pH-Regulation of acid phosphatase of plant cell walls

An example of adaptation to the intracellular milieu

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1. INTRODUCTION

Plant cell walls contain several hydrolases [1—4]. Some of them play a central role in cell extension by promoting local hydrolysis of acidic cell wall polysaccharides. Others control hydrolysis and transport of extracellular metabolites in the cell. In sycamore (*Acer pseudoplatanus*) cells cultured in vitro, the most abundant of these enzymes is an acid phosphatase whose activity may be detected at the outer surface of unbroken cells [5].

Owing to the polyanionic nature of primary plant cell walls, the local pH within the cell-wall matrix may be quite different from the one prevailing in the bulk phase. This difference may be as large as several pH units [6]. Moreover, the Donnan potential in the cell wall matrix, generated by negative fixed charges of polygalacturonates, is tightly controlled by ionic strength of the outer bulk phase; that is, the ionic strength of the solution outside the cell. Therefore depending on the experimental conditions, the local pH in the cell wall may vary by several pH units.

To understand the behaviour of cell-wall enzymes in situ it is therefore necessary to know already the pH-dependence of these enzymes in free solution. This work studies pH-regulation of acid phosphatase isolated and purified from sycamore cell walls.

2. MATERIALS AND METHODS

Sycamore (*Acer pseudoplatanus*) cells were cultured in vitro in liquid medium under sterile conditions, as in [5]. Cell disruption was done in a French press under 1000 kg/cm^2. Cell wall preparations free of cytoplasmic contaminations were obtained as in [5]. About 55% of cell-wall acid phosphatase activity was solubilized by raising the ionic strength of the cell wall fragment suspension. The enzyme was purified to homogeneity from this soluble extract as in [5]. Acid phosphatase on cell wall fragments does not result from an artefact created by cell disruption because the same enzyme may be obtained by raising the ionic strength of a suspension of intact unbroken cells. This enzyme is a monomeric glycoprotein of $\sim 100\,000$ M_r [5].

Rate determinations were effected by following *p*-nitrophenylphosphate hydrolysis with a Beckman Acta MVII spectrophotometer. Substrate was varied from 1.33×10^{-4}—10^{-3} M. Incubation mixtures contained 0.033 M succinate, Tris—HCl, MES [2-*N*-(morpholino) ethane sulfonic acid] or TES [*N*-Tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid] buffers (pH 3.3—7.8). Enzyme and substrate were incubated at 28°C for 10 min, the reaction was stopped by adding 1 N NaOH to the reaction medium and read at A_{400}. In all the conditions tested the quantity of *p*-nitrophenol formed was linear with time for ≥ 30 min.

p-Nitrophenylphosphate, *p*-nitrophenol, orthophosphate were obtained from Sigma (St Louis MO).

3. RESULTS AND INTERPRETATION

Sycamore cell-wall acid phosphatase follows classical Michaelis—Menten kinetics (fig.1A):

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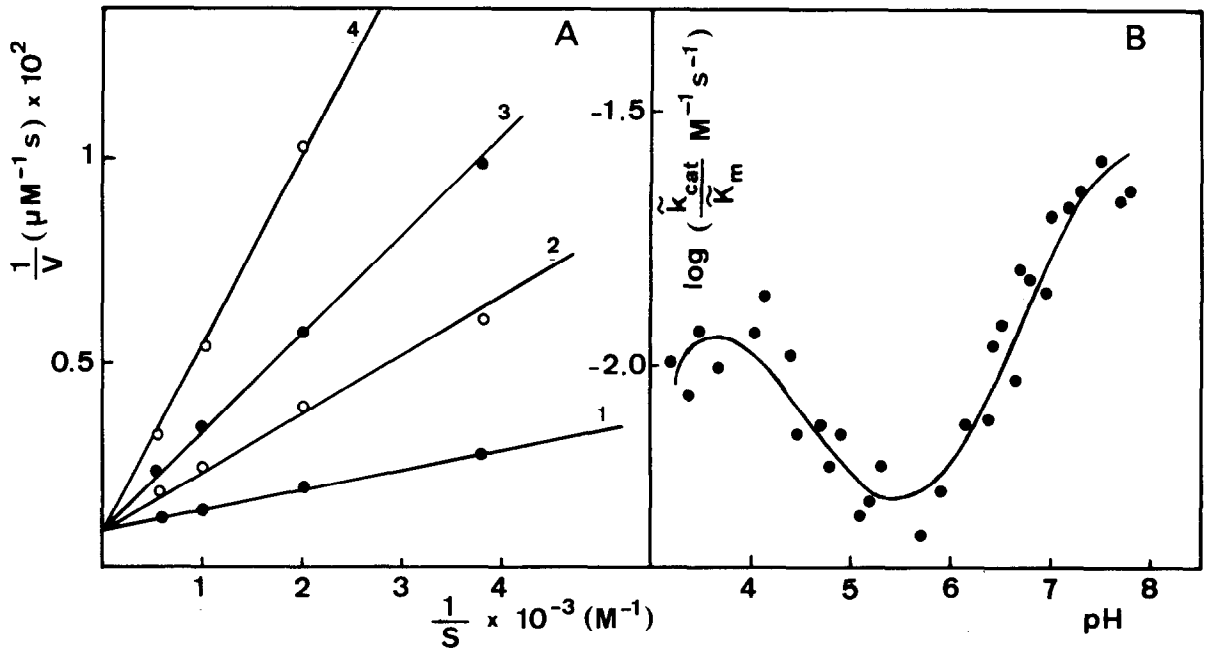

