中 文 摘 要

蔗糖合成酶 (SS) 是植物澱粉在合成代謝中的關鍵酵素。水稻 SS 可以生化方法純化得到近乎均質 (SS-5) 或完全均質 (SS-6)。經測定其生化性質，得知其原態分子量爲 400,000，次體分子量爲 90,000，NH_2-端只得一種胺基酸 (Arg)，故可能爲相同次體構成的四元體。另測得等電點爲 5.2，分子消光係數 ($E^{1\%}_{1cm}$, 280 *nm*) 爲 9.2。其胺基酸百分組成與玉米或綠豆之 SS 很相似。以水稻 SS-5 對兎子免疫，很容易產生抗體，抗血清經純化得免疫球蛋白後，可供進行酵素免疫分析 (ELISA)。由雙向擴散圖譜看來，玉米 SS 與水稻 SS 具有交叉抗原性，但亦有不同的抗原決定基 (epitopes)。若以蛋白酶部分水解做成胜肽圖譜，二者比較極爲相似。這些結果顯示，水稻與玉米的SS在生化構造與免疫性質上有相似性 (homology)，但亦有其不同之處。

以 SS-5 免疫 BALB/c 小鼠，取其脾臟細胞與 NS-1 骨髓癌細胞進行融合，經篩選後得到六株融合細胞，其中四株 (A12, D8, D9, C3) 爲抗 SS 之單株抗體，其餘兩株 (A1, A10) 則對抗一未知蛋白質 (P56)。以 ELISA 進行單株抗體的檢定，把抗原 SS 吸附到固相時，其吸附溶液的 *p*H 會影响最後呈色之强弱。可能吸附時的 *p*H 影响抗原吸附量的多寡；或者更可能地，影响吸附到固相上抗原的抗原性。另以ELISA測定各單株抗體的相對親和度，得知 A12 只能與水稻 SS 結合，對玉米 SS 幾無親和性；其餘三株則對兩種 SS 均有親和性，但 D8 對水稻 SS 結合較强，而 D9 和 C3 反而對玉米 SS 結合較强。以 Clonal selection 理論可以解釋爲何由水稻 SS 誘導出來的單株抗體反而對玉米 SS 有較强親和性。再以各單株抗體分別對水稻與玉米的胜肽圖譜進行免疫染色，結果得到不同的呈色圖譜，可以檢示二種 SS 中具有專一性抗原決定基的胜肽片段。此法同時可利用來判定兩單株抗體是否源自不同的細胞株。

A1 與 A10 所能結合的 P56 蛋白質，爲 SS-5 中之微量雜夾物，但由於其抗原性極强，故可在融合細胞進行 ELISA 篩選時被篩選出來。水稻與玉米都含有 P56，且二者均有相同的四級結構，其胜肽圖譜亦相似。雖然 P56 與 SS的胺基酸百分組成有相似之處，但二者沒有免疫學上的關係。P56 的生理功能仍有待探討。

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.