SHORT COMMUNICATIONS

BBA 63399

δ-Aminolevulinate dehydratase from ox liver and tobacco leaves*

The enzyme δ-aminolevulinate dehydratase (δ-aminolevulinate hydro-lyase, EC 4 2.1 24) has been purified from animal[1], as well as from plant, tissue[2] This enzyme isolated from ox liver[1] was found to be completely inactive in Tris buffer, although it was active in a buffer mixture of Tris and phosphate, arsenate or bicarbonate buffers at the same pH The optimum pH value for the enzyme from ox liver was 6 7 in phosphate buffer

δ-Aminolevulinate dehydratase was isolated from tobacco (*Nicotiana tabacum* L var Havana-38) The tobacco enzyme was similar in characteristics to the ox liver enzyme It was active in a buffer mixture of Tris with phosphate, arsenate or bicarbonate buffers but not in Tris–HCl alone Purification and general properties of the tobacco leaf enzyme are reported elsewhere[2] The experiments reported here illustrate that δ-aminolevulinate dehydratases from both ox liver and tobacco leaf were active in Tris–HCl buffer but at a different pH than in phosphate buffer

Enzyme activity was measured according to the method described by MAUZERALL AND GRANICK[3] A standard reaction mixture contained 10 mM each of GSH, of δ-aminolevulinate hydrochloride (neutralized with NaOH) and of Mg^{2+} in addition to buffer (pH 7 4), and enzyme was extracted in a final volume of 1 0 ml. Incubation was at 37° for 30 min The reaction was stopped by the addition of an equal volume of trichloroacetic acid containing $HgCl_2$ (10% trichloroacetic acid and 0 1 M $HgCl_2$ (4 1, by vol)) Protein was determined by the method of LOWRY *et al.*[4] A unit of enzyme activity is defined as that amount of enzyme which produced 1 nmole of porphobilinogen in 60 min, and specific activity is the number of units per mg protein

Purified tobacco leaf δ-aminolevulinate dehydratase (50-fold) did not exhibit activity when it was dialyzed against 2 mM Tris–HCl buffer (pH 7 4) The use of Sephadex G-25 gel filtration equilibrated with 1 mM Tris–HCl containing 1 mM cysteine also gave inactive fractions However, the dialyzed extracts or those eluted from Sephadex exhibited good activity in a mixture of Tris–HCl and phosphate buffers at the same pH (pH 7 4)

Arsenate and bicarbonate buffers behaved similar to the phosphate buffer in this respect In order to determine whether the activation by phosphate was due to an anion effect, a cation effect or a reversal of inhibition by Tris buffer, 25-fold purified tobacco leaf δ-aminolevulinate dehydratase was incubated with different phosphate buffers Enzyme activity was found only in reaction mixtures containing phosphate (see Table I) In separate experiments it was found that enzyme activity was pro-

* Utah Agricultural Experiment Station paper No 888

TABLE I

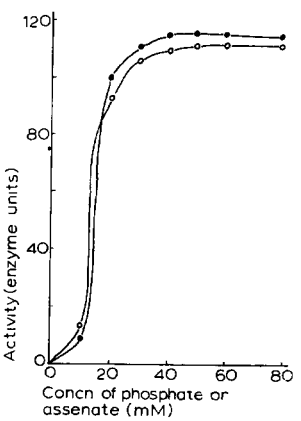
ACTION OF PHOSPHATE ON ENZYME ACTIVITY

0 3 ml dialyzed, 25-fold purified tobacco leaf enzyme (0 81 mg protein per ml) incubated at 37° for 30 min, with 10 mM each of GSH, Mg^{2+}, δ-aminolevulinate and 30 mM buffer at pH 7 4

Compound used	*Concn (mM)*	*Activity (enzyme units)*
Tris–HCl	30	6
NaCl	30	7
KCl	30	6
Na_2HPO_4–NaH_2PO_4	30	127
K_2HPO_4–KH_2PO_4	30	131

portional to the phosphate concentration and was independent of the Tris concentration When Tris–HCl buffer was compared with Tris–H_3PO_4, potassium phosphate and sodium phosphate buffers, enzyme activity was found to be the same regardless of the source of phosphate Neither Na^+, K^+, Tris nor Cl^- were activators. This is in contrast to the reported behavior of δ-aminolevulinate dehydratase from *Rhodopseudomonas spheroides*[5,6], in that the bacterial enzyme exhibited a monovalent cation requirement and did not exhibit a phosphate requirement

