SUMMARY

1. It has been established that the method of isolating ribosomes by precipitation with ethanol in the presence of Mg^{2+} can be used in the isolation of ribosomes from the seeds of other plants. The isolated 5S rRNAs have the same electrophoretic mobility, while the 5S rRNAs from plants differ sharply from the 5S rRNAs of yeast in electrophoretic mobility which is apparently due to differences in their secondary and tertiary structures.

2. In the sprouting of cotton seeds, the amount of total rRNAs first increases (two-day shoots) and then falls (seven-day shoots).

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METABOLITES OF THE PATHOGENIC FUNGUS *Verticillium dahliae*

VIII. INTERACTION OF THE PHYTOTOXIC METABOLITE PKZh-1 WITH Na^+,K^+-ATPase

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We have previously reported the isolation and the physicochemical properties and biological activity of a number of phytotoxic metabolites from the fungus *V. dahliae* [1-3]. In the present paper we have the results of a study of the interaction of the phytotoxic pigment PKZh-1 (I) and its chromophoric moiety (2,5,7-trihydroxy-1,4-naphthoquinone) (II) with Na^+,K^+-ATPase from various materials of plant and animal origin.

It is known that the active and passive transport of ions between cell and medium, and also between various elements of the cell is an important link in the regulation of the functional state of various tissues. Voluminous information exists on the active transport of Na^+ and K^+ ions through the plasmatic membranes of animal cells with the participation of Na^+,K^+-ATPase [4, 5]. For plant cells, proofs of the existence of transport systems, especially those bound to the plasmalemma, is more meager. Biochemical investigations of this type on plant material are complicated by the presence of cell walls in plants that interfere with the separation of the membranes [6]. The information in the literature is, in part, of contradictory nature and does not permit the drawing up of a general scheme as has been done for the Na^+,K^+-ATPase of animal materials [7].

Results have been obtained in a number of investigations which show the existence of Na^+,K^+-ATPase in plant materials [8-10], including cotton-plant roots [12] and its possible participation in the active transport of cations. On the other hand, it is known that the phototoxins produced by phytopathogenic microorganisms causir diseases of higher plants interfere with the active transport of Na^+, K^+, and H^+ ions in a number of cases [13, 14]. It has been established for the toxin from *Helminthosporium maydis* that this is due to its inhibiting action on K^+-dependent ATPase [15] which, in the opinion of the authors, is closely connected with the mechanism of the action of this toxin.

In the light of what has been said above, it appeared of interest to study the interaction of the phototoxic metabolite PKzh-1 isolated from the culture liquid of the fungus *V. dahliae* and its chromophoric moiety with th Na^+,K^+-ATPase of the root hairs of the cotton plant.

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TABLE 1. Inhibiting Action of Different Concentrations of I and II on the Activity of Na^+, K^+-ATPase from Materials of Animal Origin

Na^+,K^+-ATPase source	Na^+,K^+-ATPase activity for different concentrations of I and II in the incubation medium, % of source									
	$1\cdot10^{-4}$ M		$1\cdot10^{-5}$ M		$1\cdot10^{-6}$ M		$1\cdot10^{-7}$ M		$1\cdot10^{-8}$ M	
	I	II	I	II	I	II	I	II	I	II
Ox cerebral cortex	0	60,0	38,0	84,0	65,0	95,0	84,0	100,0	94,0	100,0
Bovine kidney	0	68,0	52,5	86,5	84,0	96,5	90,5	100,0	100,0	100,0
Rat cerebral cortex	0	62,3	45,4	83,7	79,8	96,7	89,0	100,0	96,0	100,0
Frog cerebral cortex	0	67,0	50,0	82,0	76,0	97,0	80,0	100,0	95,6	100,0
Tarantula ganglion	0	71,0	30,0	85,0	59,5	94,0	84,0	98 0	93,0	100,0

