CHARACTERIZATION OF THE SWEET-TASTING PROTEIN FROM *DIOSCOREOPHYLLUM CUMMINSII* (Stapf) DIELS*

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1. Introduction

In an earlier publication [1] we showed that the intensely sweet-tasting substance present in the fruit of *Dioscoreophyllum cumminsii* (Stapf) Diels is a carbohydrate-free protein. Morris and Cagan independently confirmed our findings [2] and named the protein Monellin.

We now report on the further characterization of this material. A comparison with the only other known sweet-tasting protein 'thaumatin' (from the fruit of *Thaumatococcus daniellii* Benth [3, 4]) reveals a number of similarities: both are basic, lack histidine and free-SH groups, do not contain carbohydrate and have approximately the same sweetness intensity. Denaturation of the proteins reveals that their tertiary structures are essential for their sweet taste.

2. Materials and methods

The sweet protein of *D. cumminsii* was obtained according to [1]. Electrophoresis was performed on 15% polyacrylamide gel in 0.03 M potassium acetate buffer pH 5.0 [5] for 4 hr at 75 V, using Amido Black 10B as staining agent. Protein was estimated by the biuret method [6] with bovine serum albumin as standard. Determinations of SH-groups were done by Ellman's method [7]. Amino acid analysis was carried out on a Beckman Unichrom Analyzer. Ion-exchange chromatography was performed on a SE-Sephadex C-25 column with a linear salt gradient of 0–0.25 M NaCl in 0.02 M Tris-HCl buffer pH 7.65. The fractions were concentrated and desalted by ultra-filtration on a UM 2 Diaflo membrane (Amicon). Isoelectric focusing was carried out in a 110 ml focusing column (LKB 8108) [8] using a linear sucrose density gradient (50–0%) [9] containing 0.83% carrier ampholytes (Ampholine, LKB Stockholm) being isoelectric in the range pH 8–10. The molecular weight was determined according to Andrews [10] on a column of Sephadex G-50 medium eluted with 0.1 M NaCl using trypsin (M.W. 23,300), myoglobin (M.W. 17,200), pepsin (M.W. 34,500), lysozyme (M.W. 14,700), cytochrome *c* (M.W. 13,000) and glucagon (M.W. 3,500) as markers. The sweetness intensity was determined by a panel in ranking tests.

3. Procedure and results

For an exact determination of the isoelectric point (formerly estimated as 8.7 [1]) a preparative isoelectric fractionation was performed (at 4° and a constant load of 1 W). To this end 27.4 mg of the purified protein was dissolved in 20 ml of the less dense sucrose solution [9] and applied onto the column. After a constant current had been obtained (36 hr), the electrofocusing was continued for 48 hr. Then the column was drained and monitored at 280 nm (fig. 1). Fractions of 1.6 ml were collected and the pH of each of them was determined at 4° with an Electrofact 36200/N pH-meter. The isoelectric points are indicated in fig. 1. The fractions, pooled in accordance with the peaks in fig. 1 were submitted to gel filtration on Sephadex G-25 (column 25.6 × 2.5 cm) using 0.1 M NaCl as eluant to remove the ampholytes and sucrose followed by ultrafiltration. The fractions 40–46 from

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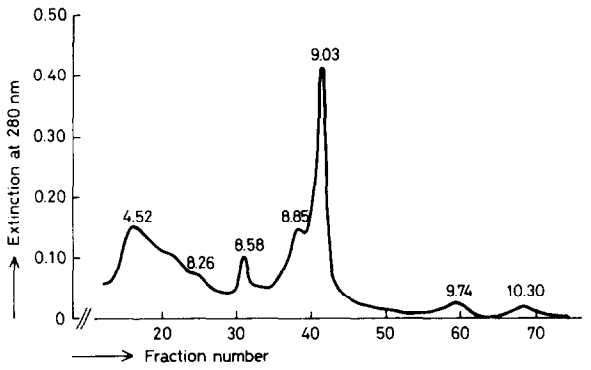

Fig. 1. Elution diagram after isoelectric focusing (pH 8–10) of the purified protein from *D. cumminsii*; flow rate 96 ml/hr, fraction volume 1.6 ml. The isoelectric points of the different fractions are indicated in the diagram.

the focusing column corresponding to an isoelectric point of 9.03 tasted intensely sweet. From 27.4 mg, applied to the column, 22.2 mg was recovered in these sweet tasting fractions. Fractions 35–39 gave a gave a very weak sweet-taste sensation while the others did not taste sweet at all. The molecular weight of the purified protein, as determined by gel filtration is 11,500 ± 1,000.

Ion-exchange chromatography of the purified protein (see [1]) on SE-Sephadex C-25 (fig. 2a) gave minimal extra purification (92% of the protein applied eluting in the sweet-tasting fractions 96–108), as was confirmed by the polyacrylamide gel electrophoresis pattern (fig. 2b).

The amino acid composition of the ion-exchange purified protein is compared with that of thaumatin in table 1. It is remarkable that neither protein contains histidine (see table 1) nor free SH-groups as determined by the method of Ellman.

The values given in table 2 for the amount of ammonia liberated on hydrolysis and for the carboxylic acid groups indicate that nearly 64% of these groups are in the amide form.

The sweetness intensity of the purified protein before and after ion-exchange chromatography was 2,000 (formerly estimated at 1,500, see [1]) respectively 2,500 times sucrose on a weight basis or 6.8×10^4 respectively 8.5×10^4 times on a molar basis.

