

Research on Phosphatases of Belladonna Leaves and Their Purification

PART I

by

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In the course of the earlier research (2) we have been able to demonstrate the existence, in several leaves of phosphatases active in acid medium.

A fraction of this phosphatase is not extractible by water. The soluble proportion of the enzyme varies. For example, in the case of chestnut leaves it is negligible, but in the case of ivy leaves it is very high.

The action of effectors on the enzyme of these leaves reveals the presence in each case of two distinct acid phosphatases:

The first, of type II according to classification proposed by Folley and Kay-Roch and Courtois (3) (8), with a pH optimum 5,0 to 5,2. This phosphatase reacts like other phosphatases of the same type with various effectors.

The second phosphatase of pH optimum 4,0 to 4,2, belonging to the type III of the mentioned classification, differs from other phosphatases III previously described by being strongly activated by various bivalent cations such as Mg, Zn, Mn, Ni, Co, etc.

In the case of *belladonna leaves* we have noted that there is an appreciable phosphatase activity easily extractible by water.

To clarify this exposal we shall designate the soluble and non-soluble fraction in water according to terminology of Wilstatter by the names of *lyo-enzyme* and *desmo-enzyme*.

We have studied in detail the properties of this phosphatase notably: the solubilization of the desmo-enzyme into lyo-enzyme, the action of different metallic effectors, the action of the lyo-enzyme on several phosphoric compounds and finally we have attempted the purification of the lyo-enzyme by fractionation (2) (4) (7).

Test o. the solubilization of the desmo-enzyme.

The desmo-enzyme is obtained from the powder of belladonna leaves by prolonged mechanical shaking and then centrifugation of their suspension. The clear supernatant liquid contains the lyo-enzyme which we have precipitated by acetone (60% at 0°) as a substance soluble in water.

By employing various methods which have given satisfactory results with other desmo-enzymes, we have not succeeded in obtaining a complete solubilization of the desmo-enzyme.

The digestion by papaine has produced a slight solubilizing effect; on the other hand extraction by a 10% solution of sodium chloride or by a dilute solution of ammonium chloride, which was efficient in the dissolution of the proteins of leaves studied by Lugg and Weller (5); Clagett, Tolbert and Burris (1); Wildman, Cheo and Bonner (11), has not given any satisfactory result.

A mixture of glycerol and water which gives favorable results in solubilization of desmoesterase of animal tissues was also inadequate. Only a small quantity of the phosphatase was made soluble by autolysis (at pH 6.5) in presence of toluene and ethyle acetate.

It appears therefore that the non-soluble fraction of the belladonna leaves phosphatase is strongly retained by the cellular constituents. The results obtained permit us to suppose that it is strongly attached to cell walls.

The action of various metallic effectors.

Our tests were made on the two pH where the optimum activity of other phosphatases is manifested.

Table No. 1

Action of different effectors on the lyo-enzyme of belladonna leaves obtained by water-extraction and acetonc precipitation.

pH of the test	Molecular concentration of the effector in the medium	Nature of the effector					
		MgSO ₄	ZnSO ₄	MnSO ₄	FeSO ₄	CaCl ₂	C ₂ O ₄ H ₂
3.84	2.10 ⁻³	232	181	113	33	90	90
	4.10 ⁻³	316	219	116	33	83	80
	2.10 ⁻²	384	181	140	26	77	32
5.10	2.10 ⁻³	139	69	78	32	93	116
	4.10 ⁻³	169	63	78	17	83	113
	2.10 ⁻²	181	42	75	8	66	43

As in the case of tables No.2,3 and 4 the figures represent the proportional activity: millg. of H₃PO₄ liberated in the presence of effectors/millg. of H₃PO₄ liberated in the absence of effectors in the same operational condition (this proportion being multiplied by 100). Based on this fact the activity of the enzyme without effectors has been fixed arbitrarily at 100. In the absence of effectors the phosphatase hydrolysis 270/o of the substrate (20 ml. of glycerophosphate M/25) at pH 3.84 and 520/o at pH 5.10.

Table No.1 shows the action of different effectors on the lyo-enzyme of belladonna leaves. We have the two optimum pH and molecular concentration of the effector. The phosphatase II is activated by Mg-ions but inhibited by Zn-and Mn-ions, whereas the phosphatase III is strongly activated by Mg, Zn, and Mn-ions. The activation increases with the increase of the molecular concentration of the effector.

Table No. 2
Action of various effectors on the desmo-phosphatase of the
belladonna leaves.

pH of the test	Molecular concentration of the effector in the medium	Nature of the effector							
		Mg SO ₄	Zn SO ₄	Mn SO ₄	Fe SO ₄	Co SO ₄	Ni SO ₄	C ₂ O ₄ H ₂	Na CN
3.84	2.10 ⁻³	131	131	112	37	117	116	87	100
	4.10 ⁻³	150	150	118	37	123	121	81	96
	2.10 ⁻²	187	150	137	25	141	131	43	93
5.10	2.10 ⁻³	108	79	75	33	102	104	120	100
	4.10 ⁻³	116	70	75	16	104	104	125	100
	2.10 ⁻²	133	62	66	8	114	110	133	100

