Action of actinomycin D on auxin-induced growth of *Avena* coleoptile section

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Abstract:

Double-stranded DNA and in a lesser degree ATP reduce the inhibitory action of actinomycin D on auxin-induced elongation growth. The reduction of inhibition by DNA is significant in the initial phase of elongation up to the 4th hour of growth. The effect of ATP is similar but less pronounced. Both substances applied jointly do not completely abolish the inhibitory action of actinomycin D.

The foregoing data and the kinetic characteristic of the auxin-induced elongation in the presence of actinomycin D suggest that this antibiotic inhibits growth by blocking synthesis of RNA essential for elongation growth and causes, moreover, certain distinct cellular changes which cannot be explained by the inhibition of DNA-dependent RNA synthesis.

INTRODUCTION

It is known that low actinomycin D (act. D) concentrations selectively inhibit cellular (Reich, Franklin, Shatkin and Tatum 1962) and enzymatic DNA-dependent RNA synthesis (Goldberg and Rabinowitz 1962; Hurwitz, Furth, Malamy and Alexander 1962). It also seems that the mechanism of inhibition as such has been elucidated. Reich, Goldberg and Rabinowitz (1962), Goldberg and Reich (1964), Haselkorn (1964) and Reich (1964) demonstrated, namely, that act. D makes RNA synthesis on native DNA template impossible since it forms a complex with the amine group of desoxyguanine in DNA.

A number of authors have demonstrated that act. D inhibits auxin-induced elongation of *Avena* coleoptile (Nooden and Thimann 1966; Masuda and Wada 1966; Buczek 1969a), soya and sunflower hypocotyls (Key 1964; Buczek 1969b) and of pea shoots (Bendana, Gakston, Kaur-Sawhney and Penny 1965; Penny and Galston 1966). The above named authors explained this fact by the dependence of the action of auxin (IAA) on simultaneous RNA synthesis.

The aim of the present study was the investigation whether inhibition by act. D of the IAA-induced elongation of *Avena* coleoptile may be explained by the sole

influence of this antibiotic on the DNA-dependent RNA synthesis, or whether act. D exerts some additional effect on elongation independently of RNA synthesis inhibition.

MATERIAL AND METHODS

The procedure for obtaining etiolated oats (*Avena sativa*, L. cv. Udyecz Żółty) has been described in a recent paper (Buczek 1969a). Preparation of sections for investigation was as follows: sections 5 mm in length were cut from the region 3 mm below the tip of a 3 cm long coleoptile. The primary leaves were removed and cylinders slipped on to small capillary tubes. The tubes with sections were soaked for about 3 hrs in redistilled water. Then the length of 20 sections was immediately measured under a binocular microscope. The remaining sections were placed in shallow Petri dishes containing 5 ml solution of 0.01 M K-phosphate buffer pH 6.1 and 0.01 M NaCl plus 10^{-5} M IAA or a buffer and NaCl plus IAA and the substances under investigation. After an appropriate time, the length of the sections was measured and their increment (Δl) calculated. All manipulations were carried out under weak green light (540–620 nm). Each sample consisted of 20 sections; all experiments were performed at least three times.

The following substances were used: 10^{-5} M indol-3-yl acetic acid (IAA), 10 $\mu\text{g/ml}$ actinomycin D (act. D), guanine and its derivatives (sodium salts) — (Calbiochem), 200 $\mu\text{g/ml}$ double-stranded deoxyribonucleic acid (DNA) ex calf-thymus (Koch-Light), 75 $\mu\text{g/ml}$ of low-polymerised deoxyribonucleic acid (Loba Chemie), 100 $\mu\text{g/ml}$ of beef pancreas deoxyribonuclease (DNAase) (Sigma). The enzyme was dissolved in 0.01 M K-phosphate buffer (pH 6.1) plus 0.01 M NaCl with addition of 0.005 M $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$.

