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Effect of *p*H and Surface Charge on Cell Uptake of Auxin

THE uptake of the auxin indol-3-yl acetic acid (IAA) into plant cells is of interest not only because this compound is a hormone, but also because its movement across the plasma membrane is probably involved in the polar transport of auxin[1]. The plasma membrane contains auxin binding sites and may be a primary site of hormone action[2].

IAA partitions into non-polar solvents from acidified aqueous solutions[3] because the undissociated acid is more soluble in such lipid solvents than in water. There is known to be a passive, non-metabolic component of the uptake of IAA and of the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) into plant tissue which has been ascribed to the diffusion of the undissociated acid across the plasma membrane[4–7]. A carrier-mediated mechanism for auxin anion uptake is also possible but has not been conclusively demonstrated[5,7].

Uptake by the diffusion mechanism is linearly related to the concentration of the undissociated acid which is a function of the acid's *p*K and the *p*H of the incubation medium. Assuming that undissociated molecules are the only permeant species, the equilibrium distribution of a weak acid or base between cells and incubation medium is given by[8]

$$\frac{\text{total concentration acid/base inside}}{\text{total concentration acid/base outside}} = \frac{1+\text{antilog}_{10}(p\text{H}_{\text{inside}}-p\text{K})}{1+\text{antilog}_{10}(p\text{H}_{\text{outside}}-p\text{K})} \quad (1)$$

If the *p*H of the medium is lower than that of the cells, the cells accumulate weak acid; the equation requires that the concentration of undissociated acid should be the same in each compartment. Thus, the relation between the initial rate of uptake and *p*H should resemble a dissociation curve with a midpoint at the *p*K of the weak acid. This prediction is realized

for the uptake of benzoic acid ($pK = 4.2$) by yeast but not by the bacterium *Proteus vulgaris*[9], when, although the curve is still that of a dissociation, its midpoint is displaced by 1 *p*H unit above the *p*K of benzoic acid. Such displacement seems fairly widespread. By collating data from ninety experiments on *p*H dependence of biological effects of weak acids, a composite curve is obtained relating *p*H to log concentration of acid required to give a standard response[10]; the midpoint of the curve is at a higher *p*H than the *p*K. Data on IAA and 2,4-D uptake reveal a similar effect[4–7]. Here we suggest an explanation of this displacement which may be of general biological significance.

In previous investigations of auxin uptake, the use of tissue segments or slices[5,6] meant that interpretation of the results was complicated by polar movement of auxin and the presence of cut surfaces. To avoid these problems we used cells of tissue cultures grown in liquid suspension. Crown gall cells were chosen because, unlike most tissue cultures, they do not depend on a supply of exogenous auxin for their growth[11]. Uncertainties due to metabolism of IAA were kept to a minimum by the use of radioactive material which, together with the rapid uptake shown by the cells, enabled incubation times of minutes to be used, rather than of hours as in previous work.

At *p*H 6.4 and 1 μM IAA the rate of uptake was much less than at *p*H 4.8 and remained linear for a shorter period (Fig. 1). The *p*H remained constant throughout the incubation. After 5 min, at both *p*H values, 90% of the radioactivity in the cells was still in IAA, although after 80 min this had fallen to 20%. The longest time used for measurement of initial rates was 5 min; 3 min incubations were routinely used. At *p*H 4.3, there was a linear relationship between uptake and IAA concentration (Fig. 2) which continued up to 500 μM with no sign of saturation. After 5 min uptake from a 1 μM IAA solution, the concentration of labelled IAA in the total cell water is approximately 2 μM. It is unlikely, however, that auxin is uniformly distributed within the cell. The *p*H of vacuolar sap is about 5; the *p*H of the cytoplasm is near neutrality[12]. A *p*H-dependent distribution of auxin across the tonoplast between these compartments would lead to a much higher concentration of IAA in the cytoplasm than in the vacuole. Calculated on the basis of equation 1, the cytoplasmic concentration would be about sixty times higher.

