

# Crystallization and Preliminary X-Ray Analysis of the Highly Thermostable Sweet Protein Mabinlin II

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**Abstract:** Mabinlin II is a thermostable sweet protein isolated from the mature seeds of *Capparis masaikai* Levl. grown in the subtropical region of the South of China. The Mabinlin II has been crystallized and diffraction data were collected to 1.7 Å resolution. The crystal belongs to space group C2 with unit cell parameters a = 80.11 Å, b = 51.08 Å, c = 47.34 Å,  $\beta = 122.77^\circ$ .

**Keywords:** Sweet protein, Mabinlin II, Heat-stable protein, *Capparis masaikai* Levl., crystallization.

## INTRODUCTION

Sweet proteins, as low-calorie sweeteners, are good potential replacement for artificial sweeteners [1]. As it is well known that low-caloric sweeteners are widely needed by persons affected by disease related to the consumption of sugar, such as obesity, diabetes, hyperlipemia and caries. In recent years the prevalence of these diseases has increased dramatically. For example, the patients of diabetes mellitus have been 50 millions populations by 2004 and the population will continue to increase by 1.5 – 2 millions per year in China [2]. A series of artificial sweeteners like Saccharin, Aspartame and Cyclamate are used world-wide as low calorie sweeteners for these patients. So far most popular sweeteners are small molecular weight compounds, which possess some site effects such as psychological problems, mental disorders, bladder cancer and heart failure [3-7]. We now know that there are also sweet macromolecules, i.e., sweet proteins, which are acting as natural, low calorie sweeteners without triggering a demand for insulin in these patients whereas sucrose does. Therefore, there are good perspective to use sweet proteins in the low calorie sweetener industry like the food, cola, snacks and chocolate industries. For the effective applications it is need in-depth understanding of the structure-function relationship of sweet proteins.

Until now, six sweet proteins, including mabinlin, have been identified, namely thaumatin [8], monellin [9], brazzein [10], curculin [11], pentadin [12] and mabinlin [13]. Among them, two crystal structures, thaumatin [14] and monellin [15], and one NMR structure of brazzein [16], have been determined. Interestingly, they exhibit no significant similarities in amino acid sequences and structures. Recently the human receptors for sweet test T1R2/T1R3 have been discovered [17]. Though a hypothesis about interaction of sweet protein with T1R2-T1R3 receptor was proposed [18], the

structural basis and molecular mechanism of the sweet taste of sweet proteins remain unknown. Here we report the crystallization and preliminary X-ray analysis of a sweet protein Mabinlin II with the highest known thermostability, which will lead to a novel 3D-structure of sweet proteins to be determined.

Mabinlin II (Mab II) is isolated from mabinlang, the plant of *Capparis masaikai* Levl. Growing in the subtropical region of the Yunnan province of China and bearing fruits of tennis-ball size [13]. The mature seed of mabinlang elicits a sweet test and used as a traditional medicine in China. Native commonly chew the seeds for their sweetness. Hu Zhong and his coworkers derived four sweet proteins, named Mabinlin I, II, III, IV, from these seeds [13, 19-21]. Among others, Mab II possesses most interesting properties. Its sweetness was estimated to be around 400 times that of sucrose on weight basis [13]. The sweetness of Mab II is unchanged after 48 hour incubation at boiling point [21]. It is now known that Mab II consists of an A chain with 33 amino-acid residues and a B chain with 72 amino-acid residues, with a total molecular weight of 12.4 KDa [22]. The B chain contains two intrachain disulfide bonds and is connected with A chain through two interchain disulfide bridges [23]. The full sequence of Mab II showed that there is no obvious homologue with that of all sweet proteins reported so far (Fig. 1). Evidently, Mab II is distinct with those known sweet proteins. Therefore it is very interesting to elucidate the 3D structure of Mab II for in-depth investigation of its structure-function relationship. As a first step here we report the crystallization and preliminary X-ray analysis of Mab II.