Fig.1. pH effects in cell wall acid phosphatase reaction.

(A) Competitive inhibition of the reaction by orthophosphate: (1) no orthophosphate; (2) orthophosphate 2.6×10^{-4} M; (3) 6.6×10^{-4} M; (4) 16.6×10^{-4} M; (pH 7.9) Tris–HCl buffer. Qualitatively similar results are obtained between pH 3.5–8.

(B) Variation of the corrected log ($\tilde{k}_{cat}/\tilde{K}_m$) as a function of pH. Points are averages of experimental values. No specific effect of the buffer was observed on the kinetic measurements. The curve is theoretical and corresponds to the equation:

$$\frac{\tilde{k}_{cat}}{\tilde{K}_m} = \frac{\alpha_0 + \alpha_1 [H] + \alpha_2 [H]^2 + \alpha_3 [H]^3 + \alpha_4 [H]^4}{\beta_0 + \beta_1 [H] + \beta_2 [H]^2 + \beta_3 [H]^3 + \beta_4 [H]^4}$$

after a log–log transformation.

The numerical values of the coefficients are:

$\alpha_0 = 0.66 \times 10^{-15}$	$\beta_0 = 4.41 \times 10^{-4}$
$\alpha_1 = 2.42 \times 10^{-7}$	$\beta_1 = 6.90 \times 10^{-6}$
$\alpha_2 = 0.48$	$\beta_2 = 1.10 \times 10^{2}$
$\alpha_3 = 0.29 \times 10^{5}$	$\beta_3 = 2.08 \times 10^{6}$
$\alpha_4 = 5.3 \times 10^{-2}$	$\beta_4 = 1.09 \times 10^{9}$

These values are given without dimension, for dimension depends on the nature of the model. The dimension of the ratio $\tilde{k}_{cat}/\tilde{K}_m$ is $M^{-1} \cdot s^{-1}$.

during hydrolysis of *p*-nitrophenylphosphate, orthophosphate behaves as a competitive inhibitor (fig.1A), whereas the other product, *p*-nitrophenol, has no significant inhibitory effect, under these conditions. These results are consistent with the view that *p*-nitrophenol is the first product to be released, whereas orthophosphate is formed afterwards. The step of *p*-nitrophenol release must be nearly irreversible in practice, since *p*-nitrophenol cannot inhibit the overall reaction process to a significant extent. The same kind of result has been obtained under different pH conditions. This situ-

ation is by no means novel and has been found to apply to different acid phosphatase [7,8].

Since the enzyme follows Michaelis–Menten kinetics between pH 3.3–7.8, it is possible to determine the apparent catalytic and Michaelis constants as well as their ratio in the pH-range investigated.

