INFLUENCE OF GROWTH REGULATORS ON LIPASE ACTIVITY II.

M. M. Rakhimov, É. I. Aizikov, and P. Kh. Yuldashev

UDC 577.15.04+577.153

In a preceding paper [1] it was shown that in the sprouting of seeds growth regulators change the level of lipase activity in them. These changes are not connected with the action of the growth regulators on lipolytic enzymes and bear a mediated nature.

A number of chemical compounds act directly on lipases and thereby change the rate of lipolysis. The present paper describes the regulator properties of fatty-acid derivatives.

Figure 1 (curve 1) shows the action of different concentrations of phenylbutyric acid on the rate of hydrolysis of tributyrin catalyzed by cottonseed lipase [2]. Three zones differing in the nature of the change in lipase activity as a function of the concentration of the phenylbutyric acid can be clearly seen. The first zone is inhibitory; additions of phenylbutyric acid up to a concentration of $5 \cdot 10^{-5}$ M (minimum in the lipase activity) lead to a decrease in the rate of lipolysis; then the activity rises, reaching its initial level ($1.8 \cdot 10^{-4}$ M). The second zone is activatory; the activity of the lipase rises sharply, reaching a maximum value at a concentration of phenylbutyric acid of $4.2 \cdot 10^{-4}$ M. Above this concentration, the activity decreases and an inhibition of the lipase takes place (third zone).

This nature of the influence of phenylbutyric acid on the rate of lipolysis shows that it can exert a stimulating or an inhibiting effect depending on the concentration of the growth regulator. At a given concentration of the growth regulator, an inhibition of growth is observed for some plants and a stimulation for others. This is due, on the one hand, to difference in the lipases of these plants and, on the other hand, to differences in concentrations between the inhibitory (1st) zone and the stimulatory (2nd) zone for the chemical agent investigated.

Table 1 gives the concentrations of some compounds with inhibiting (C_{min}) and activating (C_{max}) effects, and also the differences in the concentrations between the inhibitory and stimulatory zones ($\Delta C = C_{max} - C_{min}$).

The figures given show that butyric acid (Fig. 1, curve 2) cannot be a growth stimulator. An increase in the length of the hydrophobic part of the molecule by a methyl group (Fig. 1, curve 3) leads to the appearance of an activatory zone (valeric acid). The increase in the activatory zone is greater if instead of a methyl group a phenyl group is introduced (phenylbutyric acid). When an amino group is introduced into the same position, the inhibitory zone disappears (Fig. 1, curve 4). This compound cannot be a growth inhibitor. The location of the amino group in the α position relative to the carboxy group enhances the activating capacity of the compound (Fig. 1, curve 5). Growth stimulation is possible even at far lower concentrations ($C_{max} = 8 \cdot 10^{-5}$ M). This compound also has no inhibiting zone.

With an increase in the length of the hydrophobic part of the molecule not only does the position of the activatory zone change [C_{max} decreases in the sequence phenylbutyric, n-valeric, δ-phenylvaleric, β-(4-hydroxy-3-methylphenyl)butyric acids] but the efficiency of inhibition rises [C_{min} decreases in the sequence butyric, n-valeric, γ-phenylbutyric, β-(4-hydroxy-3-methylphenyl)butyric, δ-phenylvaleric acids].

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 100-103, January-February, 1972. Original article submitted October 4, 1971.

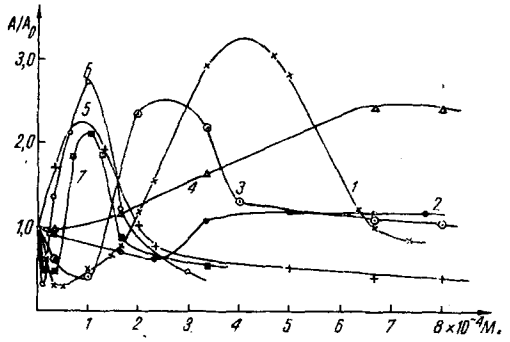


Fig. 1

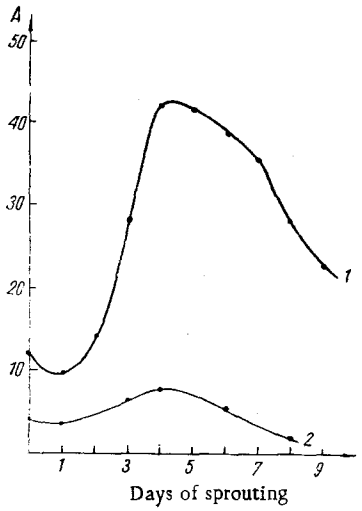
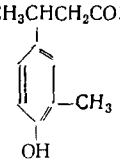


Fig. 2

Fig. 1. Influence of fatty acids on the activity of cottonseed lipase: 1) γ-phenylbutyric acid; 2) butyric acid; 3) valeric acid; 4) γ-aminobutyric acid; 5) α-aminobutyric acid; 6) δ-phenylvaleric acid; 7) β-(4-hydroxy-3-methylphenyl)butyric acid.

