

THE ALKALINE SUBSTANCES OF THE PROTEIN DERIVED FROM
LEGUME SEEDS

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When an aqueous extract of legume seeds is acidified, a precipitate of proteins is formed; when a solution of caustic soda is added to the filtrate, precipitates are obtained at various pH values. The paper chromatography of the com-

Mean Value of the pH for the Formation of Precipitates (Cotyledons without Embryos)

Species	Fraction No. and pH value						
	Phytin	1	2	3	4	5	6
<i>Phaseolus aureus</i> Roxb.	7.5-7.6	9.3	9.5-9.7	10.3-10.5	—	11.7	12.5
<i>Glicine hispida</i> Max.	7.6	8.5-9.2	9.5-9.8	10.5	—	11.9	—
<i>Vigna sinensis</i>	—	9.0	9.7-9.8	—	11.2	11.8	12.4
<i>Cicer arietinum</i> L.	7.8	—	—	10.5	11.1	—	12.1

plete hydrolysates of these precipitates has shown the presence in them of a whole set of amino acids, particularly alkaline ones. Similar substances with these properties and composition have been isolated from pea and soya shoots [1, 2]. In some types of beans, only the presence of nitrogenous substances which are neither albumins nor globulins has been reported [3]. Individual workers [4, 5] have isolated alkaline proteins at pH 9.0-12.5 from the previously separated nucleoproteins. Because the alkaline protein substances that we have isolated are present in legumes in only small amounts, we worked with large samples of flour (about 120 g).

Experimental

The investigation was carried out with seeds from the Central Asiatic Experimental Station of VIR [All-Union Scientific-Research Institute of the Plant Industry]: *Phaseolus aureus* Roxb. (mung bean), variety Pobeda 104; *Glicine hispida* Max (soya), variety Kubanskaya 276; *Cicer arietinum* L. (hick pea), variety Azerbaidzhanskaya 583; and *Vigna sinensis* (vigna or cowpea), variety Gibrinaya 7. The seeds were freed from skin and germ, finely ground, and extracted with ether, and then converted into flour and exhaustively extracted with ether and acetone. The resulting flour was extracted with water three times in ratios of 1:5, 1:4, and 1:3. From the combined aqueous extracts, 4% hydrochloric acid at pH 3.9-4.1 precipitated proteins provisionally called phosphoproteins [6], and the filtrate from these was subjected to stepwise alkalinization with 4% caustic soda solution.

After 20-30 min, the slightly turbid solution deposited a loose flocculent precipitate. The addition of more alkali to impart a higher pH to the solution (by 0.5-1.0 pH units) led to the appearance of turbidity which changed after 15-30 min into a flocculent precipitate, i.e., precipitation was stepwise (table).

It was observed that the increase in pH was accompanied by a change in the color of the solution (from yellow to deep orange). In order to free them from salts, some of the precipitates obtained were passed through a column (9 × 300 mm) filled with Dowex 50 × 2 in the H form. Some of the alkaline fractions were suspended in 1-2 ml of water and dissolved by the addition of a few drops of 4% hydrochloric acid. The column was washed with water until chloride ions had been completely eliminated and was then eluted with 0.1 N ammonia until the ninhydrin reaction was negative. The eluates were concentrated in vacuum at 55-60° C in a water bath to small bulk and were dried in a vacuum desiccator over phosphorus pentoxide. The total yield of all the fractions was 0.1-0.2% of the weight of the flour. The yellowish dry samples, readily soluble in dilute hydrochloric acid, gave positive ninhydrin and biuret reactions and a negative reaction for tryptophan and partially passed through a colloidal membrane prepared from 7% collodion, which shows their heterogeneity. Their nitrogen content was high: mung bean (fraction 2) 17.20%, (fraction 3) 17.5%; (fraction 2) 17.25%, (fraction 3) 16.40%.

To elucidate the nature of the precipitates, the individual fractions were hydrolyzed (5.7 N hydrochloric acid, 24 hr, sealed capillary, boiling water bath), and the hydrolysate was studied by two-dimensional chromatography with the solvents phenol-o-cresol (1:1) saturated with water, and butan-1-ol-acetic acid-water (4:1:5). All the protein amino acids were detected, the alkaline ones being particularly plentiful.

Conclusions

1. Substances containing a large amount of hexone bases and containing no tryptophan, which permits them to be assigned to the alkaline proteins of the histone type, have been isolated for the first time from legume seeds at pH 9.0-12.5 by a method developed for the purpose.

2. The amino acid composition and some of the properties of these substances have been determined by chromatography.

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