Structural relationship between barley (*Hordeum vulgare*) trypsin inhibitor and castor-bean (*Ricinus communis*) storage protein

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A significant sequence homology was found between barley (*Hordeum vulgare*) trypsin inhibitor and castor-bean (*Ricinus communis*) seed glutamine-rich storage protein. This appears to suggest a divergent evolution of the two different classes of seed proteins and to support a view that plant proteinase inhibitors may also act as storage proteins.

Many plant seeds contain relatively large amounts of proteinase inhibitors, and their major biological role appears to be a defence mechanism of plants against various pests and parasites (Ryan, 1973). Some inhibit extracellular proteinases produced by phytopathogens, such as *Fusarium solani* (Mosolov *et al.*, 1976), possibly to prevent invasion and growth of the micro-organisms. On the other hand, many of these inhibitors inhibit digestive enzymes of animals and insects and thereby act as an anti-nutrient (Ryan, 1973). The most abundant proteins in seeds, however, are the storage proteins. They are formed during the seed development and internalized to protein bodies. On germination they are rapidly degraded to amino acids and transported to the growing parts of the seedling, thus serving as the most important nitrogen source (for reviews see Millerd, 1975; Ashton, 1976; Derbyshire *et al.*, 1976).

Recently we have determined the complete amino acid sequence of barley (*Hordeum vulgare*) trypsin inhibitor (Odani *et al.*, 1983), which was first isolated and characterized by Mikola & Suolinen (1969). Formerly, we reported the unexpected sequence homology of this protein with wheat α -amylase inhibitor at the *N*-terminal region (Odani *et al.*, 1982), and this was confirmed by the complete sequence determination. Campos & Richardson (1983) also found that a bifunctional trypsin/ α -amylase inhibitor from ragi (*Eleusine coracana*) is highly homologous with the barley and the wheat inhibitors. These results established a new family of enzyme inhibitors in cereal seed proteins.

Recently the complete amino acid sequence of

glutamine-rich storage protein of castor-bean (*Ricinus communis*) seeds has been reported by Sharief & Li (1982). We found that the barley trypsin inhibitor shows a significant sequence homology with this protein, which consists of two polypeptide chains, a large subunit of 6600 daltons and a small subunit of 4300 daltons, linked by disulphide bridges. As shown in Fig. 1, the small and the large subunits of the castor-bean storage protein can be aligned to residues 1–34 and 36–94 respectively of the barley trypsin inhibitor. Out of 95 residues 26 are common between the two proteins (27% identity), with five half-cystine residues being conserved. This homology is particularly strong in the *N*-terminal 74 residues of the barley inhibitor (35% identity). Significance of this homology was tested by the Moore–Goodman test (Moore & Goodman, 1977), which is based on a count of minimum mutation distances (Fitch & Margoliash, 1967) between two sequences to be compared. The minimum mutation distance value for this 73-alignment (residues 1–74, with two gaps) is 76, giving a ‘significance level’ (matching probability) of 0.1886×10^{-6} , a value that defines an alignment as ‘highly significant’ (Moore & Goodman, 1977; Vogel, 1978). This result strongly suggests a common ancestry for the two proteins, at least for the *N*-terminal 74 residues, and also suggests that the castor-bean storage protein has been synthesized as a single polypeptide precursor having the small subunit at the *N*-terminal portion. The trypsin-reactive (-binding) site of the barley inhibitor is Arg₍₃₃₎-Leu₍₃₄₎ (Odani *et al.*, 1983), which undergoes limited proteolysis by trypsin or other proteinases on interaction. It is noteworthy in the alignment of the two proteins that the polypeptide chain of the castor-bean storage protein appears to be separated

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Fig. 1. Comparison of the primary structures of barley trypsin inhibitor and castor-bean glutamine-rich storage protein. Barley, barley trypsin inhibitor (Odani *et al.*, 1983); Castor bean, castor-bean glutamine-rich storage protein (Sharief & Li, 1982). The identical residues are in boxes. The arrow indicates the trypsin-reactive site (Arg₍₃₃₎-Leu₍₃₄₎) of the barley trypsin inhibitor. The castor-bean protein consists of two polypeptide chains of 34 and 61 residues. A gap is included to the barley inhibitor sequence.

into two subunits at the site next to the locus that is equivalent to the reactive site of the barley trypsin inhibitor. This also suggests possible trypsin-inhibitory activity of the precursor (single-chain) castor-bean protein, because the critical arginine residue (Arg-33) at the trypsin-reactive site of the barley inhibitor is conserved in the castor-bean protein (Arg-33 of the small subunit) and some trypsin inhibitors possess Arg-Arg at the reactive site (Norioka & Ikenaka, 1983).

Sharief & Li (1982) pointed out partial sequence homology of their castor-bean storage protein with other plant proteins, i.e. the small subunit of the sweet protein thaumatin I (*Thaumatococcus daniellii*) (Iyenger *et al.*, 1979) and the large subunit of a Bowman-Birk-type proteinase inhibitor of lima bean (*Phaseolus vulgaris*) (Stevens *et al.*, 1974). The significance level of this comparison by the Moore-Goodman test (Moore & Goodman, 1977) is 0.1118×10^{-2} for the first alignment ('marginally significant'; Vogel, 1978) and 0.2713×10^{-6} for the second comparison ('highly significant'). However, these two proteins are entirely unrelated in biological activity, molecular mass and overall primary structure. Therefore the present homology with a single protein (i.e. barley trypsin inhibitor) appears to be more conceivable than that proposed by Sharief & Li (1982). Of course this does not exclude the possible evolutionary relationship of the short

segment of lima-bean trypsin inhibitor to parts of the barley and the castor-bean proteins.

Major storage proteins of plant seeds are large (7–11S) complex globulins consisting of multiple subunits (Derbyshire *et al.*, 1976). Others are relatively small molecules (less than 30000 daltons) containing exceptionally high proportions of glutamine residues (Gerristen, 1956; Houston & Mohammad, 1970; Platt & Kasarda, 1971; Lönnnerdal & Janson, 1972; Li *et al.*, 1977). The above-mentioned castor-bean protein is typical of the latter group of storage proteins. Although many plants utilize arginine as a main storage form of nitrogen, the amide residues can also serve as a nitrogen source, and these low-molecular-mass glutamine-rich proteins may be regarded as the reserve proteins for nitrogen (Lönnnerdal & Janson, 1972; Li *et al.*, 1977). The present finding of sequence homology between a trypsin inhibitor and a storage protein may not only suggest an interesting divergent evolution of two different classes of seed proteins but also support a view that proteinase inhibitors (and possibly inhibitors of other enzymes) also act as storage proteins (Ryan, 1973).

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