A sudden break occurs at pH 5.4 on the curve expressing the variation of $p\tilde{K}_m$ as a function of pH (not shown), and above this pH-value the $p\tilde{K}_m$ continuously declines when pH is raised. Since 5.4 is precisely a pK of *p*-nitrophenylphosphate, one must conclude that it is a protonated form of the substrate (the substrate monoanion) which reacts with the enzyme. This kind of experimental situation has been found to occur with any acid phosphatase studied so far. Therefore the apparent $p\tilde{K}_m$ and log $(\tilde{k}_{cat}/\tilde{K}_m)$ have to be corrected to take into account of the substrate ionization when the pH is raised. A plot of log $(\tilde{k}_{cat}/\tilde{K}_m)$ *vs* pH is shown in fig.1B. The ratio $\tilde{k}_{cat}/\tilde{K}_m$ is directly related to the free energy difference between the initial ground state and the central transition state. It therefore expresses the ease with which the enzyme reaction occurs. The complex curve-shape of fig.1 may be simulated or fitted by the ratio of 2 polynomials in $[H^+]$[4].

If an enzyme exists under different ionization states and if product desorption occurs in 2 consecutive steps, as it occurs with hydrolases, the general reaction scheme may be depicted as shown in fig.2. The general expression of $\tilde{k}_{cat}/\tilde{K}_m$ is of the form:

$$\frac{\tilde{k}_{cat}}{\tilde{K}_m} = \frac{\sum_{i=1}^{n} {}^{i}k_1 [H]^{i-1} \prod_{j=i}^{n-1} K_j}{\sum_{i=1}^{n} [H]^{i-1} \prod_{j=i}^{n-1} K_j} \times \frac{\sum_{i=1}^{n} {}^{i}k_2 [H]^{i-1} \prod_{j=i}^{n-1} K'_j}{\sum_{i=1}^{n} ({}^{i}k_{-1} + {}^{i}k_2) [H]^{i-1} \prod_{j=i}^{n-1} K'_j} \quad (1)$$

Contrary to [9], the expression of $\tilde{k}_{cat}/\tilde{K}_m$ not only involves in theory ionization constants of free enzyme K_j but also those of the enzyme–substrate complexes K'_j. However if:

$$\frac{{}^{1}k_2}{{}^{1}k_{-1} + {}^{1}k_2} = \ldots = \frac{{}^{n}k_2}{{}^{n}k_{-1} + {}^{n}k_2} \quad (2)$$

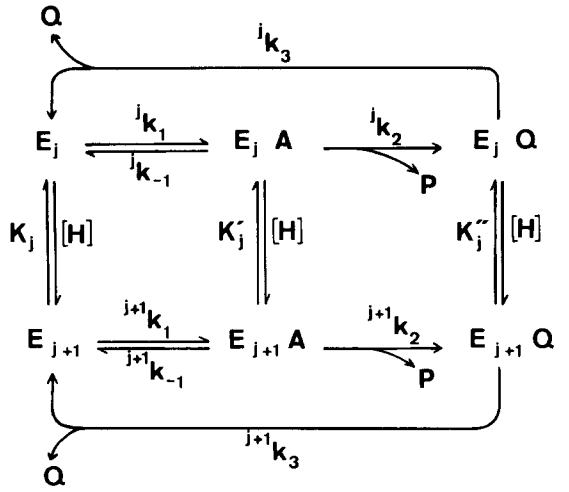

Fig.2. General reaction scheme that provides an explanation for the pH-effects of hydrolases. E_j and E_{j+1} represent 2 ionization states of the enzyme. The K-values are the ionization constants and the k-values the rate constants; A is the substrate, P and Q the 2 products. Care must be taken that rate constants k_3 are apparent rate constants that include water concentration.

eq. (1) assumes a much simpler form, namely:

$$\frac{\tilde{k}_{cat}}{\tilde{K}_m} = \frac{\sum_{i=1}^{n} {}^{i}k_1 [H]^{i-1} \prod_{j=i}^{n-1} K_j}{\sum_{i=1}^{n} [H]^{i-1} \prod_{j=i}^{n-1} K_j} \times \frac{k_2}{k_{-1} + k_2} \quad (3)$$

and then expression of log $(\tilde{k}_{cat}/\tilde{K}_m)$ solely depends upon the pK-values of the free enzyme. Condition (2) is quite likely and implies that ionization or protonation of the enzyme–substrate complex controls substrate and product desorption in the same manner. Since for most, if not all, hydrolases studied so far $\tilde{k}_{cat}/\tilde{K}_m$ is a curve of low degree in [H], it is tempting to consider that in those cases conditions (2) must hold.

Since the data in fig.1 can be simulated or fitted by the ratio of 2 fourth-degree polynomials in [H], in the light of the above theoretical considerations, two possibilities exist. Either the acid phoshatase from cell walls undergoes 2 ionization steps that control the reaction rate and condition (2) does not hold, that is eq. (1) applies with $i = 1, 2, 3$, or condition (2) holds and eq. (3) applies with $i = 1, 2,$

3, 4, 5, which implies that the free enzyme undergoes 4 ionization steps.

