6 −25 Mungbean Leghemoglobin. II. Amino Acid Sequence of Mungbean Leghemoglobin

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The amino acid sequences of leghemoglobin from several legumes such as soybean, lupin, kidney bean, pea, broad bean, alfalfa, and sesbania rostrata have been determined. But nothing had been done yet in the case of mungbean (Vigna radiata L.) which is also an important leguminous crop particularly in Asia. We therefore attempted to establish the N-terminal amino acid sequences of major leghemoglobin components from this legume using cv. Pagasa 3. To attain our objective, 50 g of six-week old mungbean root nodules collected from the Philippines and 25-ml of 20 mM Tris-HCl buffer (pH 8.8) containing 10 uM p-APMSF protease inhibitor were homogenized on a chilled mortar. The resulting suspension was centrifuged at 15,000 rpm and 4°C for twenty minutes to recover the supernatant.

Total protein content of the supernatant was analyzed by following the procedures described in the Bio-Rad Protein Assay Kit. For initial purification of leghemoglobin, the supernatant was loaded into Sephadex G-75 column (50 cm x 2.5 cm) equilibrated previously with 20 mM ammonium carbonate buffer (pH 8.9). Fractions containing leghemoglobin were pooled, freeze-dried, dissolved in 20 mM ammonium carbonate, and then reloaded into Sephadex G-75 column using the same buffer for elution. Final purification of the different leghemoglobin components was done by ion exchange chromatography on a Whatman DE52-cellulose column (10 cm x 0.5 cm) using the acetate buffer (pH 5.2, 0.01 M; pH 5.2, 0.1 M) linear gradient elution procedure. The purified major components were subjected to isoelectric focusing (IEF). Immediately after IEF, the tube gel was subjected to SDS-PAGE directly. Protein on the slab gel was blotted onto Immobilon-P PVDF transfer membrane. The membrane was stained with Coomassie brilliant blue, destained with methanol, and rinsed with distilled water. Spots of major leghemoglobin components were cut from the membrane and used for the determination of N-terminal amino acid sequences with a gas-phase sequencer (Applied Biosystems, USA).

Results of analyses revealed that one of the major leghemoglobin components from the root nodules of mungbean has valine as the N-terminal amino acid residue which is similar to that of soybean leghemoglobin a. Furthermore, the amino acid sequences of major leghemoglobin components from mungbean seem to exhibit greater homology with the leghemoglobin components from soybean rather than those from the other legumes.

6 −26 ダイズ根より分泌するレクチン様物質の特性

異なるダイズ根より分泌するレクチン様物質のヒト赤血球と根粒菌に対する凝集活性

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ダイズ品種の根粒菌に対する親和性を規制している生理的機構を検討するため、遺伝的特性の異なるダイズ品種（Rj品種など）を無菌的に水耕栽培し、培養液中に根から分泌されたレクチン様物質を抽出し、ヒト赤血球及びRj品種との親和性の異なる根粒菌に対する凝集活性を調べた。

ダイズ品種 Hill(Rj$_4$), IAC-2(Rj$_2$Rj$_3$), アキシロメ(non-Rj), Peking（？）の種子を殺菌し、滅菌した水耕栽培容器に播種、栽培（N-free Fahraeus medium, 25℃）した。その後、培養液を回収し、濃縮した後、ゲルろ過し、それにより得られた分子量約5000以上の画分をレクチン様物質の粗抽出液とした。この粗抽出液を Micro assay plate 上でヒト赤血球(A, AB, B, O 型)、または Rj 品種に対する親和性の異なるBradyrhizobium japonicum(Is-1, Is-21, Is-80) とRhizobium fredii (USDA-191)の菌体と混和し、凝集反応の有無を調べた。

その結果、各々の粗抽出液の赤血球凝集活性は、アキシロメではA, AB, B型、IAC-2 とPekingでは全血液型、Hillでは A型で認められた。赤血球凝集活性に対する単糖（Glc, Gal, Man, Methyl-α-D-glucoside）の阻害能を調べた結果、アキシロメ、IAC-2 、Hillの粗抽出液では、Methyl- α-D-glucoside で阻害された。根粒菌に対する凝集活性はアキシロメ, Hillでは供試４菌株の菌体で認められ、IAC-2 では、それと親和性の高いIs-21 のみが反応した。

これらの結果から、Rj遺伝子保有品種に対する根粒菌の親和性の差異と、抽出したレクチン様物質による凝集反応の有無との関係は明確にはできなかった。