 MHz) spectrometers at both 22 and 37 °C , except where otherwise noted. All data sets

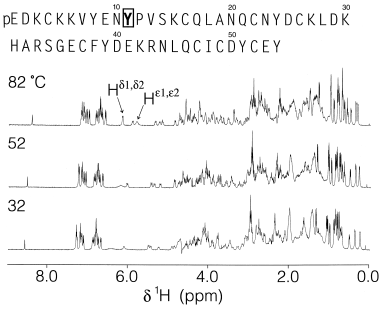

Figure 1. One-dimensional ^{1}H NMR spectra (600 MHz) of 2 mM brazzein at pH 5.18 in D_2O as a function of temperature (as indicated in the figure), with the ring protons of Tyr11 indicated by arrows. The sequence of brazzein appears at the top, with pyroglutamic acid represented by pE, and Tyr11 highlighted with a box.

* These data have been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 4067.
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were zero-filled to the nearest power of two and Fourier transformed using FELIX software version 2.3 or 95.0 (Molecular Simulations Inc., San Diego, CA, U.S.A.), followed by baseplane correction of 2D data sets, when necessary, using Facelift version 1.2 (Chylla and Markley, 1993).

Variable temperature (32–82 °C) studies of brazzein were performed at 600 MHz on a pH 5.2 sample in D_2O. The NMR spectra (Figure 1) changed very little over this temperature range. The only significant alteration was due to Tyr11, whose aromatic ring rotates slowly on the NMR time scale at low temperatures (lifetime $\tau > 10$ ms), becomes more mobile above 32 °C, and rotates rapidly above 52 °C ($\tau < 2$ ms). The absence of other spectral changes with temperature indicates that the overall structure of brazzein remains essentially unchanged up to at least 82 °C.

The assignment of ^{1}H spin systems was based on comparisons of TOCSY spectra with NOESY and double-quantum filtered COSY spectra (Wüthrich, 1986). These spin systems were given sequential assignments on the basis of H_i to H_{i+1} resonances observed in NOESY spectra collected with a 100 ms mixing time. ^{13}C chemical shift assignments were obtained from a gradient-enhanced HSQC spectrum (Bodenhausen and Ruben, 1980; Bax and Pochapsky, 1992) collected at 37 °C on a Bruker DMX750 spectrometer from a sample of 4 mM brazzein, pH 5.2, at natural isotopic abundance.

Stereospecific resonance assignments were determined for the H^β methylene protons (Hyberts et al., 1987) of 15 residues and for the H^γ methyl groups (Zuiderweg et al., 1985) of Val7 using established criteria. $^3J_{\alpha\beta}$ couplings were measured from an E. COSY spectrum (Griesinger et al., 1987) collected from a 2 mM sample of brazzein in D_2O at 37 °C on a Bruker DMX750 with 8K points in T_2. These were correlated with intraresidual H^N cross peaks from a ROESY (Griesinger and Ernst, 1987) spectrum collected at 22 °C with a 45 ms mixing time using a 3-9-19 water suppression sequence (Sklenar et al., 1993).

Extent of assignments and data deposition

Complete ^{1}H resonance assignments were obtained at all conditions for all nonexchangeable protons and for nearly all backbone and side-chain amide and amine protons. Resonance assignments for the H^δ and H^ϵ ring protons of Tyr11 were determined only at 22 °C, because rotation of the aromatic ring renders those resonances too broad to be observed between 32 and 52 °C, as discussed above (Figure 1).

^{13}C resonance assignments were determined for 91% of the C^α carbons and 89% of the C^β carbons. Resonance assignments determined for the side-chain carbons of several residues are less complete owing to overlapping resonance frequencies in the ^{1}H dimension of the ^{1}H-^{13}C HSQC.

The ^{1}H and ^{13}C chemical shifts for brazzein under each of the conditions mentioned above have been deposited in the BioMagResBank under BMRB accession number 4067.

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