

## Precise investigation of salt effect on solubility of seed storage proteins

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## Summary

Major storage proteins of many seeds are 7S and 11S globulins. Although each globulin shares similarity of amino acid sequence among plant species, their solubilities are not uniform. Solubility of seed proteins is one of the fundamental properties required for food usage. Therefore, it is desired to elucidate precisely the effects of salt on solubilities of 7S and 11S globulins. Then, we investigated solubility-pH dependence of mungbean 7S globulin and rapé and soybean 11S globulins at ionic strengths 0.5 and 0.08 using *Escherichia coli* expression system.

Solubility of recombinant wild-type mungbean 7S globulin 8SA subunit was compared with that of soybean 7S globulin  $\beta$  subunit. Although 8SA has structural similarity with  $\beta$ , their solubilities were quite different to each other at ionic strength 0.08. Thus, 8SA was soluble at pH6-8, but  $\beta$  was insoluble. We demonstrated that this difference is able to be explained by the distribution profile of charged residues on their molecular surfaces.

Generally, 11S globulins have five variable regions. The variable region IV is rich in negatively charged residues. The length of the variable region IV is different among subunits, and those of A1aB1b and A3B4 are composed of 42 and 70 residues. On the other hand, among  $\alpha$ ,  $\alpha'$  and  $\beta$  subunits of soybean 7S globulin,  $\alpha$  and  $\alpha'$  have an extension region at their N terminus, which is rich in negatively charged residues. The extension region of  $\alpha'$  is composed of 142 residues. Then, we prepared modified rapé 11S globulin BNC1 subunit and soybean 11S globulin A1aB1b subunit. The modifications were insertion, addition or replacement of the variable region IV of A1aB1b and A3B4 or  $\alpha'$  extension region into or with the variable region II or III or the C terminus. The solubilities of the modified 11S globulins were compared with those of the wild-type. We obtained the following results: ① 11S globulins of mungbean and soybean exhibited different and similar profiles at ionic strengths 0.5 and 0.08, respectively; ② solubilities of modified versions at ionic strength 0.5 were similar to those of the wild-type; ③ solubilities of modified versions at ionic strength 0.08 were affected by the length and the position of the introduced hydrophobic peptides.

As a conclusion, the effect of salt on solubilities of seed storage proteins is affected by distribution profile of charged residues on molecular surfaces as well as amino acid compositions. Therefore, determination of the three dimensional structure of each protein is desired.