At *p*H 6.5, the uptake/concentration curve is non-linear at low IAA concentrations (Fig. 2) and is the resultant of diffusion of the undissociated acid and of carrier mediated uptake of the IAA anion which becomes saturated with increasing concentration. Carrier mediated IAA transport will be described elsewhere.

When we examined the effect of a range of *p*H values on the initial rate of IAA uptake using external concentrations of 1.0 and 50.0 μM IAA, the general shape of the curves resembled that of IAA dissociation, but with a midpoint displaced from the *p*K of IAA at *p*H 4.75 (Fig. 3). Uptake is expressed as a percentage of the uptake at *p*H 3, taken as the maximum uptake, and obtained by extrapolation. At 1.0 μM IAA, the displacement became more pronounced as the *p*H increased because of the carrier mediated transport of IAA anions. At 50 μM IAA, the carrier is saturated leaving diffusion as the major uptake mechanism so that the curve runs more nearly parallel to the dissociation curve of IAA with a midpoint at *p*H 5.1. The *p*K of IAA is taken as 4.75. This is the highest value reported in the literature. Our own measurements indicate that the *p*K is nearer 4.6, in agreement with Albaum *et al.*[13]. Increased ionic strength (0.2 M LiCl) decreased the apparent *p*K very slightly.

We performed similar experiments on a range of cells and tissues in order to see if this displacement was of general occurrence. *p*H uptake curves for 3 mm *Avena sativa* mesocotyl sections from 5 d old dark-grown plants and for single, giant internodal cells of the alga *Chara australis* resembled dissociation curves and were again displaced to more alkaline *p*H values than the dissociation curve of IAA (mid point at *p*H 5.2 and *p*H 5.0, respectively). This contrasts with early observations