## 1. PURIFICATION

The Mab II used in crystallization was purified from the mature seeds of *C. masaikai* plants grown in the south of Yunnan in China according to the method described previously [13]. The seed powder, defatted with petroleum ether (boiling point 303-333K), was extracted with 50% aqueous acetone at room temperature and then precipitated at pH 10.0. After the sediment had been dissolved at pH 6.0, the

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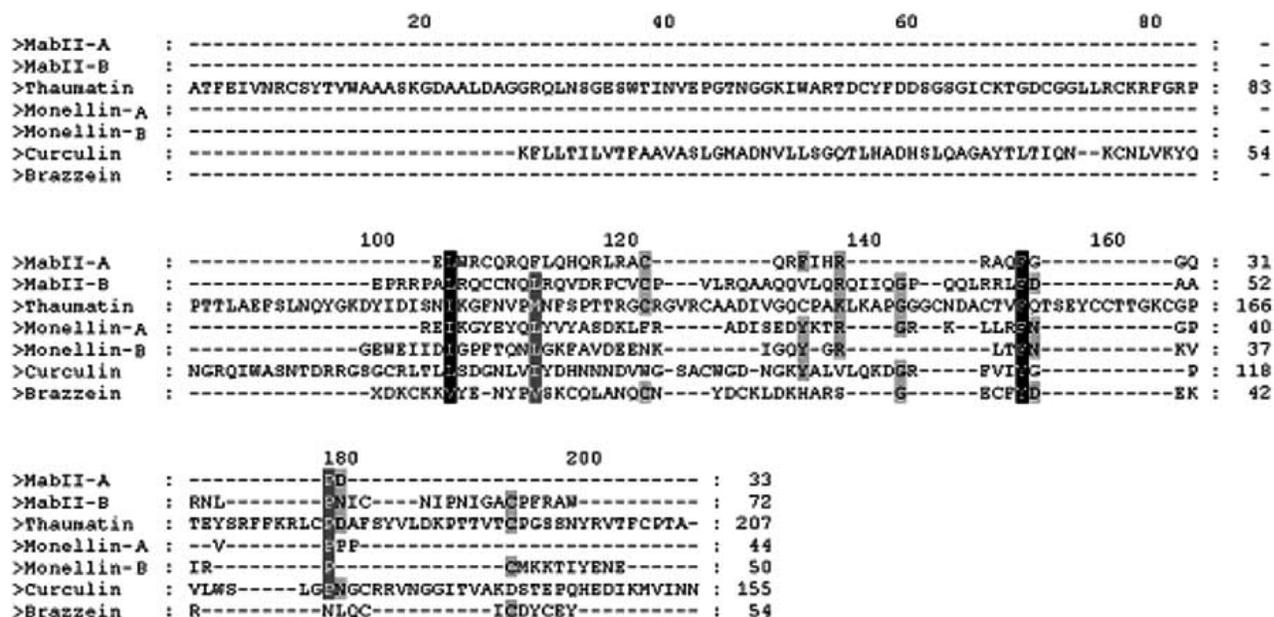


Figure 1. Sequences alignment of Mab II and other four sweet proteins with CLUSTAL X (1.8) multiple sequence alignment.

sample was applied to a carboxymethylcellulose CM-53 (Whatman Inc.) column eluted with a stepwise gradient of NaCl. Mab II eluted out at 0.45M NaCl. After desalting, the sample used in the crystallization was obtained. When analyzed on a C8-reverse-phase column (5 $\mu$ m, 4.6x250 mm, the solvents were 0.1% TFA: buffer A, and 80% acetonitrile containing 0.1% TFA: buffer B) on an AKTA Purifier system (Amersham Pharmacia Biotech), the protein showed a high degree of purity (Fig. 2).

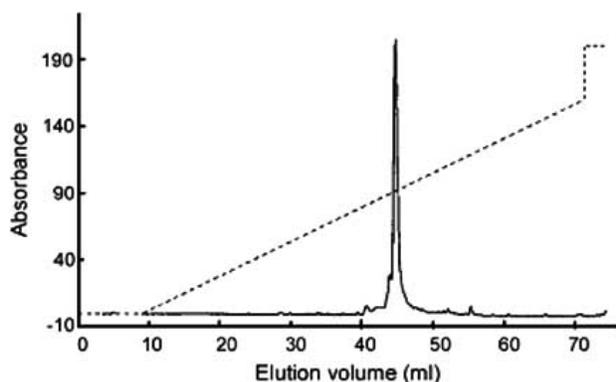


Figure 2. Chromatography of mablin II on an analytical reverse-phase C8 column (5 $\mu$ m, 4.6x250 mm) on an AKTA Purifier System.