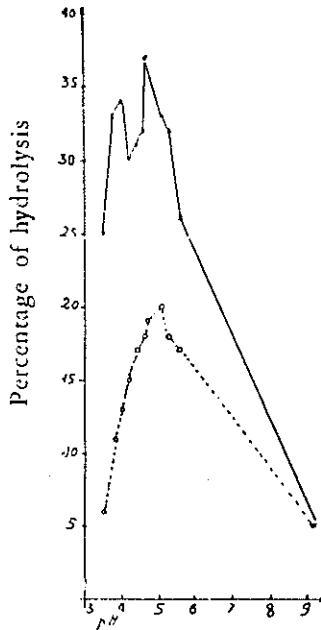
The figures of the table represent the proportional activity calculated as in the case of table No. 1. In the absence of effectors the phosphatase hydrolysis 280/o of the substrate (20 ml. of glycerophosphate M/25) at pH 3.84 and 420/o at pH 5.10

Table No. 2 shows the action of various effectors on the desmo-phosphatase. The phosphatase II is activated by Mg, Ni and Co- ions and the phosphatase III is activated by Mg, Zn, Mn, Ni and Co - ions in the same manner as mentioned above.

Finally we have used a preparation of the desmo-enzyme obtained by treating the belladonna powder first with distilled water and then by a solution of a complexe forming agent such as cyanid. The cyanid was used here to eliminate the metallic ions present which could provoke an activation of the enzyme.

Chart No. 1

The activity pH-curve of the washed desmo-enzyme of belladonna leaves.



..... Without Mg

———— with Mg at a concentration of 1.10^{-2} M in reactional medium

Operational technic: 10 ml. of a 20/o suspension of the desmo-enzyme are put in contact for 48 hours at 37° with 20 ml. of glycerophosphate $M/25$, buffer of appropriate pH and distilled water a sufficient quantity, to make 50 ml.

The activity pH-curve of this preparation in presence of Mg-ions shows two optima: the first at pH 4.0 and the second at pH about 5.0.

Table No. 3
Action of various effectors on the desmo enzyme of belladonna leaves washed with cyanid

pH of the test	Molecular concentration of the effector in the medium	Nature of the effector				
		MgSO ₄	ZnSO ₄	NiSO ₄	CoSO ₄	C ₂ H ₂ O ₄
3.84	2.10 ⁻³	190	155	130	130	75
	4.10 ⁻³	200	185	145	140	70
	2.10 ⁻²	230	160	170	160	20
5.10	2.10 ⁻³	133	83	108	114	106
	4.10 ⁻³	136	77	114	118	130
	2.10 ⁻²	147	72	123	132	82

The figures of the table represent the proportional activity calculated as in the case of table No. 1. In the absence of effectors the phosphatase hydrolysis 17% of the substrate (20ml. of glycerophosphate M/25) at pH 3.84 and 30% at pH 5.10.

This table shows the action of several effectors on this preparation. We have the same kind of activation with phosphatase II and III as in the case of two above mentioned preparations.

From these results we may conclude that first, the three studied preparations react exactly in the same way with the various chemical effectors, and second, that they contain the same two phosphatase systems:

First: the phosphatase active at pH 5.0 which by its comportment with effectors permit us to conclude that it has properties identical to those of phosphatases II of the other leaves that we have studied previously. The phosphatases II of leaves which have been studied are activated by Mg-ions; this activation is somewhat rare for this type of enzyme and very often the Mg-ions inhibit the enzyme of this type. Except for this activation the phosphatases II of leaves do not show a

marked difference to those of other origins (2).

Second: The phosphatases III of leaves, active at pH 4.0, on the contrary shows a marked difference from the other phosphatases of the same type previously described such as, ascomycets, grains or animal tissues. According to Nguyen-Van-Thoai (6), the Mg-inhibition was common to all these enzymes of different origin. On the contrary, in the three phosphatase preparations of belladonna as well as in the case of all other leaves which have been studied, we have observed a very marked activation by Mg-ions (2) (4).

Therefore, the sensitiveness to Mg-inhibition which has been considered up to now as being specific characteristic of the very acid phosphatases of the type III is not observed in the case of the enzyme of leaves.

Now taking the properties as a whole, it is evident that the most acid phosphatase of leaves presents a somewhat exceptional character being at the same time inhibited by the most specific inhibitors of acid phosphatases such as sodium fluoride or molybdic acid and being activated by the most specific activators of alkaline-phosphatases.

SUMMARY

Belladonna leaves as well as all other studied leaves contains two distinct phosphatase fractions belonging respectively to types II and III; the major parts of these enzymes is extractible by water.

It was not possible to extract the non soluble fraction which is solidly retained by the cellular constituents.

Phosphatase II does not differ from other phosphatases of the same type. Whereas phosphatase III is distinctly different from enzymes of the same type of vegetal or animal origins. It is activated by bivalent metallic ions which are specific activators of the alkaline phosphatases: Mg-Zn-Ni and Co.

RÉSUMÉ

Les feuilles de belladone renferment deux fractions phosphatases distinctes appartenant respectivement aux types II et III; la majeure partie de ces deux enzymes est directement extractible par l'eau; il n'a pas été possible d'extraire, d'une façon satisfaisante, la fraction insoluble de ces deux enzymes qui est fortement retenue par les constituants cellulaires.

La phosphatase II ne diffère pas d'une façon très marquée des autres phosphatases de ce type. La phosphatase III se différencie nettement des enzymes de ce type obtenus à partir d'autres matières premières que les feuilles. Elle est fortement activée par les ions métalliques bivalents qui sont les activateurs les plus spécifiques des phosphatases alcalines: Mg, Zn, Ni et Co.

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