Exploring the Zn-Binding Properties of the Storage Proteins in Rice

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INTRODUCTION

Recent evidence strongly indicates that Zn is primarily bound to proteins in cereal grains. Increasing the overall protein content is positively correlated with a higher Zn content, not only in the whole grain, but also in the endosperm (Cakmak *et al.* 2010). However, the Zn binding properties of endosperm proteins is poorly investigated. This is crucial information for plant breeders aiming to increase the transport and loading of Zn into the endosperm. It is also important in many other aspects, such as in improving the bioavailability of Zn to humans and animals, improving the baking quality of wheat and increasing the lysine content of feeds (Shewry and Halford 2002, Peck *et al.* 2008). Therefore, the Zn speciation in the storage proteins of the endosperm, which to date is virtually unknown, is emerging as a key factor with respect to biofortification of cereal grains with Zn.

METHODS

At the Faculty of Life Sciences in Copenhagen, we combine state of the art Zn, S, Fe and P detection by ICP-MS, as well as separation and identification of metal-binding compounds by HPLC and ESI-TOF-MS. Currently, we are investigating ferritine, phytic acid, metallothioneins, the water soluble albumins and globulins, as well as the alcohol soluble prolamins of the rice endosperm. Also, we recently introduced two novel techniques; capillary-reverse phase-LC-ICP-MS for the speciation of hydrophobic proteins, and tryptic digest-nanoLC-ESI-Iontrap-MS/MS for the identification of Zn-binding proteins.

RESULTS AND DISCUSSION

Using enzymatic extractions in the endosperm of rice, it was observed that incubation with phytase, the enzyme responsible for degradation of phytic acid, increased the extractability of Fe and P, but not that of Zn and S (Fig. 1.). Using protease XIV, which degrades proteins, approximately 70% of the total Zn was extractable. Hence it was shown that phytic acid binds a majority of Fe, whereas the majority of the Zn is protein bound; supporting the results in the articles cited above.

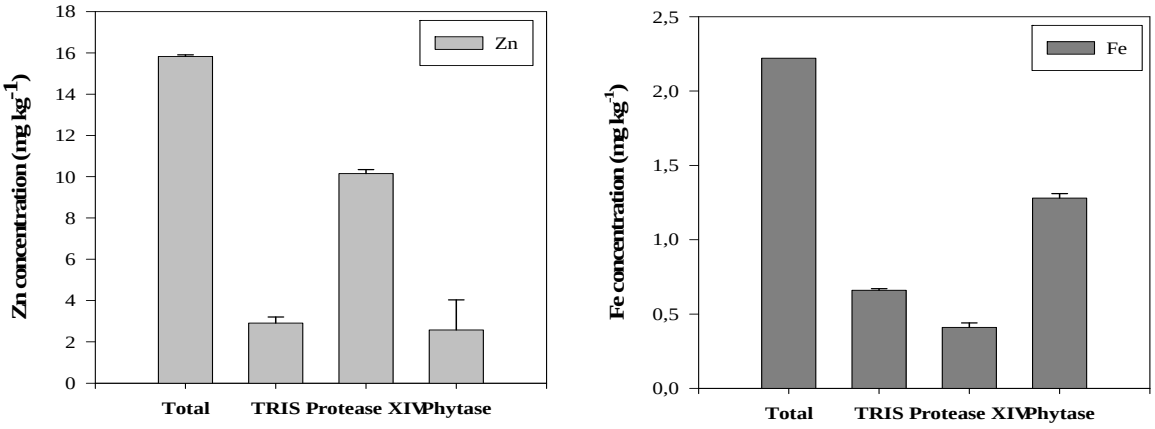

Fig. 1. Zn and Fe concentrations in rice endosperm and extraction from rice endosperm with 50 mM TRIS, Protease XIV and phytase, respectively. Bars represent SE (n=5).

The speciation of water-soluble albumin and globulin proteins shows that a majority of the extracted Fe is bound to phytic acid, whereas the extracted Zn elutes as low molecular complexes.

However, water or salt solutions can only extract approximately 15% of the total Zn, and <10% of the total proteins. The prolamin fraction, on the other hand, contains two Zn-binding proteins with apparent molecular sizes of 45 kDa and 29 kDa, both co-eluting with Cu and S (Fig. 2.). We are currently analyzing these fractions by capillary-reverse phase-LC-ICP-MS and by nano-LC-ESI-Iontrap-MS. These data will be presented at the conference.

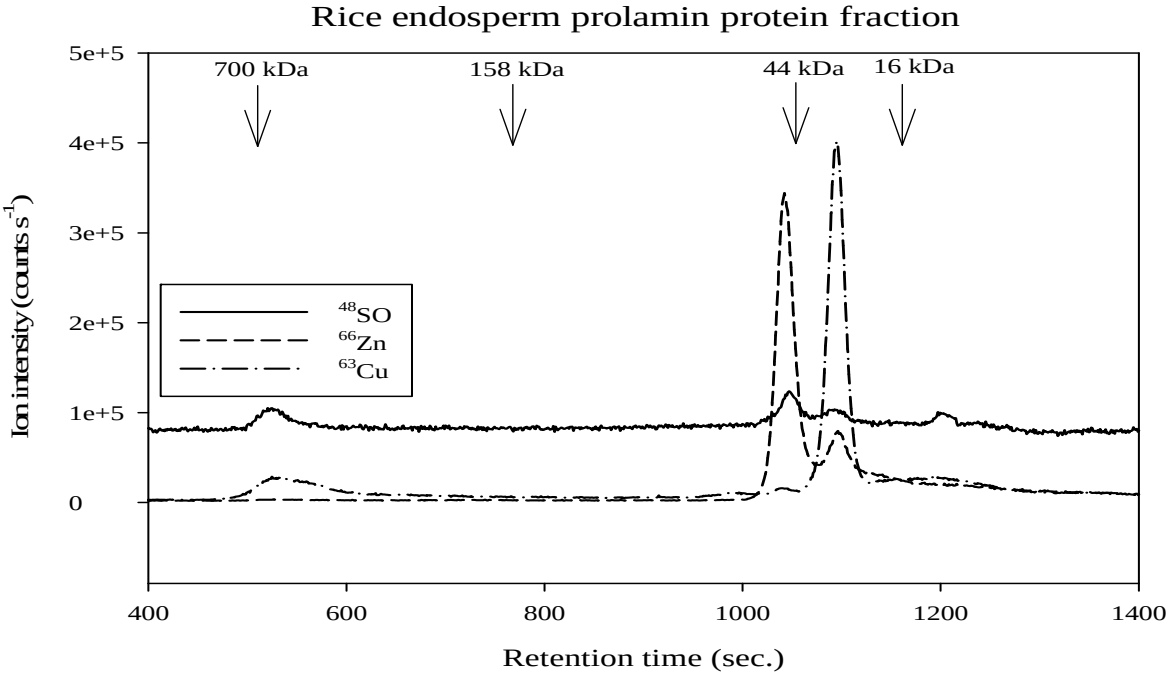

Fig. 2. SEC chromatography of prolamins in the rice endosperm. Two Zn binding fractions with the approximate sizes 45 and 29 kDa, co-elutes with Cu and S.

CONCLUSIONS

Zinc is mainly bound to proteins in the endosperm of rice. The speciation of Zn, *i.e.* the Zn binding properties of these proteins, is, however, virtually unknown. We present here the first data on Zn speciation in the albumin, globulin and prolamin protein fractions from the rice endosperm. These results will greatly improve breeding and biofortification work aiming at increasing the bioavailable Zn pools in any cereal-based foods.

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