In either case, however, the behaviour of this cell wall phosphatase appears exceptional with respect to that of other hydrolases. It is therefore tempting to relate this pH regulation to enzyme location in the cell wall and to the properties of the cell wall as well.

4. DISCUSSION

For most enzyme reactions studied so far and even for acid phosphatases [7,8,10] the plot of log $(\tilde{k}_{cat}/\tilde{K}_m)$ *vs* pH is a smooth curve which can be fitted by an equation of low degree in [H]. Therefore, it is usually believed, either implicitly or explicitly [11], that whatever the number of reaction steps, a plot of log $(\tilde{k}_{cat}/\tilde{K}_m)$ reflects enzyme ionizations. To be so, ionizations of the enzyme–substrate complexes must control in similar ways substrate and product desorption, and it is likely that this condition does indeed occur for most hydrolases. Owing to curve-shape complexity of the plot log $(\tilde{k}_{cat}/\tilde{K}_m)$ *vs* pH, sycamore cell-wall acid phosphatase is a remarkable exception in that respect.

Since $\tilde{k}_{cat}/\tilde{K}_m$ represents the ease with which the enzyme reaction occurs, it is tempting to assume that the complex pH-profile depicted in fig.1 represents a complex regulation imposed on the enzyme by the very peculiar structure of the plant cell wall. One can make the sound speculation that this complex shape allows the maintainance of organic phosphate hydrolysis and transfer within the cell constant, or nearly constant. The local pH in the cell wall, which varies between 3–5 under most conditions, strongly depends on organic phosphate concentration in the outer bulk phase. When its concentration is raised, the Donnan potential $\Delta\Psi$ in the cell wall drops and the local pH raises from 3–5 [6,12], inducing in turn a decrease of phosphatase activity (fig.1). Above pH 6, the concentration of protonated reactive substrate markedly declines, owing to its ionization, and this decline is correlated with an increase of enzyme activity. Indeed this complex behaviour tends to buffer rates of substrate hydrolysis and transport against possible variation of organic phosphate concentration and of the pH in the external bulk phase.

Therefore, it is extremely tempting to consider that the complexity of the plot of log $(\tilde{k}_{cat}/\tilde{K}_m)$ *vs* pH has been selected by neo-Darwinian evolution as best suited to the peculiar physical structure of the plant cell wall to perform a given type of work. Therefore, it represents a remarkable adaptation to a given type of cellular compartmentalization and function. One may indeed expect that a similar behaviour will be observed with other enzymes of the plant cell wall.

REFERENCES

[1] Lamport, D.T.A. (1965) Adv. Bot. Res. 2, 151–218.
[2] Darvil, A., McNeil, M., Albersheim, P. and Delmer, D. (1980) in: The Biochemistry of Plants (Stumpf, P.K. and Conn, E.E. eds) vol.1, pp. 91–162, Academic Press, New York.
[3] Aspinall, G. (1980) in: The Biochemistry of Plants (Stumpf, P.K. and Conn, E.E. eds) vol. 3, pp. 473–500, Academic Press, New York.
[4] Lamport, D.T.A. (1980) in: The Biochemistry of Plants (Stumpf, P.K. and Conn, E.E. eds) vol. 3, pp. 501–541, Academic Press, New York.
[5] Crasnier, M., Noat, G. and Ricard, J. (1980) Plant, Cell and Environment 3, 217–224.
[6] Ricard, J., Noat, G., Crasnier, M. and Job, D. (1981) Biochem. J. 195, 357–367.
[7] Hickey, M.E., Waymack, P.P. and Van Etten, R.L. (1976) Arch. Biochem. Biophys. 172, 439–448.
[8] Alvarez, E.F. (1962) Biochim. Biophys. Acta 59, 663–672.
[9] Fersht, A. (1977) Enzyme Structure and Mechanism, Freeman, New York.
[10] Van Etten, R.L. and McTigue, J.J. (1977) Biochim. Biophys. Acta 484, 386–397.
[11] Brocklehurst, K. and Dixon, H.B.F. (1976) Biochem. J. 155, 61–70.
[12] Noat, G., Crasnier, M. and Ricard, J. (1980) Plant, Cell and Environment 3, 225–229.