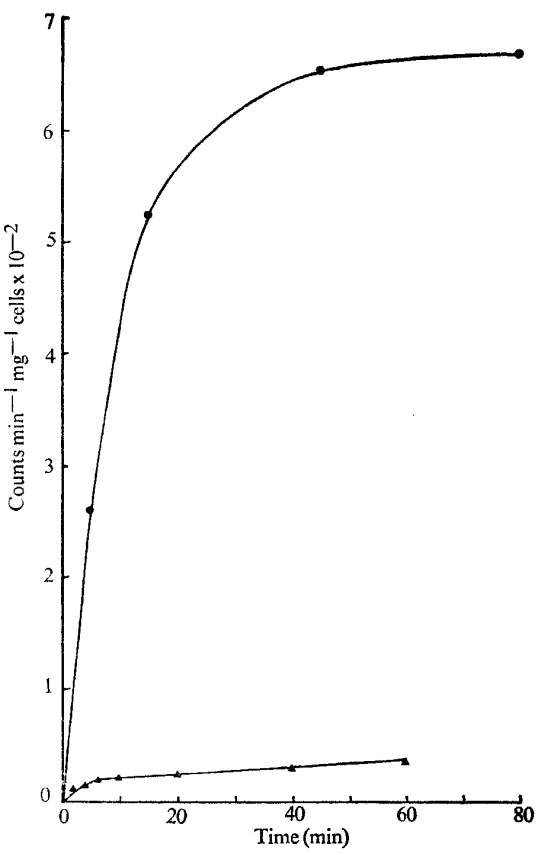

Fig. 1 Progress curve for IAA uptake by crown gall cells from a 1 μM solution at *p*H 4.8 and *p*H 6.4. The growth and maintenance of the *Parthenocissus tricuspidata* crown gall culture has been described elsewhere[11]. Two-week-old cultures were used, the cells being washed with distilled water, suspended in fresh growth medium (80 mg fresh wt ml^{-1}) and pre-incubated for 1 h at 25° C in a water bath fitted with a reciprocal shaker (120 strokes min^{-1}) before removing aliquots for uptake studies. Individual incubations forming part of a series were performed in random order. Four ml aliquots were removed from the stock suspension of cells in fresh medium and transferred to boiling tubes containing 1 ml glass distilled water and 1 ml buffer (0.2 M Na_2HPO_4 +0.1 M citric acid which, mixed in the appropriate proportions, provides a set of buffers covering the *p*H range 2.8 to 8.2). The *p*H of the incubation mixture was rapidly measured using a Beckman Research *p*H meter and the cells returned to the shaking water bath for 3 min preincubation before addition of 0.31 μCi 1-^{14}C-IAA ammonium salt (specific activity 52 mCi $mmol^{-1}$; 31 μl of a stock solution of 10 μCi ml^{-1}). Uptake was terminated after various incubation times by rapid filtration under reduced pressure through a Whatman glass fibre C 2.5 cm disk using a 'Millipore' castle apparatus. The cells were maintained under suction for 30 s, weighed on a piece of 'Parafilm', transferred to 20 ml scintillation bottles and 10 ml scintillant added. The scintillant was 3.5 g, 2,5-diphenyloxazole and 50 mg 1,4-bis-(5-phenyloxazol-2-yl) benzene in 1 l of S-free toluene+'Triton' X-100 (2:1 by vol). The samples were counted in a Philip's Liquid Scintillation Spectrometer using an external standard to enable corrections for quenching due to the presence of the cells to be made. It was found preferable to apply a correction for the presence of radioactive incubation medium in the free space outside the cells rather than to allow for the considerable efflux which occurs if the cells are washed. ●, *p*H 4.8; ▲, *p*H 6.4.

on the closely related *Nitella* by Albaum *et al.*[13], who used a crude colorimetric method without measuring initial rates and obtained an uptake curve coinciding with the IAA dissociation curve (*p*K 4.6). Using resting baker's yeast cells (*Saccharomyces cerevisiae*) suspended in distilled water, we found no indication of the operation of a carrier and, as for benzoic acid[9], the uptake curve coincided exactly with the dissociation curve of IAA (Fig. 4).

This difference in behaviour between plant cells and resting yeast may be related to their differing wall compositions. The primary cell walls of higher plants and of the Characeae[14]

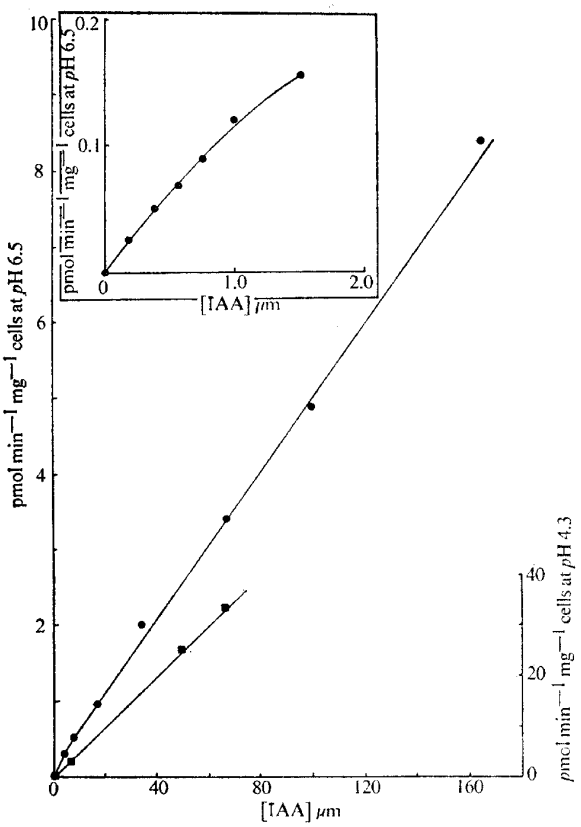

Fig. 2 Relationship between IAA concentration and uptake by crown gall cells at *p*H 4.3 and *p*H 6.5. The experimental procedure was as described in Fig. 1. Three min incubation times were used. IAA concentrations up to 2.0 μM were achieved by addition of undiluted radioactive IAA. For higher concentrations, the radioactive IAA (0.31 μCi) was first diluted with the potassium salt of unlabelled IAA (British Drug Houses Ltd., twice recrystallized from water). The total volume of the incubation was kept constant at 6 ml by adjusting the amount of water added. The inset shows uptake at *p*H 6.5 for 0 to 2.0 μM IAA. ●, *p*H 6.5; ■, *p*H 4.30.

