CI. ON THE SPECIFIC ACTION OF PLANT PROTEASES

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THE problem of the specific action of ferments which is one of great general importance for biology in its application to plant proteases has a comparatively short history. Aron and Klempin [1908] and Bialosuknia [1909] were the first to detect that the ferment preparations from the seeds of wheat, rye and oats acted with great activity on proteins of cereals and vetch. The same preparations were without action on proteins of animal origin. Jacobson [1912] stated that the proteases of alfalfa seeds hydrolysed caseinogen and Witte-pepton, but left egg-albumin, serum-albumin, legumin and congrutin intact. At about the same time B. Issatschenko [1917] published the results of his investigations on the specific proteoclastic action of antibodies, which are produced in the organism of the rabbit by parenteral injection of various plant proteins. The application of Abderhalden's ninhydrin reaction showed that the proteoclastic action was strictly specific and antibodies of the proteins from flax seed, wheat, hazel-nut and oats were active only in relation to their own proteins, but not to the others. My present experiments give a new confirmation of these facts.

In order to isolate proteins from seeds I used extraction with 5 % NaCl solution at 60° (10 litres of solution for 1 kilo. of ground seeds). After filtration the globulins were precipitated by diluting the sodium chloride extract with pure water. After having been filtered and washed with water, the globulins were treated with alcohol and ether or with acetone and placed in a vacuum exsiccator over sulphuric acid.

As ferment preparations were taken, except in the first series of experiments, the ground seedlings of the same plants as gave the material for the extraction of the globulins. Sørensen's formaldehyde titration method was employed for quantitative determination of the amino-groups, liberated in the process of protein hydrolysis. The results are presented either as milligrams of amino-nitrogen or in the form of the so-called amino-index (the ratio of the total nitrogen of the protein to its amino-nitrogen). All the experiments were parallel and the results given in the tables present the average of two determinations.

In the first series of experiments the proteins were dissolved in 5 % NaCl solution. For the ferment preparation the fresh seedlings were ground in a mortar with the same NaCl solution, pressed and filtered.

For the determination of the influence of NaCl on proteases the following control experiment was carried out. Seedlings of *Phaseolus Mungo* L., aged one day, were divided into eight equal parts, ground and diluted each with 40 cc. of solvent (water or 5 % NaCl solution). Two samples of each group were titrated by Sørensen's method immediately, two others kept with toluene at room temperature and titrated after 24 hours. The divergence of results in each series was within the limits of possible experimental error.

	Amino-nitrogen in mg.					
	Initial			After 24 hours at room temperature		
	1	2	Average	1	2	Average
Water	15.0	14.6	14.8	24.3	25.2	24.8
5 % NaCl solution	15.0	15.0	15.0	23.7	24.5	24.7

Exp. 1. The press-juice of seedlings of *Cannabis sativa* L. as ferment. The globulins of *Cannabis sativa* and other plants, 1 g. for each sample. Toluene as antiseptic. The acidity in all flasks only showed a very small difference at the end of the experiment: no greater than 2 cc. of *N*/5 NaOH for 50 cc. of reacting liquid. The globulins without ferment after standing showed no distinct change.

	Amino-nitrogen in mg.	
	Initial	Final
Globulin of <i>Cannabis sativa</i>	1.8	1.8
" <i>Brassica rapa</i>	3.6	3.6
" <i>Lupinus luteus</i>	3.8	3.8
" <i>Phaseolus Mungo</i>	2.1	2.1

The content of amino-nitrogen in the ferment changed at the same time from 14.3–25.2 mg.

	Amino-nitrogen in mg.			
	Initial	Final	Calculated*	Difference
<i>Cannabis</i> ferment + <i>Cannabis</i> globulin	16.1	30.1	27.0	3.1
" " + <i>Brassica</i> "	17.9	28.8	28.8	—
" " + <i>Lupinus</i> "	18.1	29.0	29.0	—
" " + <i>Phaseolus</i> "	16.4	27.3	27.3	—

* From data for globulin and ferment separately.

The preference for its own protein by the ferment is evident.

Exp. 2. The ferment from seedlings of *Phaseolus Mungo* L.

The amounts of globulins were greater than in the first experiment. The duration of the experiment was 65 hours at 26–28°. The globulins without ferment did not change. The amino-nitrogen content of the ferment solution changed from 9.5–16.5 mg.

	Amino-nitrogen in mg.			
	Initial	Final	Calculated	Change
<i>Phaseolus</i> ferment + <i>Phaseolus</i> globulin	15.7	33.4	22.7	10.7
" " + <i>Cannabis</i> "	13.4	28.0	20.4	7.6
" " + <i>Lupinus</i> "	13.6	24.7	20.6	4.1
" " + <i>Brassica</i> "	14.1	23.0	21.1	1.9

Exp. 3. The ferment from seedlings of *Brassica rapa* L.

The conditions of the experiments were the same as in *Exp. 2*. The amino-nitrogen content of the ferment solution changed from 3.1–4.4 mg.

	Amino-nitrogen in mg.			
	Initial	Final	Calculated	Change
<i>Brassica</i> ferment + <i>Brassica</i> globulin	7.7	13.4	9.0	4.4
" " + <i>Lupinus</i> "	7.2	11.7	8.5	3.2
" " + <i>Phaseolus</i> "	9.3	10.6	10.6	—

Exp. 4. The ferment from seedlings of *Lupinus luteus* L.