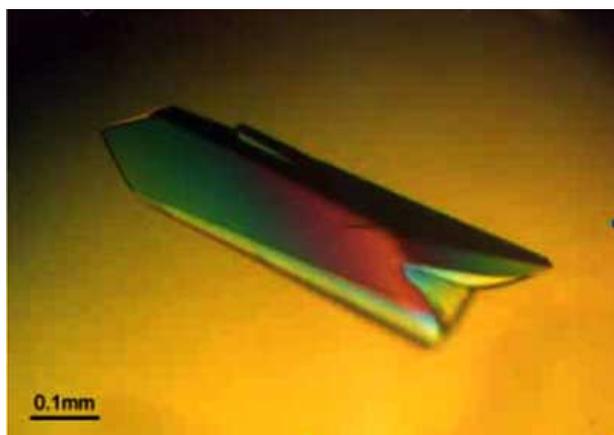
## 2. CRYSTALLIZATION

The crystallization of Mab II had been obstructed for some time in gaining the crystals qualified to the X-ray structural analysis. We have got its crystals once, but they were not suitable for X-ray diffraction data collection and could also not be repeatable. After some improvements of the sample treatment and more widely searching for novel crystallization conditions, the qualified crystals of Mab II were finally obtained for the structural analysis. In the present

crystallization, Mab II was dissolved in pure water at a concentration of 10 mg/ml for crystallization. Initial screening was performed using a sparse matrix method [24] with Hampton Research Crystal Screen<sup>TM</sup> and Index<sup>TM</sup> at 293 K. The crystallization experiments were performed by using hanging-drop vapour-diffusion method. Each drop contained equal amounts (1  $\mu$ l) of protein (10 mg/ml) and reservoir solutions equilibrated against 500  $\mu$ l of reservoir solution in the well. In the initial screen, small crystals as rod clusters appeared from a condition. Through optimization, large single crystals suitable for X-ray diffracting were obtained under the following conditions: drops formed by mixing equal volumes (2 $\mu$ l) of 10 mg/ml protein in water and reservoir solution at 310 K. The reservoir solution contained 0.1 M Sodium Acetate Trihydrate buffer (pH 4.6) and 1.4M Sodium malonate. The crystals of Mab II appeared in two weeks and grew to their full size within 2 months. They were inclined columnar in shape with dimensions of 0.15x0.15x0.6 mm<sup>3</sup> (Fig. 3). The growth of Mab II crystals was sensitive to temperature. Decreasing temperature would cause the appearance of rod clusters or phase separation.

## 3. DATA COLLECTION AND CRYSTALLOGRAPHIC ANALYSIS

X-ray diffraction data from Mab II crystals were collected on a Rigaku R-Axis IV<sup>++</sup> image plate using Cu K radiation ( $\lambda = 1.5418 \text{ \AA}$ ) from a rotating anode operating at 40 kV and 20 mA with 0.1 mm confocus incident beam diameter. Data were collected at 85K with a crystal-to-detector distance of 100 mm,  $\theta = 1^\circ$  and 300 s exposure time. A total of 180 frames were collected. The crystals were briefly soaked in paraffin oil (Hampton Research) used as cryoprotectant and then flash-cooled in a nitrogen-gas stream. Data sets were processed by *mosflm* 6.2.3 program and scaled using CCP4 program *SCALA* [25].



**Figure 3.** Crystals of Mab II. The length of the bar is equal to 0.1 mm.

**Table 1.** Data-Collection Statistics.

Values in parentheses are for the highest resolution shell (1.79-1.70 Å).

Wavelength (Å)	1.5418
Space group	C2
Unit-cell parameters (Å)	a=80.11, b=51.08, c=47.34, =122.77
Molecules per AU	2
Matthews coefficient (Å <sup>3</sup> Da <sup>-1</sup> )	1.6
Solvent content (%)	24.5
Resolution limit (Å)	20.35-1.70
Total reflections	62800(8865)
Unique reflections	17686(2555)
Average redundancy	3.6(3.5)
Average I/ (I)	6.0(2.6)
Completeness (%)	99.5(100)
Rsym (%)	5.3(26.6)

Statistics of data collection and processing are shown in Table 1. The crystal of Mab II belongs to space group C2. The unit cell parameters are a =80.11 Å, b =51.08 Å, c =47.34 Å, = 122.77°. Assuming that the Matthews coefficient,  $V_m$  [26], is 1.60 Å<sup>3</sup>Da<sup>-1</sup>, there are two Mab II molecules in the asymmetric unit with a solvent content of 24.50%. Crystals of Mab II can well diffract to X-rays and a data set

to a resolution of 1.7 Å under in-house X-ray source has been collected with good quality (Table 1). The perfect crystallographic property of Mab II found a sound basis for 3D structure analysis, in turn, the investigation of the structure-function relationship of the novel sweet protein. In fact, the structural determination with above data is in good progress now.

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