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Wheat grain softness protein (Gsp1) is a puroindoline-like protein that displays a specific post-translational maturation and does not interact with lipids

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ABSTRACT

Compared to puroindolines a and b, grain softness proteins (Gsp1) remained up to now the less characterized members of this wheat specific seed protein family. Due to their low expression levels, their purification remains relatively arduous. We report in the present work on the purification of wheat Gsp, Gsp1b and its post-translational maturation. We showed that Gsp1b underwent different proteolytic cleavages both in the N and C-terminal extremities of the preproprotein. Especially, after the putative signal peptide, a 29 mer peptide is highlighted that is highly truncated in puroindolines (8–9 mer). We also showed that Gsp1b and puroindolines display similar secondary structure but Gsp1b does not interact *in vitro* with lipids. In addition, by using the iterative threading assembly refinement (I-TASSER) methods, we showed that Gsp1b three-dimensional structure prediction is however identical to both those of puroindolines and of the dicotyledon 2S storage proteins. In contrast with puroindolines, all these data make us question about the role of Gsp1 proteins in endosperm texture and the previously suggested function in plant defense lent to these proteins.

1. Introduction

Puroindolines are cationic proteins, characterized by a tryptophan-rich domain and an α-helical fold stabilized by five disulfide bonds (Bhave and Morris, 2008; Douliez et al., 2000). Two major proteins have been characterized, puroindoline-a (Pin-a) and puroindoline-b (Pin-b). Actually, the relationship between puroindolines and grain hardness is well established and has generated an abundant research literature on these particular wheat proteins. Indeed, *Pin-a* and *Pin-b* genes are located on the hardness locus (on the short arm of chromosome 5D) and analysis of the protein polymorphism in hexaploid cultivars showed that the absence of either *Pin-a* or *Pin-b* or a mutation in the *Pin-b* leads to a hard phenotype (Bhave and Morris, 2008). However, when protein expression levels are taken into account, the relationship turns out to be not obvious (Igrejas et al., 2001). Consequently, the physicochemical mechanisms underlining the role of these proteins in the adhesion strength of the protein matrix to starch granule, remain an enigma. It has been however suggested that puroindoline–lipid interactions may interfere with such an adhesion. This hypothesis, mainly based on the lipid binding properties of puroindolines *in vitro* (Dubreil et al., 1997; Le Guerneve et al., 1998), was reinforced by the co-localization of the hardness QTL, the QTL of lipids extractable with non-polar solvents ("free lipids") (Morrison et al., 1989) and by the significant differences of lipid binding properties among Pin-b variants (Clifton et al., 2008, 2007). However, if such assertions are true, it remains extremely hard to conciliate them with the fact that most of the synthesized puroindolines are not associated with the starch granule surface, but embedded in the protein matrix of the dried endosperm (Dubreil et al., 1998). This fact is in full agreement with the puroindoline localization, since these proteins are exclusively localized in the protein bodies of developing endosperm (Lesage et al., 2011). In addition *ab initio* modeling studies have established that puroindolines are structurally related to the 2S albumin family of storage proteins of dicot seeds (Lesage et al., 2011). Analysis of near-isogenic lines differing by their hardness and the presence or absence of Pin-a leads to the conclusion that puroindolines and the hardness phenotype could also be related to the development kinetics of wheat endosperm. In particular, the programmed cell death occurs more rapidly in the endosperm of the hard line than in the endosperm of the soft one (Lesage et al., 2012). It is noteworthy that these discrepancies are also correlated with the aggregation level of storage proteins

