SOMMAIRE

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Research on Phosphatases of Belladona Leaves and Their Purification*

PART II

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Now we come to action on various substrates: We have attempted to find out if both types of phosphatases of leaves react indentically or differently on various phosphric esters used as substrate.

We have used different phosphoric compounds. The results of our tests shown in table No. IV indicate that no great difference is evident between the reaction of the two associated phosphatases in belladona leaves as is the case in all other studied leaves.

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Table No. IV.

Action of Phosphatases of Leaves on Various Substrates.

Substrate	Lyo-enzyme of Ivy Leaves		Lyo-enzyme of Belladona Leaves		Desmo-enzyme of Chestnut Leaves	
	pH of the Test	Percentage of Hydrolysis	pH of the Test	Percentage of Hydrolysis	pH of the Test	Percentage of Hydrolysis
Pyrophosphoric acid	4.07	42	3 84	17	4 0	31
	4.95	65	5.10	38	5.2	42
Glycerophosphoric acid	4 07	8	3.84	9	4.0	9
	4 95	22	5·10	20	5.2	18
Hexosediphosphoric acid	4.07	13	3 84	11	4.0	10
	4 95	19	5.10	21	5.2	16
Saccharose-diphosphoric acid	4.07	3	3.84	2	4 0	2
	4.95	8	5.10	7	5.2	6
Phospho-glycolic acid	4 07	7	3.84	6	4 0	8
	4.95	17	5.10	18	5.2	16
Benzyl-phosphoric acid	4 07	26	3 84	35	4.0	26
	4 95	61	5 10	62	5.2	35
Inositol-hexaphosphoric acid	4.07	0	3.84	0	4.0	0
	4.95	0	5.10	0	5 2	0
Phenylphosphoric acid	4.07	7	3 84	5	4 0	5
	4.95	54	5.10	50	5.2	29

The figures of the table represent the percentage of hydrolysis of 5 ml.

This table shows that the studied leaves have hydrolysed more or less rapidly all indicated phosphoric compounds, but none of the studied leaves have any action on phytic acid. Therfore, they contain no phytophosphatase. The only active phytase known in leaves is the fraction separated from spinach studied by Wildman and Bonner (7) (10).

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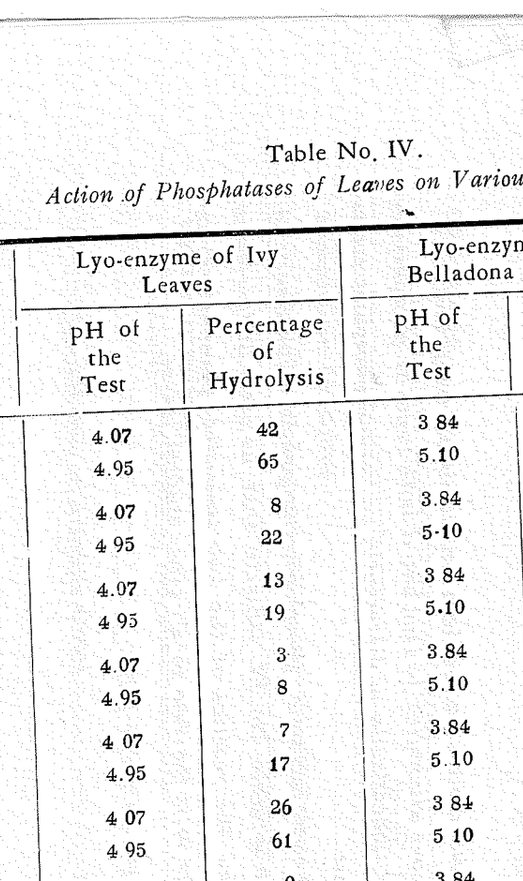
Finally we come to the test of purification of the lyo-enzyme.

We have attempted to purify lyo-enzyme of belladona leaves by fractionation with ammonium sulfate. We have obtained three fractions corresponding to three different concentrations of ammonium sulfate The first precipitate was obtained by 33°/₀ of saturation, the second obtained by 66°/₀ and third obtainedby 100°/₀ of saturation of ammonium sulfate.

Chart No.2.-*The Activity pH-Curves of the three Samples with and without Magnesium*



Operational technic :

1) 2 ml. of a 0.25°/. enzymic solution of the sample 33,
2) 3 ml. of a 0.25°/. enzymic solution of the sample 66,
3) 4 ml. of a 0.25°/. enzymic solution of the sample 100, are put in contact for 8 days at 37° with 30 ml. of glycerophosphates solution M/25, buffer of appropriate pH and distilled water, a sufficient quantity to make 50 ml.

Dotted line curve : without magnesium.

Solid line curve : in presence of magnesium sulfate at a concentration of 1.10^{-2} in the reactional medium.

Cart No. 2 shows the activity pH-curves of the three mentioned samples with and without magnesium. As we see, the phosphatase III is found in the first precipitate; with the preparation obtained by 66°/₀ of saturation we can find only one pH optimum. On the curve of the preparation obtained by 100°/₀ of saturation with magnesium we see an inflextion of the curve toward pH 4.0 which allows us to beleive that it contains a small amount of phosphatase III combined with phosphatase II.

The small yield of the first fraction indicates that a large proportion of the phosphatase III of the crude enzyme had been destroyed during de fractionation.

The fragility of this enzyme is pointed out by all authors having studied the phosphatases III. The sample 66 is the most important fraction in the weight. It contains almost exclusively the phosphatase II as we see on the activity curve.

