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STIMULATION OF INDOLEACETIC ACID OXIDASE OF BEAN PLANTS BY NAPHTHENATES

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Abstract—The roots of 13-day-old etiolated bush beans plants (*Phaseolus vulgaris* L. cv Top Crop) were immersed in 100 ppm solutions of potassium naphthenate, potassium cyclohexanecarboxylate (KCHC), or potassium cyclopentylacetate (KCPAc). After 24 hr, each treatment resulted in significant increases in the *in vivo* activity of IAA oxidases in the epicotyls. However, the greatest stimulatory effect was obtained with the use of KCHC and KCPAc. Enzyme activity was not affected significantly by the addition of either KCHC or KCPAc to cell-free extracts of untreated plant tissue. The results suggest that the stimulation of enzyme activity was associated with the presence of naphthenate metabolites rather than with the free acids, and treatment may influence plant growth by affecting auxin metabolism.

INTRODUCTION

Alkylated alicyclic monocarboxylic acids present in alkaline extracts derived from crude petroleum and several of its by-products are collectively referred to as the naphthenic acid fraction [1]. Despite the complex nature of this petrochemical mixture, attempts to resolve individual components of the mixture have been successful [1, 2]. Further, Goldstein and Waddams [3] and others [4] have stated that the mixture contains a variety of low MW derivatives of cyclopentane and cyclohexane, as well as compounds with MW's of over 1000. Stimulation of growth and metabolism of many plant species following the application of the naphthenic acid mixture has been well documented [5–7]. Increased growth and metabolic rates of bean plants which were treated with the individual naphthenic acid, cyclohexanecarboxylic acid, have also been reported [8].

In a recent publication Wort *et al.* [9] reported that the mechanism of plant growth stimulation by naphthenic acid involved an enhancement of metabolism at both genetic and metabolic levels. Increased protein and nucleic acid content, as well as increases in the sp. act. of numerous enzymes, supported this view. Other authors have also observed that naphthenate treatment can augment photosynthesis and dark respiration [5], phosphorus assimilation [10, 11], and glucose uptake and metabolism [12].

In 1966, Babaev [13] reported that soaking cotton seeds in a 10 ppm solution of naphthenic acid, and the application of the acid to the soil (20 mg/kg dry soil) stimulated peroxidase activity in the roots. More recently, Loh [14], utilizing the root initiation and straight-growth bioassays, demonstrated that the naphthenic acid mixture possessed auxin-like properties. However, results of the two

auxin bioassays indicated that the auxinic properties of naphthenic acid were less than either IAA or indolebutyric acid. The immersion of the seeds of *Phaseolus vulgaris* in a 100 ppm naphthenate solution for 12 hr prior to sowing resulted in a 140% stimulation of IAA synthesis determined in the epicotyls of 14-day-old bean seedlings [15]. The present paper describes the effects of the naphthenic acid mixture and two individual naphthenic acids on the activity of IAA oxidases in etiolated bush bean seedlings.

RESULTS

The mean activity of IAA oxidases measured in the epicotyls of plants whose roots were immersed in a potassium naphthenate (KNap) solution for 24 hr was greater than the mean control value by 4·1% in 15 determinations, significant at the $P = 0{\cdot}01$ level.

For the *in vivo* effects of two individual naphthenic acids, viz. potassium cyclohexanecarboxylate (KCHC) and potassium cyclopentylacetate (KCPAc), on the activity of IAA oxidases, both naphthenate treatments significantly ($P = 0{\cdot}05$) increased the sp. act. of IAA oxidases in etiolated epicotyl tissue. The sp. act. of IAA oxidases in plants treated with KCHC was greater by 23·4%; the increase observed in KCPAc-treated plants was 19·3%. The greater stimulative effect was obtained with the use of the cyclohexyl derivative. The *in vitro* enzyme activity was not affected significantly by the addition of either KCHC or KCPAc to the cell-free extract of epicotyl tissue of untreated plants.

That the naphthenate anions, and not the K^+ cation were responsible for the observed increases in the sp. act. of IAA oxidases was determined by assaying the enzymes in a cell-free extract of 14-day-old plants whose roots were immersed in KCl ($7{\cdot}8 \times 10^{-4}$ M) for 24 hr in the dark. This finding supports the observation of Wort [6]. In this experiment the author reported that no stimulation was obtained in bean plants which had received KCl in the form of a foliar spray.

DISCUSSION

Biosynthesis of IAA oxidases in higher plants is regulated by IAA [16], and their activity increases to a certain extent with an increase in IAA concentration [17]. By virtue of their role in limiting the endogenous level of IAA within the plant, IAA oxidases have been recognized as plant growth regulatory enzymes [18]. The significantly greater IAA oxidase activity in KNap-, KCHC- and KCPAc-treated plants (4, 19 and 23%, respectively) is in agreement with the observed increases in peroxidase activity in the roots of seed-treated cotton plants [13], and in the foliage [19]. On a percentage basis the greater stimulation of IAA oxidase activity in KCHC-treated bean plants suggests again that this naphthenic acid is the most biologically active form of the individual acids tested to date (Severson, unpublished data). Similar results have been reported for the reproductive growth of bean plants [8], and glucose metabolism in bean root tips [12].