contain a high proportion of negatively charged pectic polysaccharides but the cell wall of resting, non-flocculent, baker's yeast consists predominantly of neutral glucan and mannan with only a small proportion (up to 0.3%) of phosphorus[15]. The charge at plant cell surfaces is likely to be considerably more negative than at the surfaces of resting yeast cells. The local ionic concentrations near the surface of a particle are determined by the zeta potential. The *p*H at a charged surface is different from the *p*H in the bulk phase and is a function of the zeta potential at the surface[16]. At 25° C, and measuring the zeta potential in mV

$$pH_{surface} = pH_{bulk} + \frac{\zeta}{60} \qquad (2)$$

A negatively charged surface will have a negative zeta potential and so a lower *p*H than the bulk phase because in a given time interval, more positive ions, including hydrogen ions, will be near the surface rather than far away from it giving rise to an electrical double layer[17]. Increased proportions, relative to H^+, of other cations will make the zeta potential less negative and thus decrease this *p*H difference[17,18]. Danielli has treated the phenomenon as a special case of the Donnan equilibrium[19]. Peters[20] has shown that the absorption of stearic acid at a benzene/water interface led to the *p*H at the interface being about 3 units lower than the *p*H in the bulk aqueous phase, resulting in a shift in the apparent *p*K of stearic acid from 4.8 to 7.5. *p*H shifts of this type can be simply demonstrated[21] by shaking with benzene an aqueous solution (*p*H 7.4) of the acidic indicator bromthymol blue (*p*K 7.1) which has a sulphonic acid group as well as the carboxyl group responsible for its *p*H-dependent colour changes. As an emulsion is formed, the colour changes from blue to yellow and back again on separation of the two phases. This represents a *p*H shift of about 1.5 units at the interfacial due to the adsorbtion of negatively charged indicator molecules.

Although surface *p*H effects have been discussed in relation to the properties of enzymes[21,22], no account has previously been taken of them in the interpretation of uptake of weak acids and bases by cells. Indeed, the general biological significance of surface *p*H effects does not seem to be widely realized. For instance, although it has been suggested[23] that teichoic acids may help maintain bacterial surface enzyme systems in an appropriate environment of Mg^{2+}, their influence on the surface *p*H may also be important. In the experiments described here, the displacement of the uptake curve from the theoretical dissociation curve of IAA could simply be due to the *p*H within the cell wall adjacent to the plasma membrane being about 0.5 units lower than the bulk *p*H resulting in an increase in the apparent *p*K of IAA. The corresponding zeta potential would fall within the range found for cell surfaces by electrokinetic measurements[18]. Pectin is 50% dissociated around *p*H 2.95 (ref. 24) so that its contribution to the zeta potential, and