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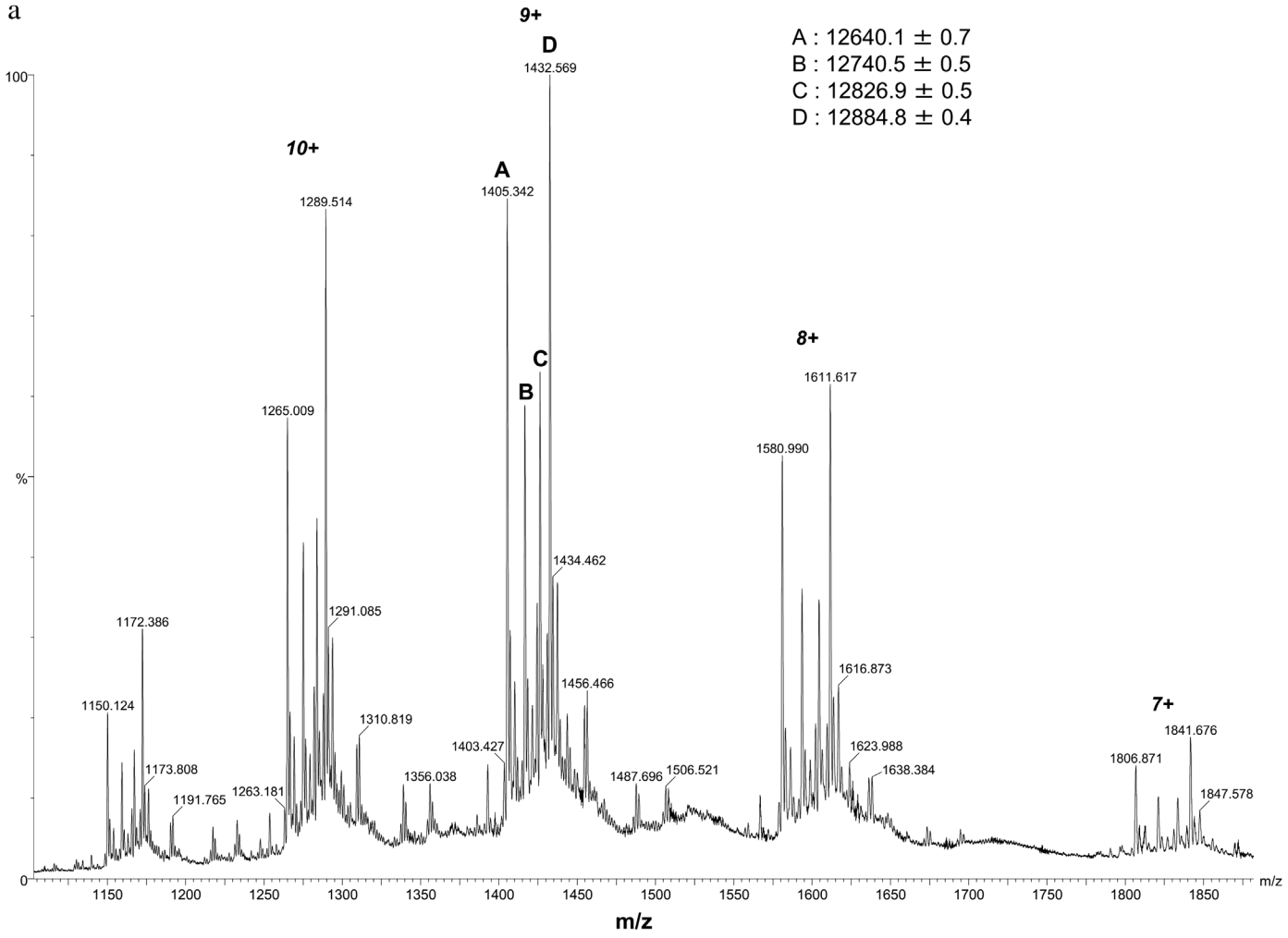
a



b

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Gsp1a  MKTFFLLAFLALVVSTAIAQYAEVPSPAAQRPTADGFGEWVAIAPSASGSENCEEEQPKVDSCSDYVMDRCVMKDMPLSWFF 82
Gsp1c  MKIFFLLAFLALVVNTAIAQYAEVPSPAARAPTADGSGEWVAIAPSASGSENCEEEQPKLDSCSDYVMDRCVTKDMPLSWVF 82
Gsp1b  MKTFFLLAFLALVVSTTIAQYAEVPSPAMQAPTADVFGEWVAIAPSASGFEDCEEEHPKLDSCSDYVMDRCVMKDMPLSWIF 82
       ** ***********.*:*********** : ****  ************ *:****:**:************ *******.*

Gsp1a  PRTWGKRSCEEVRNQCCKQLRQTTSRCRCKAIWTSIQGDLSGFKGLQQGLKARTVQTAKSLPTQCNIDPKFCNIPITSGYYL 164
Gsp1c  PQTWGKRSCEEVQNQCCQQLRQTTPRCRCKAIWTSIQGDLSGFKGLQQGLEAKMVQTAKSLPSKCNIDPKYCNIPITSGYYW 164
Gsp1b  PRTWGKRSCEEVRNQCCQQLRQTTPRCRCKAIWTSIQGDLSGFKGVQQGLKARTVQTAKSLPSKCNIDPKYCNIPITSGYYW 164
       *:**********:****:******.*******************:****:*: ********::******:**********
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12894.81 (12884.8) D
GFEDCEEEHPKLDSCSDYVMDRCVMKDMPLSWIFPRTWGKRSCEEVRNQCCQQLRQTTPRCRCKAIWTSIQGD
LSGFKGVQQGLKARTVQTAKSLPSKCNIDPKYCNIPITSG

12837.76 (12826.9) C
GFEDCEEEHPKLDSCSDYVMDRCVMKDMPLSWIFPRTWGKRSCEEVRNQCCQQLRQTTPRCRCKAIWTSIQGD
LSGFKGVQQGLKARTVQTAKSLPSKCNIDPKYCNIPITS

12750.68 (12740.5) B
GFEDCEEEHPKLDSCSDYVMDRCVMKDMPLSWIFPRTWGKRSCEEVRNQCCQQLRQTTPRCRCKAIWTSIQGD
LSGFKGVQQGLKARTVQTAKSLPSKCNIDPKYCNIPIT

12649.58 (12640.1) A
GFEDCEEEHPKLDSCSDYVMDRCVMKDMPLSWIFPRTWGKRSCEEVRNQCCQQLRQTTPRCRCKAIWTSIQGD
LSGFKGVQQGLKARTVQTAKSLPSKCNIDPKYCNIP

Fig. 1. a) ESI-Mass spectrum of the purified Gsp1 protein and calculated molecular mass of the different major isoforms A, B, C and D), b) The primary structures of the three major mature Gsp1 (GSP1b in bold) proteins with deduced cleavage sites of Gsp1b leading to the four observed isoforms (A, B, C and D) and c) MALDI-TOF/TOF of identified tryptic