The thermal lability of the sweet protein was found to be pH dependent: a 0.01% aqueous solution with a pH of 2.0 (with HCl) at 20° has no sweet taste while

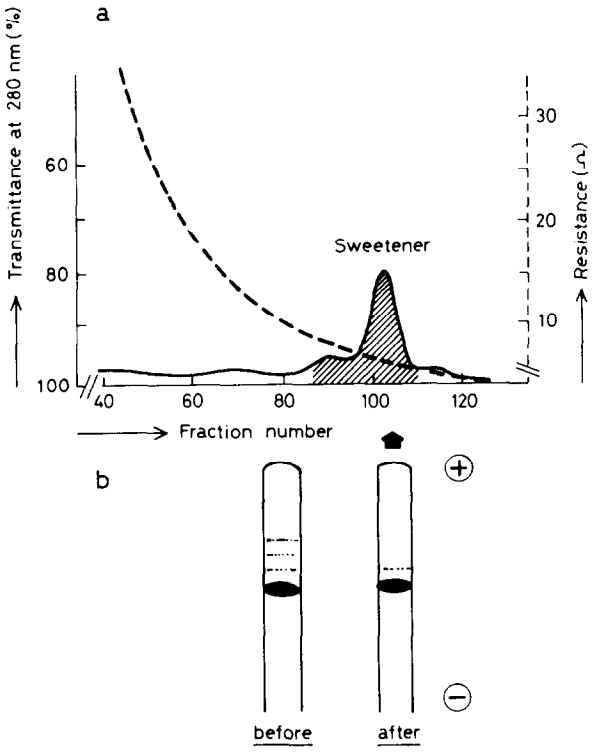

Fig. 2. a) Ion exchange chromatography of 26 mg of the purified protein from *D. cumminsii* in 2 ml 0.02 M Tris-HCl buffer pH 7.65 on SE-Sephadex C-25, using a linear salt gradient; bed 1.5 × 61 cm, flow rate 8.2 ml/hr, fraction vol 1.6 ml. b) Polyacrylamide gel electrophoresis of the purified protein before and after ion exchange chromatography.

solutions with a pH of 3.2, 5.0 and 7.2 lose their sweetness above 50, 65 and 55°, respectively. Thaumatin behaves almost identically [13] (see table 3). Complete loss of sweetness was observed on splitting the disulphide bridges in the molecules with 0.5 M cysteine or ethanethiol.

4. Discussion

The sweet tasting proteins from *D. cumminsii* and *T. daniellii* show many similarities (see table 3). Which characteristics are essential for the sweet taste remains to be determined. As the sweetness intensity on a molar basis is approximately the same for these proteins, we may tentatively conclude that these molecules contain similar sweet-tasting sites.

Korver et al. [13] have shown by means of circular

Table 1

Amino acid composition of the ion-exchange purified protein from *D. cumminsii* and thaumatin in g residues/100 g protein.

Amino acid	Protein from *D. cumminsii*	Thaumatin
Aspartic acid	10.6	10.9
Glutamic acid	14.4	6.3
Threonine	3.3	8.5
Serine	1.6	4.4
Proline	5.4	5.8
Glycine	4.3	6.1
Alanine	2.1	5.0
Valine	3.8	4.1
Methionine	1.6	0.8
Isoleucine	7.2	5.1
Leucine	6.2	3.9
Tyrosine	7.2	6.2
Phenylalanine	7.3	7.2
Lysine	11.5	6.6
Histidine	–	–
Arginine	10.4	9.4
Half-cystine*	1,3	6,6
Tryptophan**	1.9	3.1

* As cysteic acid after performic acid oxidation [11].
** Spectrophotometric estimation [12].

The values are extrapolated to hydrolysis time t = 0, derived from hydrolysis times of 24, 48, 72 and 96 hr. The hydrolysis took place in 6 N HCl at 110° in vacuum with norleucine as internal standard.

Table 2

Amount of basic and acidic groups found in ion exchange purified protein of *D. cumminsii.*

Group	mmoles/g protein
Free basic	1.29
Acidic	1.68
Acid amide (NH_3)	1.07
Free carboxylic	0.61
Excess free basic	0.68

Table 3

Some properties and characteristics of the purified proteins from *D. cumminsii* and *T. Daniellii.*

Criterium	Protein from *D. cumminsii*	Thaumatin
Isoelectric point	9.03	near 12
Carbohydrate content (%)*	⟨1	⟨1
Free –SH groups	0	0
Histidine	0	0
M.W.	11,500 ± 1000	20,400 ± 600**
Sweetness intensity (on molar basis)	8.4×10^4	1×10^5
Temperature (°) at which sweetness disappears***		
at pH 3.2	⟩ 50	⟩ 55
at pH 5.0	⟩ 65	⟩ 75
at pH 7.2	⟩ 55	⟩ 65

* Carbazol method using glucose as a standard.
** Ultracentrifugal data.
*** in a 0.01% aqueous solution.

dichroism that both proteins show similar conformational changes on heating and changing the pH. These changes coincide with a loss of sweetness of both proteins; this indicates that a similar structural entity is directly or indirectly responsible for the sweet taste. The loss of sweetness on splitting the disulphide bridges in these proteins indicates that the tertiary structure is an essential factor for the sweet taste.

The instability against heating and the loss of sweetness at low pH restricted the use of these proteins as a sweetener.

Acknowledgement

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