STUDIES ON SUCROSE SYNTHETASE FROM RICE

Sucrose synthetase (SS) plays an important role in the carbohydrate metabolism of plants. The enzyme was purified to apparent (SS-5) or absolute homogeneity (SS-6). The molecular weight of the native enzyme was 400,000, and that of the subunit was 90,000. Arginine was determined as the sole NH_2-terminus, indicating that the native molecule might be composed of four identical subunits. Other biochemical properties, e. g. isoelectric point (5.2), extinction coefficient ($E^{1\%}_{1cm}$=9.2, 280 nm), and amino acid composition, had been determined. All the SS from rice, maize, and mung bean showed similiar amino acid compositions. The conventional antiserum against rice SS was raised by immunizing rabbits with SS-5. The purified immunoglobulin was used in establishing a method of enzyme linked immunosorbent assay (ELISA). Results from double diffusion test showed that maize SS cross-reacted with the antiserum raised against rice SS, but the spur on the precipitin pattern suggested that these two enzymes also contained different antigenic determinants (epitopes). Partial proteolysis of SS from rice and maize showed similar peptide maps. These results indicated that SS from rice and maize had structural homology.

To study the structural differences in SS, the monoclonal antibodies (mAb) against rice SS were prepared. Spleen cells from SS-5 immunized mouse were fused with NS-1 myeloma cells using PEG 1500 as the fusing agent. After screening and subcloning, six clones were obtained. Four clones (A12, D8, D9 and C3) produced mAb against the rice SS. Other clones (A1 and A10) were identified to secret mAb against a minor protein contaminant (P56) of the antigen used. When ELISA was used in detecting mAb, the *p*H of the coating solution

influenced the degree of color development for the enzyme test. This might be due to the influence of *p*H on the amount of antigen bound on the solid phase, or more possibly, the modification of antigenicity under a high *p*H condition (in this case *p*H 9.7). When the relative affinities of these mAb against SS were measured by ELISA, A12 reacted with rice SS only, and showed no affinity at all toward maize SS. Other three clones reacted with both rice and maize enzymes, and D8 showed stronger affinity toward rice SS. To our surprise, D9 and C3 reacted more strongly with maize SS than rice. It seemes that "clonal selection theory" can explain why a mAb induced by rice SS will be endowed with a stronger affinity toward maize SS. When the peptide maps of rice and maize SS were probed with these mAb, different patterns were obtained. Since the immunostaining revealed the peptides containing the antigenic determinant specific to the mAb in use, it was possible to establish the identity of mAb producing cell clones by comparing the immunostaining patterns of a protein partial hydrolysate probed with mAb produced by the cell clones in question.

The unknown protein P56 identified by mAb A1 and A10 was a trace contaminant in SS-5. With so strong an antigenicity to the mouse, it induced considerable number of mAb producing B cells and thus the hybridoma cells derived from them could be selected with good yield by using SS-5 as the solid phase antigen. Both rice and maize contained P56. They had similar quaternary structures and similiar peptide maps. Although P56 and SS showed very similiar amino acid compositions, immunological tests proved that they were unrelated.