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Structural factors affecting salt-dependence of solubility of seed proteins

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Summary

Major storage proteins of many kinds of plant seeds are salt-soluble globulin. Globulins are classified into 7S and 11S globulins according to their sizes. Each globulin exhibits high amino acid sequence similarity among various plant species, but variable solubility. Prior to this study, we had compaired pH-dependence of solubility of 7S and 11S purified from various seeds, and found that 7S from French bean exhibits excellent solubility at low ionic strength where the other 7S and 11S are insoluble. French bean 7S is glycosylated at two positions in analogy with soybean 7S α and α'. One of the two positions is close in both 7S, but the other is completely different. In this study, we studied which determins the excellent solubity of French bean 7S, protein or carbohydrate moieties.

We can prepare recombinant French bean 7S having no carbohydrate moiety (recombinant 7S) using *E. coli* expression system of its cDNA. So we cloned its cDNA by RT-PCR method based on reported nucleotide seguence, and attempted to construct *E. coli* expression system using direct expression vector pET-21d. However, we could not get expressed proteins from this system even though we have many experiences in constructing expression system using pET-21d. Then, we attempted to construct an expression system using fusion expression vector pGEX-6P-1 as a fusion protein with glutathione S-transferase, and succeeded in getting soluble expressed proteins at a high level. The recombinant 7S cleaved from a fusion protein formed a correct conformation. Solubility of the recombinant 7S was compaired with that of the native 7S from French bean. At ionic strength 0.5, both 7Ss were completely soluble at any pHs examined. At ionic strength 0.08, the recombinant 7S was insoluble at pH 5-7 although the native 7S was completely soluble. Surface hydrophobicity of the recombinant 7S was much higher than that of the native 7S. These strongly suggest that the carbohydrate moiety cover regions with high hydrophobicity on the molecular surface, resulting in high solubility. In fact, the three dimensional structures demonstrate that regions with high hydrophobicity is located near the N-glycosylation site in French bean 7S but not in soybean 7S.