Finaly the fraction obtained by 100°/₀of saturation is very small and not very active. It contains a small proportion of phosphatase III combined with a relatively important quantity of phosphatase II.

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SUMMARY OF PART I AND PART II

Through experimentation with several leaves it has been possible for us to point out the existance of two different acid phosphatases. We have studied in more detail the phosphatases of belladona leaves (Atropa Belladona L. Solanacees). The great part of the phosphatase activity is water extractable. We have compared the activity of the soluble fraction with that not directly extractable by means of water.

The insoluble fraction could not be solubilized in a satisfactory manner.The digestion by papaine produced a slight solubilizing effect; on the other hand salt solutions, neutral or alkaline, or water glycerol mixtures had no solubilizing effect on the enzyme.

It has been possible to demonstrate the existence of two different phosphatases in the insoluble fraction: the first of the type II,

optimum pH about 5 0; the second of type III, optimum pH about 4.0 . Whereas phosphatase II is only slightly activated by ions magnesium, nickel and cobalt, phosphatase III is markedly activated by the bivalent ions and particularly by Mg++, Mn++, Zn++, Ni++, and Co++. Both phosphatase fractions are inhibited by the fluorides and molybdates.Washing the insoluble phosphatase with a solution containing a complex forming agent like cyanide increases the sensitivity of these desmo-phosphatases to activation by bivalent ions.

The soluble fraction also contains phosphatases II and III. These two enzymes are capable of hydrolyzing a certain number of phosphoric esters, but they are without action on phytic acid. The phosphatase II which is activated by the magnesium ion, is inhibited by the Zn++, Mn++, Fe++, Ca++ ions. The phosphatase III is markedly activated by the Mg++, Zn++, Mn++, Ni++, Co++ ions, and inhibited by Fe++ and Ca++

We have tried to separate the two enzymes by salting out with ammonium sulfate. The phosphatase III can be identified in the precitate obtained with 33°/₀ of saturation. This phosphatase is quite unstable, for it is substantially destroyed during this fractionation. The fraction obtained with in a saturation of between 33 and 66°/₀ consists almost entirely of a phosphatase II, the characters of which are similar to those other phosphatases II of vegetable origin.

The activation by bivalent ions, a frequent phenomenon observed with alkaline phosphatases, is a very rare occurrence with acid phosphatases.

In the case of the enzyme of belladona leaves, this capacity for activation seems to be a characteristic feature of the very acid phosphatases contained in them.

The different preparations which have been obtained of various degrees of purity have all shown a capacity for activation.

RÉSUMÉ DES PREMIÈRE ET SECONDE PARTIES

Au cours des recherches antérieures, nous avons pu démontrer l'existence, dans différentes feuilles, de deux phosphatases acides distinctes. Nous avons étudié, en détail, les phosphatases des feuilles de belladone (Atropa Belladonna L. Solanacées). La majeure partie de ces deux enzymes est directement extractible par l'eau. Nous avons comparé l'activité de la fraction non directement extractible par l'eau.

Il n'a pas été possible d' extraire, d'une façon satisfaisante, la fraction insoluble. L'action de la papaïne a permis d'obtenir un très faible effet solubilisant; d'un autre côté, les solutions d'électrolyte neutres ou alcalines et les mélanges de glycérol et d'eau furent inefficaces.

Il a été possible de démontrer l'existence de deux différentes phosphatases dans la fraction insoluble : la première de type II, avec un pH optimum d'environ **5.0**; la seconde de type III, à pH optimum de **4.0** environ. Alors que la phosphatase II est seulement légèrement activée par les ions magnésium, nickel et cobalt, la phosphatase III est fortement activéepar les ions bivalents et particulièrement par Mg^{++}, Mn^{++}, Zn^{++}, Ni^{++}, et Co^{++}.

Les deux fractions phosphatasiques sont inhibées par les fluorures et les molybdates. L'épuisement de la phosphatase insoluble par une solution contenant un agent formateur de complexes, comme le cyanure, accroît la sensibilité des desmo-phosphatases à l'activation des ions bivalents.

La fraction soluble contient aussi des phosphatases II et III. Ces deux enzymes sont capables d'hydroliser un certain nombre d'esters phosphoriques, mais ils sont sans action sur l'acide phytique. La phosphatase II, qui est activée par l'ion Mg^{++}, est inhibée par les ions Zn^{++}, Mn^{++}, Fe^{++}, Ca^{++}. La phosphatase III est fortement activée par les ions Mg^{++}, Zn^{++}, Mn^{++}, Ni^{++}, Co^{++}, et inhibée par les ions Fe^{++}, Ca^{++}.

Nous avons cherché à séparer les deux enzymes en relarguant par le sulfate d'ammonium. La phosphatase III peut être identifiée dans le

précipité obtenu à **33** % de saturation. Cette phosphatase étant très fragile, elle se trouve en partie détruite au cours du fractionnement. La fraction obtenue entre **33** et **66** % de saturation est presque exclusivement formée de phosphatase II, dont les propriétés sont les mêmes que celles des autres phosphatases II d'origine végétale.

L'activation par les ions bivalents, phénomène fréquemment observé avec les phosphatases alcalines, est très rare avec les phosphatases acides.

Dans le cas de l'enzyme des feuilles de belladone, cette capacité d'activation semble être une propriété due aux phosphatases acides qu'elles contiennent.

Les préparations obtenues à différents degrés de pureté ont toutes montré une capacité à l'activation par les ions bivalents.

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