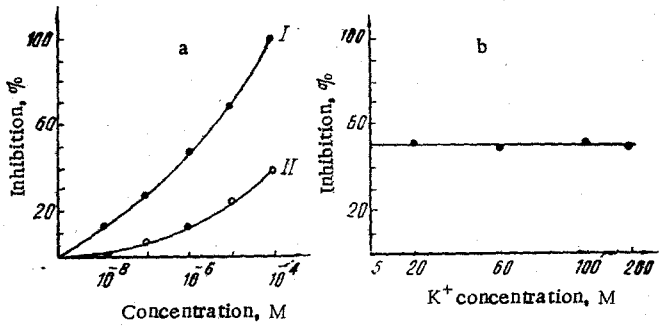

Fig. 1. Action of various concentrations of the phytotoxin PKZh-1 (I) and of 2,5,7-trihydroxy-1,4-naphthoquinone (II) on the ATPase activity of the microsomal fraction of the root hairs of the cotton plant (a) and absence of the influence of an increase in the concentration of K^+ in the incubation medium on the inhibition of the Na^+,K^+-ATPase by the phototoxin PKZh-1 (b).

A microsomal fraction possessing Na^+,K^+-ATPase activity was isolated from the root hairs of 10-day shoots of the cotton plant of variety 108-F by a modification of Skoy's method [16]. The ATPase activity was determined from the splitting off of inorganic phosphate from ATP, and the inorganic phosphate was determined by the Fiske–Subbarow method [17].

Figure 1a, shows the dependence on their concentrations in the incubation medium of the inhibiting action of (I) and (II) on the Na^+,K^+-ATPase of cotton-plant root hairs. It can be seen from this that high concentrations of (II) ($1\cdot10^{-4}$ M) suppress the activity of the Na^+,K^+-ATPase by 25-30% without at the same time affecting Mg^{2+}-ATPase; with a decrease in the concentration, this effect diminishes. At the same concentration, compound (I) causes 100% inhibition of Na^+,K^+-ATPase and partial inhibition of Mg^{2+}-ATPase (15-20%).

In order to determine the specificity of the action of (I) and (II) we have studied the interaction of these metabolites with the Na^+,K^+-ATPase of the microsomal fractions of the cells of the cerebral cortexes of the ox, the frog, and the rat, the bovine kidney, and tarantula ganglion.

As can be seen from Table 1, the nature of the interaction of (I) and (II) with the Na^+,K^+-ATPases from materials of animal origin is similar to that considered above, and in all cases (I) is more active than (II), which is apparently due to the presence of the peptide section in (I). The results obtained indicate the absence of any specificity in the interaction of (I) and (II) with Na^+,K^+-ATPase, the inhibiting action of (I) apparently being due to its capacity for disorganizing the phospholipid part of the complex, changing the conformation of the enzyme, and thereby inactivating it.

It is known that cardiac glycosides inhibit the activity of Na^+,K^+-ATPase, while this effect is overcome by increasing the concentration of K^+ ions in the medium, since cardiac glycosides and K^+ ions interact with the cation-sensitive center of the enzyme on the outer surface of membranes. To compare the nature of the action of (I) with that of cardiac glycosides it appeared of interest to study the influence of (I) on Na^+,K^+-ATPase with various amounts of K^+ in the incubation medium. In experiments with the Na^+,K^+-ATPase of the micro-

somal fraction of frog brain cells it was established that an increase in the concentration of K^+ does not reduce the inhibiting action of (I), which shows the absence of competition between (I) and K^+ cations (Fig. 1b).

The results obtained are similar to those on the action of a pathotoxin from Helminthosporium maydis on K^+-dependent ATPase of the microsomal fraction of the cells of maize root hairs and of the direct hemolytic factor (DHF) from snakes [18] on Na^+,K^+-ATPase.

Thus, the inhibiting action of the phototoxin PKZh-1 from the fungus V. dahliae on Na^+,K^+-ATPase of cotton-plant root hairs that we have established possibly leads to a disturbance in the normal ion metabolism in vivo and is one of the causes of its toxic action.