Fig. 2. Action of different concentrations of phenylbutyric acid on the lipase activity of sprouting seeds. 1) $9.6 \cdot 10^{-5}$ M; 2) $5.0 \cdot 10^{-4}$ M.

TABLE 1

Compound	C_{min}, M	C_{max}, M	ΔC, M
$CH_3CH_2CH_2COOH$	$2{,}2 \cdot 10^{-4}$	None	—
$NH_2CH_2CH_2CH_2COOH$	None	$6{,}6 \cdot 10^{-4}$	—
$CH_3CH_2CH(NH_2)COOH$	None	$8{,}5 \cdot 10^{-5}$	—
$C_6H_5CH_2CH_2CH_2COOH$	$5 \cdot 10^{-5}$	$4{,}2 \cdot 10^{-4}$	$37 \cdot 10^{-5}$
$CH_3CH_2CH_2CH_2COOH$	$7 \cdot 10^{-5}$	$2{,}7 \cdot 10^{-4}$	$20 \cdot 10^{-5}$
$C_6H_5CH_2CH_2CH_2CH_2COOH$	$0{,}8 \cdot 10^{-5}$	$1{,}10 \cdot 10^{-4}$	$9{,}2 \cdot 10^{-5}$
CH_3CHCH_2COOH	$3 \cdot 10^{-5}$	$1 \cdot 10^{-4}$	$7 \cdot 10^{-5}$
			

Thus, with an increase in the length of the hydrophobic part of the molecule of a growth regulator the selectivity of the inhibitory and stimulatory effects rises. In this sense, a phenyl radical is preferable to a methyl radical, since the activatory zone is characterized by a higher stimulating action. Simultaneously with the increase in selectivity, the efficiency of the action of the compound rises. A lower concentration is sufficient to show the same degree of inhibition. Here an alkyl radical is preferable to the voluminous phenyl radical.

The presence of hydrophilic groups in the hydrophobic part of the molecule [the OH group in β-(4-hydroxy-3-methylphenyl)butyric acid and the NH_2 group in γ-aminobutyric acid] leads to a decrease in the efficiency of the inhibitory action (compare with phenylvaleric and butyric acids, respectively). However, the introduction of hydrophilic groups into the α position with respect to the carboxy group enhances the efficiency of the stimulating action. (Compare α-aminobutyric acid with butyric acid.)

Consequently, on the basis of the facts given above it is possible actually to predict not only whether a chemical compound is a growth stimulator but also the concentration of the solutions at which stimulation is possible.

Below experiments are described with sprouting seeds according to the results of a study of the behavior of the lipase in the presence of phenylbutyric acid. It follows from Table 1 that C_{max} for phenyl-

butyric acid is $4.2 \cdot 10^{-4}$ M. To ensure such a concentration in the seeds it was necessary to soak the seeds in a $9.6 \cdot 10^{-5}$ M ($\sim 1 \cdot 10^{-4}$ M) solution because of the osmotic difference in concentrations. At this concentration of phenylbutyric acid, good development of the seeds and a normal change in lipase activity was observed (Fig. 2, curve 1). Soaking the seeds at a concentration of phenylbutyric acid of $5 \cdot 10^{-4}$ M retarded their development, and the change in lipase activity was insignificant (Fig. 2, curve 2). Thus, the facts given show the existence of a correlation between the activatory action of fatty acid derivatives on the lipolytic enzymes of cotton seeds with the stimulatory action of these compounds in the sprouting of the seeds.

EXPERIMENTAL

The lipases were obtained from an acetone powder of cotton seeds as described previously [2]. The conditions for measuring the activity and the concentrations of the enzyme and substrate were the same as in the preceding work [1]; optimum pH 8.8; temperature 25°C; concentration of protein 0.33 mg/kg.

The tributyrin was purified by vacuum distillation (bp 192-195°C at 15 mm Hg).

The fatty-acid derivatives were given to us by Ch. Sh. Kadyrov. These derivatives were not additionally purified. Solutions of the required concentration were prepared in double-distilled water with the pH brought to 8.8.

The seeds soaked in solutions of phenylbutyric acid were sprouted on washed sand in the light at 25°C [3]. The conditions for the isolation of the lipases from the sprouts and the measurement of their activity were as described previously [1].

SUMMARY

1. Fatty acids inhibit or activate cottonseed lipases in dependence on their concentration.

2. The activating and inhibiting action of this class of compounds correlates with their stimulating and inhibiting activity in the sprouting of cotton seeds.

LITERATURE CITED

1. P. Kh. Yuldashev, M. M. Rakhimov, and É. I. Aizikov, Khim. Prirodn. Soedin., 7, 803 (1971).
2. M. M. Rakhimov, N. R. Dzhanbaeva, and P. Kh. Yuldashev, Khim. Prirodn. Soedin., 6, 642 (1970).
3. V. N. Vyas and K. D. Patel, J. Amer. Oil Chemists Soc., 46, 176 (1969).