The conditions were the same as in *Exps. 2* and *3*. The amino-nitrogen content of the ferment solution changed from 18.4–22.0 mg.

	Amino-nitrogen in mg.			
	Initial	Final	Calculated	Change
<i>Lupinus</i> ferment + <i>Lupinus</i> globulin	22.5	34.0	26.1	7.9
" " + <i>Phaseolus</i> "	24.2	33.9	28.2	5.7
" " + <i>Cannabis</i> "	22.3	28.9	25.9	3.0

Assuming that the maximal action for each ferment is equal to 100, the values of the other amounts are as follows:

Ferments	Globulins			
	<i>Phaseolus</i>	<i>Lupinus</i>	<i>Brassica</i>	<i>Cannabis</i>
<i>Phaseolus</i>	100	38	18	71
<i>Lupinus</i>	72	100	—	38
<i>Brassica</i>	0	73	100	—
<i>Cannabis</i>	0	0	0	100

These values indicate clearly that the proteases of various plants, acting on the different globulins, hydrolyse them to an unequal extent.

The second series of experiments gives a new confirmation of this specificity. All the globulins were newly prepared. The total nitrogen content was determined (by Kjeldahl's method) and the ratio of total nitrogen to amino-nitrogen (amino-index). The results of these determinations are given for air-dried globulins.

Plant	Total nitrogen %	Amino-nitrogen %	Amino-index
<i>Hibiscus esculentus</i>	13.8	0.518	26.3
<i>Cannabis sativa</i> L.	15.54	0.196	79.3
<i>Dolichos melanophthalmus</i> DC.	13.75	0.552	25.0
<i>Lathyrus sinensis</i>	14.94	0.515	29.1
<i>Phaseolus Mungo</i> L.	12.76	0.445	28.8

The samples of ferments and globulins in all experiments were strictly of 1 g. To each flask were added 30 cc. of 4 % NaCl solution and chloroform and toluene as antiseptics. The duration of all experiments was 168 hours at room temperature (19°).

Exp. 5. The ferment of seedlings of *Phaseolus Mungo* L.

The amino-nitrogen content in the ferment solution changed from 2.16–4.9 mg.

Origin of globulins	Amino-nitrogen in mg.	Amino-index		Extent of ferment action
		Initial	Final	
<i>Phaseolus Mungo</i>	11.5	28.8	19.03	100
<i>Dolichos melanophthalmus</i>	13.2	25.0	16.6	99
<i>Hibiscus esculentus</i>	11.3	26.3	21.2	57
<i>Cannabis sativa</i>	7.0	79.3	74.0	20
<i>Lathyrus sinensis</i>	10.1	29.1	28.7	4

Exp. 6. The ferment of seedlings of Hibiscus esculentus.

The amino-nitrogen content of the ferment at end of autolysis was 8.85 mg.

Origin of globulins	Amino-nitrogen in mg.	Amino-index		Extent of ferment action
		Initial	Final	
<i>Hibiscus esculentus</i>	20.3	26.3	11.9	100
<i>Dolichos melanophthalmus</i>	19.1	25.0	13.5	84
<i>Lathyrus sinensis</i>	14.9	29.1	24.9	26

Exp. 7. The ferment of seedlings of Dolichos melanophthalmus DC.

The amino-nitrogen content of the ferment at end of autolysis was 5.3 mg.

Origin of globulins	Amino-nitrogen in mg.	Amino-index		Extent of ferment action
		Initial	Final	
<i>Dolichos melanophthalmus</i>	14.8	25.0	14.4	100
<i>Lathyrus sinensis</i>	11.9	29.1	22.6	53
<i>Phaseolus Mungo</i>	11.5	28.8	20.6	67

Exp. 8. The ferment of Cannabis sativa L.

The amino-nitrogen content of the ferment at end of autolysis was 2.1 mg.

Origin of globulins	Amino-nitrogen in mg.	Amino-index		Energy of ferment action
		Initial	Final	
<i>Cannabis sativa</i>	7.55	79.3	28.7	100
<i>Lathyrus sinensis</i>	7.05	29.1	30.4	0
<i>Hibiscus esculentus</i>	8.4	26.3	21.6	44

The results of all experiments are summarised in the following table, similar to that for the first series:

Ferments	Globulins				
	<i>Phaseolus</i>	<i>Dolichos</i>	<i>Cannabis</i>	<i>Hibiscus</i>	<i>Lathyrus</i>
<i>Phaseolus</i>	100	99	20	57	4
<i>Dolichos</i>	67	100	—	—	53
<i>Cannabis</i>	—	—	100	44	0
<i>Hibiscus</i>	—	84	—	100	26

Though these experiments are preliminary to a detailed investigation, I am able to state that in all cases the ferment from every plant splits its own globulin more actively than the globulins of other plants. The close systematic relation of *Phaseolus* and *Dolichos* is clearly reflected in the mutual relation of their ferments and globulins. If further investigations confirm this regularity, observed in a large number of cases, Roaf's [1909] ideas on the connection of the specificity of enzyme action with the degree of development of a living organism may receive quite a new direction: for we may think that both proteins and enzymes are undergoing the same evolutionary process that causes the evolution of physiological properties in plants or animals as a result of deep changes in their internal cellular structure. Comparative physiology, whose development is quite recent, gives new evidence of an intimate connection between the structure and the physiological properties of an organism. Thus the question of the origin of systematic units seems to be reduced to a purely chemical problem of the evolution of proteins and other chemical bodies in the protoplasm.

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