EXPERIMENTAL

A microsomal fraction possessing Na^+,K^+-ATPase activity was obtained from the root hairs of 10-day shoots of a cotton plant of variety 108-F by a modification of Skoy's method [16]. The washed root hairs were homogenized in 10 volumes of an ice-cold solution containing 250 mM sucrose, 1 mM EDTA, and 25 mM Tris-HCl (pH 7.4). The homogenate was centrifuged at 6000 rpm for 10 min, the precipitate was discarded, and the supernatant was centrifuged at 12,000 rpm for 30 min and then at 20,000 rpm for 1.5 h in a TsVR-1 centrifuge. The preparation obtained was stored at 2-4°C.

The microsomal fractions of the cells of the cerebral cortex of the ox, rat, and frog, of bovine kidney, and of tarantula ganglion possessing Na^+,K^+-ATPase activity were obtained by a published method [19].

Determination of ATPase Activity. The ATPase activities of the enzyme preparations were determined from the splitting out of inorganic phosphate from ATP after incubation at 37°C for 30 min, the inorganic phosphate being determined by the Fiske–Subbarow method [17]. The basic incubation medium contained 1 mM ATP, 2 mM Mg^{2+}, 100 mM Na^+, 20 mM K^+, and 30 mM Tris-HCl (pH 7.4). Compounds (I) and (II) were added to the incubation medium in the form of aqueous solutions to final concentrations of from $1 \cdot 10^{-8}$ M to $1 \cdot 10^{-4}$ M. ATP was added after preincubation with the enzyme for 30 min. The final volume of the reaction mixture was 1.0 ml. In each variant there was a control on the nonenzymatic decomposition of the ATP (incubation medium without the addition of the enzyme preparation). The experiments were performed in duplicate.

SUMMARY

It has been established that the phototoxic metabolite PKZh-1 of the fungus V. dahliae and, to a smaller extent, its chromophoric moiety possess an inhibiting action on the Na^+,K^+-ATPase of the microsomal fraction of the cells of the root hairs of the cotton plant of variety 108-F.

The absence of specificity in the action of the phytotoxin has been shown in experiments on the Na^+,K^+-ATPase of the microsomal fractions of the cells of the cerebral cortexes of the ox, rat, and frog, bovine kidney, and tarantula ganglion.

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OPTIMIZATION OF THE PROCESS OF EXTRACTING AMYLOLYTIC ENZYMES FROM THE TECHNICAL PRODUCT "AMILORIZIN-P_x"

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In the process of obtaining the medicinal enzyme preparation "Oraz," which has been approved for use in medical practice as an agent for the treatment of diseases of the gastrointestinal tract, one of the main steps is the aqueous extraction of the enzymes from the initial raw material "Amilorizin-P_x," which is a culture of the mold fungus Aspergillus oryzae surface-grown on wheat bran [OST (All-Union Standard) 59-6-72].

As the method of extracting the active substances from this raw material we selected extraction in a battery of percolators by the countercurrent principle which, in the opinion of a number of authors [1-3], ensures the most complete extraction of the active components both from plant medicinal raw material and from surface cultures of mold fungi.

In view of the fact that this process is a complex one and depends on various factors, it appeared desirable to carry out its optimization.

To solve this problem we used the method of mathematical planning of experiments with the aid of Latin squares [4, 5]. The process of extracting the amylolytic enzyme was studied as a function of the following factors, which, according to the literature and preliminary experimental results, play a fundamental part in it: A – the selected volume of the extract, ml; B – the number of percolators in a battery; and C – the time of steeping, min. The levels of the factors mentioned are given below:

Factor	Level of the factor			
A	200	400	600	800
B	2	3	4	6
C	10	15	20	25

As constant factors of the extraction process we took the temperature of extraction (20-25°C), the method of extraction (countercurrent), the sequence of technological operations in the subsequent isolation of the "Oraz" preparation, and also the nature of the extractant (water).

For planning the experiment we used a 4 × 4 Greco-Latin square according to a method described in the literature [5].

The optimization parameters were the saccharifying power of the "Oraz" preparation and its yield calculated to unit weight of the initial raw material, the values of which, according to a Provisional Pharmaceutical Article (VFS-42-570-76) should be not less than 200 units per gram of preparation and 1%, respectively.

The matrix for the planning of the experiment is given below (the results of the investigations are given in Table 1):

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