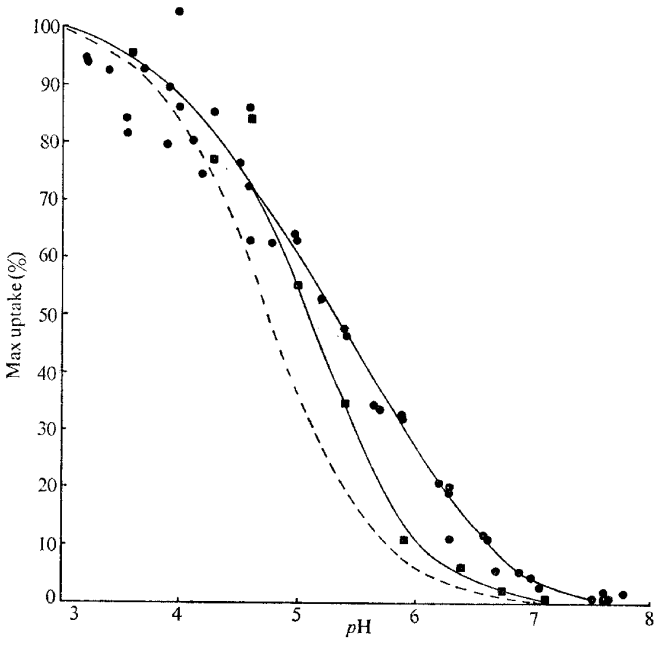

Fig. 3 The effect of *p*H on IAA uptake by crown gall cells from a 1.0 μM solution and from a 50.0 μM solution. Three min incubation times were used except for *p*H above *p*H 6.5 when the incubation time was 5 min. The uptake was measured as described in Fig. 1. ●, Experimental curve, 1.0 μM IAA; ■, experimental curve, 50.0 μM IAA; - - - , theoretial curve, *p*K 4.75.

therefore to the *p*H differential, would only start to show dependence on the bulk *p*H below about *p*H 4 (ref. 18).

Most cells, whether walled or not, have negatively charged surface groups[25]. The net charge is also dependent, presumably, on the isoelectric points of membrane proteins and on the relative amounts of different classes of phospholipid present as well as on the membrane potential. Resting yeast cells may be exceptional in having a low surface charge so that little or no displacement of the uptake curve from the IAA dissociation curve is seen. In another resting system, insect eggs[26], the *p*H dependence of the toxic effect of 3,5-dinitrocresol exactly follows its dissociation curve so that the surface charge may again be low.

At *p*H values lower than about *p*K+1, the plasmalemma is "transparent" to IAA and also to the other acidic plant hormones, abscisic acid and the gibberellic acids, whose undissociated forms are lipid-soluble. The extracellular *p*H of plant

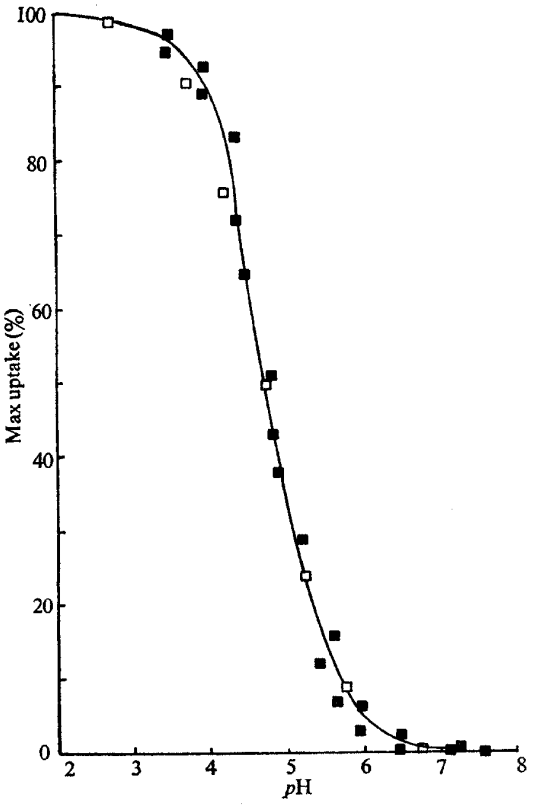

Fig. 4 The effect of *p*H on IAA uptake by resting yeast cells from a 1 μM solution. A stock suspension of pressed baker's yeast cells in distilled water (100 mg ml^{-1}) was kept at 25° C in the shaking water bath. The effect of *p*H on uptake of 1-^{14}C-IAA was determined following the procedure used for crown gall cells (Figs 1 and 3). ■, Experimental curve; □, theoretical points, *p*K 4.75.

cells *in vivo* must depend to a large extent on the acidic pectic substances in the cell wall. Hormone uptake will be affected by changes in wall *p*H due to changing concentrations of cations and also by the degree of methyl esterification of pectin which changes during development[27].

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