Fig 1 shows the effect of phosphate and arsenate buffers on tobacco leaf δ-amino-



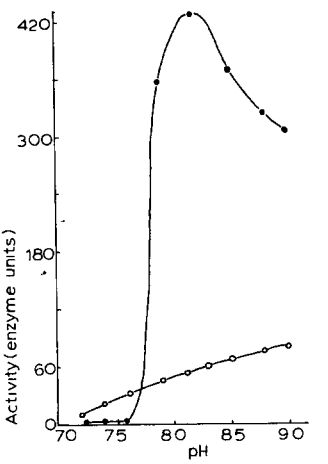


Fig 1 Tobacco leaf δ-aminolevulinate dehydratase activity as a function of phosphate and arsenate concentrations Reaction mixture contained 10 mM each of GSH, δ-aminolevulinate, Mg^{2+}, 100 mM total buffer (phosphate or arsenate as indicated and deficit made up by Tris–HCl buffer) and 0 2 ml 50-fold purified dialyzed enzyme (0 47 mg protein per ml) Incubation for 30 min at 37° and at pH 7 4 ○—○, arsenate, ●—●, phosphate

Fig 2 pH–activity curve for δ-aminolevulinate dehydratase from ox liver and tobacco leaf in Tris–HCl buffer Incubation mixture contained 40 mM Tris–HCl buffer, 10 mM each of GSH, δ-aminolevulinate, Mg^{2+} and 0 2 ml enzyme in a final volume of 1 ml Tobacco leaf enzyme used was 50-fold purified (0 47 mg protein per ml) Ox liver enzyme was 40–55% $(NH_4)_2SO_4$ precipitate of liver homogenate Incubation for 30 min at 37° and at the pH value indicated ○—○, tobacco leaf enzyme, ●—●, ox liver enzyme

levulinate dehydratase in Tris buffer (pH 7 4) Both phosphate and arsenate had a similar influence on the activity A saturation level was reached around 10 mM, indicating a critical threshold level for these anions beyond which there was a sharp increase in the activity K_m values from this figure for phosphate and arsenate were 15 and 13 mM, respectively

It was found that the tobacco enzyme was highly active in Tris buffer above pH 8 0, although it had a pH optimum of 7 4 in phosphate buffers[2] Fig 2 illustrates the results obtained in experiments designed to study the optimal pH range for tobacco leaf and ox liver δ-aminolevulinate dehydratases in Tris–HCl buffer The ox liver enzyme was found to be completely inactive in Tris buffer up to pH 7 6 Activity was exhibited beyond this value with an optimum around pH 8 2 The tobacco leaf enzyme did not show any peak but increased in activity in Tris–HCl up to a pH value of 9 0 The specific activities of the tobacco leaf enzyme in phosphate buffer (pH 7 4) and in Tris–HCl buffer (pH 9 0) were 1200 and 850 units/mg protein, respectively

From the results of this investigation, it appears that the change in activity with different buffers was a function of the pH It is probably much more complicated, since phosphate and related anions seem to activate the enzyme from both ox liver and tobacco leaf in the presence of Tris–HCl The results are suggestive that phosphate (also arsenate or bicarbonate) induce an enzyme configuration that is active This configuration is found with Tris–HCl only at high pH values To bring about such a change in the configuration, it appears that at least a minimum concentration of 10 mM phosphate is required

The pK value of Tris may also explain its effect on enzyme activity Below its pK value, Tris is predominantly in the form of its bulky quaternary ammonium form $(CH_2OH)_3CNH_3^+$ This positive ion may be complexing with the substrate δ-aminolevulinate at its free carboxyl group A free carboxyl group is known to be necessary for binding the substrate on to the active site of the enzyme[5] In the presence of phosphate, substrate and phosphate may compete for the cationic species of Tris

In conclusion, δ-aminolevulinate dehydratase from ox liver and tobacco were active in either phosphate or Tris buffers Only the pH optimum was changed

This investigation was supported in part by AEC Grant AT (11-1)-1287

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Received March 31st, 1969

Biochim Biophys Acta, 185 (1969) 458–460