THE PRIMARY STRUCTURE OF KIDNEY BEAN LEGHEMOGLOBIN

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1. Introduction

The determination of the primary structure of soybean (*Glycine max.*) leghemoglobin component *a* [1] has already shown that plant leghemoglobin is distantly related to animal hemoglobins and myoglobins. The sequences of other leghemoglobins are required before further comparisons between the globins of animal and of plant origin can be made. In this study preliminary results of kidney bean (*Phaseolus vulgaris*) leghemoglobin (PhLb) sequence are reported. The sequence contains 145 amino acids, corresponding to a molecular weight of 16 100 for the hemoprotein. A comparison of PhLb*a* with soybean Lb*a* shows a 24% difference in the primary structure.

2. Materials and methods

Leghemoglobin from kidney bean (*Phaseolus vulgaris* var. Kaiser Wilhelm) was purified by ammonium sulphate fractionation and ion exchange chromatography. Purified PhLb*a* was digested with trypsin and thermolysin, and the peptides obtained were separated by ion exchange chromatography, gel filtration, paper electrophoresis and paper chromatography. Peptide sequences were determined by the dansyl–Edman procedure. The dansyl–Edman method and hydrazinolysis were used for the N- and C-terminal analyses of the apoprotein. Details of all these procedures will be published later.

3. Results and discussion

Sixteen tryptic peptides and twelve lysine or arginine containing thermolytic peptides were obtained from PhLb*a*. It was shown that the N-terminal sequence of the protein is NH_2–Gly–Ala–Phe–Thr–Glu–, and that the C-terminal residue is alanine. The preliminary sequence presented in fig. 1 is compatible with the amino acid composition of the whole protein.

The unexpected instability of the Phe–Gly bond (66–67) during digestion with trypsin remains to be clarified. PhLb*a* appears also to split in two parts during SDS-electrophoresis, although it primarily is a single polypeptide chain, according to the N- and C-terminal analyses.

PhLb*a* lacks methionine and cysteine like soybean Lb*a*. The two histidine residues are 61 and 92. In contrast to soybean Lb*a* there is one Lys–Lys bond, which, unusual for globins, is situated at the carboxyl end.

Thirty-five residues of the total 145 (about 24%) in the PhLb*a* sequence differ from those in the soybean Lb*a* sequence. Most of the differences are point mutations. Regions corresponding to globin B- and C-helixes seem to be conserved best. Near the heme-binding histidines the mutations have been mainly conservative. However, the residue 64 in PhLb*a* is serine, whereas most globins, e.g. soybean Lb*a*, have a lysine residue in the analogous position. PhLb*a* here resembles some myoglobins in which the corresponding residue is threonine. Mutations Arg–Thr and Trp–Leu (residues 127 and 129 respectively) have a very low rate of acceptance by natural selection [2].

The residues Trp-15 and Tyr-145, which are not present in soybean leghemoglobin, are invariable for animal globins. This is a new evidence for the common origin of animal and plant globins.

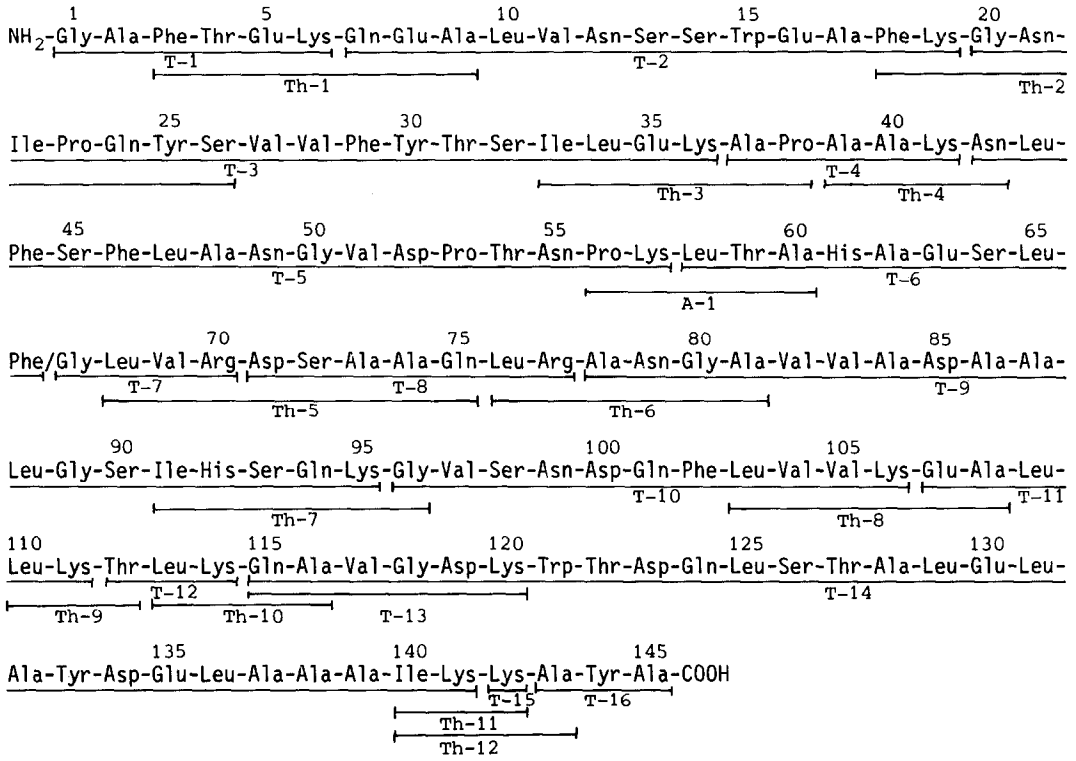

Fig. 1. The amino acid sequence of kidney bean leghemoglobin component *a*. Tryptic peptides (T), thermolytic peptides (Th) and one dilute acid hydrolysis peptide (A) are indicated by horizontal lines.

References

[1] Ellfolk, N. and Sievers, G. (1971) Acta Chem. Scand. 25, 3532–3534.

[2] Dayhoff, M. O., Eck, R. V. and Park, C. M. (1972) in: Atlas of Protein Sequence and Structure (Dayhoff, M. O., ed.), Vol. 5, pp. 89–99, National Biomedical Research Foundation, Washington.