In a study of glucose-[^{14}C] metabolism in KNap- and KCHC-treated bean root tips Severson [12] reported that the content of labeled serine was significantly greater in each case. This observation may be related to a more rapid synthesis of tryptophan, an important intermediate in the IAA biosynthetic pathway, and subsequently may account for the reported increase of IAA in KNap-treated bean plants [15]. Loh [15] reported that the sp. act. of enzymes involved in the conversion of tryptophan to IAA was significantly greater in KNap-treated bean plants. The observed increases in IAA oxidase activity in the present experiment may well be the result of augmented IAA levels. The increased IAA oxidase activity in turn regulates the endogenous effective concentration of IAA for increased growth activity. The stimulation of plant growth following the application of KNap has been demonstrated [6, 7].

That the conjugated form of naphthenate is the biologically active form *in vivo* was suggested by Severson [12] and Wort *et al.* [9]. This followed the discovery by Severson *et al.* [20] and Padmanabhan [21] that ^{14}C labeled KCHC administered to bean plants was converted to several radioactive metabolites. In these experiments it was determined that the majority of the ^{14}C label was present in the glucose ester and aspartic acid amide conjugates. Results of the present study suggest also that the metabolites of the parent naphthenate compounds may be responsible for the observed stimulation of IAA oxidase activity. The lack of *in vitro* enzyme stimulation and the same

reported by Wort *et al.* [9] lend support to this view.

It has also been demonstrated that in many plant species the level of endogenous IAA can be regulated by a chemical conjugation mechanism involving glucose [22], inositol [23], and/or aspartic acid [24]. Further, it has been reported that IAA may be physically bound forming an IAA-protein adsorption product [25]. To further our understanding of the mechanism of naphthenate action in plant growth stimulation, future research should attempt to elucidate the effect which naphthenate and its metabolites have on the formation of auxin complexes.

EXPERIMENTAL

Seeds of the dwarf bush bean (*Phaseolus vulgaris* L. cv Top Crop) were grown in flats containing vermiculite in the dark for 13 days at 23°. Plants were then removed and divided into 5 groups. Roots were treated by immersion in (1) H_2O (control), (2) 10^{-3} M (100 ppm) naphthenic acid (practical) (3) $7{\cdot}8 \times 10^{-4}$ M (100 ppm) cyclohexanecarboxylic acid (4) $7{\cdot}8 \times 10^{-4}$ M (100 ppm) cyclopentylacetic acid or (5) $7{\cdot}8 \times 10^{-4}$ M KCl in the dark. The salt of each acid was prepared by neutralization with an equimolar amount of KOH.

Effect of KNap on IAA oxidase activity. After 24 hr, 10 g etiolated epicotyls (terminal 5–8 cm) including leaves were harvested, frozen with liquid N_2 and ground to fine powder. The powder was suspended in 50 ml cold 20 mM Pi buffer at pH 6·1, stirred for 1·5 hr at 4°, and centrifuged at 18000 *g* for 10 min. $(NH_4)_2SO_4$ was added to the supernatant to 35 and to 70% saturation. The ppt containing the enzymes was recovered by centrifugation at 18000 *g* for 10 min, resuspended in 12·5 ml buffer, and dialyzed for 24 hr at 4° with 1 change of buffer. IAA oxidase activity was determined by the method of ref [26]. The reaction mixture consisted of: enzyme extract (0·5 ml), 1 mM 2,4-dichlorophenol, Na salt (0·25 ml), 1 mM IAA in 0·5 mM $MnCl_2$ (1:1, v/v) (1·0 ml), and 20 mM Pi buffer (3·25 ml) at pH 6·1. The reaction mixture was shaken for 2 hr in the dark and then 1 ml Salkowski reagent was added. After shaking for 3 hr, A at 525 nm was determined.

Effect of KCHC and KCPAc on IAA oxidase activity. After 24 hr, 30 g etiolated epicotyl tissue (terminal 10 cm) were collected from plants in each treatment group. For each treatment the finely chopped tissue was homogenized at high speed in H_2O at 4° for 3 min. The homogenate was filtered through cheesecloth and centrifuged at 10000 *g* for 15 min. Me_2CO was added to the resulting supernatant to 40% final concn. After centrifuging at 600 *g* for 15 min, the pellet was suspended in 15 ml cold 0·2 M Pi–0·1 M citrate buffer at pH 5·6. IAA oxidase activity was measured by the procedure of ref [27]. The reaction mixture contained: enzyme extract (1 ml), 200 μg IAA (1 ml), and 0·2 M Pi–citrate buffer (pH 5·6) in a total vol of 10 ml. Using Salkowski reagent, residual IAA in the reaction mixture was determined colorimetrically. On a fr. wt basis, the sp. act. of IAA oxidase is calculated in terms of μg IAA destroyed per mg protein per hr. Protein was determined by the method of ref. [28]. *In vitro* effect of KCHC and KCPAc on enzyme activity was determined by the addition of sufficient KCHC or KCPAc to assay mixtures to give final concs of 5×10^{-5} M. In these assays the cell-free supernatants were obtained from plants whose roots had been immersed in H_2O for 24 hr. There were 5 replicates within each treatment, and all measurements were made in duplicate.

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