RESULTS

The formation of a complex *in vitro* between act. D and DNA occurs between the chromophore group of actinomycin and the amine group of deoxyguanine in DNA (Goldberg et al. 1962; Hamilton et al. 1963; Reich 1964). Addition of appropriate DNA amounts to definite act. D levels produces progressive and more or less proportional changes in the spectrum up to complete saturation of the complex (Reich et al. 1962). The deoxyribonucleic acid concentration used in the present experiments was determined by measurement of changes in absorption associated with the formation of coloured complexes with a definite amount of act. D (10 $\mu\text{g/ml}$). As seen from diagram (Fig. 1) full saturation of the complex for low-polymerised DNA was obtained at a 75 $\mu\text{g/ml}$ concentration, whereas for double-strand DNA — after addition of 200 $\mu\text{g/ml}$.

In view of the ability of act. D of complex formation with DNA *in vitro* and the dependence of auxin-induced elongation on RNA synthesis, an experiment was carried out in which to a solution containing act. D in a concentration inhibiting the IAA-induced growth, deoxyribonucleic acid was added or respectively guanine

Fig. 1. Binding of actinomycin D by DNA was measured by the decrease in absorption at 440 nm after addition of a standard amount of DNA. All solutions contained 0.01M K-phosphate buffer, pH 6.1 plus 0.01 M NaCl. The concentration of act. D was 10 $\mu\text{g/ml}$.

1. Low-polymerised DNA; 2. Double-stranded DNA

and its derivatives. The author postulated that the formation of an exogenic complex with act. D should reduce the biological activity of the antibiotic. As seen from the data in Table 1, only double-stranded DNA reduced partly the inhibitory action of act. D on IAA-induced elongation. The low-polymerised DNA and guanine or its derivatives had no effect on the inhibition of elongation growth. Probably only double-stranded DNA is capable of forming a stable complex with act. D.

Table 1

Effect of 10 $\mu\text{g/ml}$ actinomycin D on growth of *Avena* coleoptile sections in the presence of 10^{-5}M IAA with or without guanine or its derivatives or deoxyribonucleic acids. Measurement after 24 hours

Treatment	Buffer Increase in length mm	Buffer+actinomycin D Increase in length mm	Inhibition %
IAA	3.16	1.54	51
IAA + guanine $5 \times 10^{-4}\text{M}$	4.68	1.78	62
IAA + guanosine $5 \times 10^{-4}\text{M}$	3.28	1.23	62
IAA + guanosine $5 \times 10^{-3}\text{M}$	3.00	1.17	61
IAA + guanosine-5'-monophosphate $5 \times 10^{-4}\text{M}$	3.85	0.96	75
IAA + guanosine-5'-triphosphate $5 \times 10^{-4}\text{M}$	4.44	1.57	65
IAA + deoxyguanosine $5 \times 10^{-4}\text{M}$	3.31	1.42	57
IAA + deoxyguanosine $5 \times 10^{-3}\text{M}$	2.98	1.28	57
IAA	3.24	0.95	71
IAA + low-polymerised DNA 75 $\mu\text{g/ml}$	2.98	1.10	63
IAA	2.66	0.98	62
IAA + double-stranded DNA 200 $\mu\text{g/ml}$	1.99	1.19	40

The data in diagram (Fig. 2a) show that the reduction of the inhibitory action of act. D on IAA-induced elongation in the presence of double-stranded DNA is almost complete in the initial phase of elongation. After 4-h incubation of the tissues

in an auxin solution with act. D and DNA added, elongation was inhibited only in 7 per cent (difference statistically not proved), whereas in the same time act. D reduced IAA-induced elongation by 47 per cent (difference statistically significant). After 8 and 24 h of elongation, only a slight but significant inhibition of growth is observed in tissues incubated in IAA solution plus act. D in the presence of DNA.

The application of DNA-ase indicates that it is actually double-stranded DNA which, forming a complex with act. D, reduces the inhibitory action of the antibiotic on elongation growth. This relation is visible in diagram (Fig. 2*b*). Act. D in the presence of double-stranded DNA and DNA-ase inhibited the IAA-induced elongation growth identically as in the solution to which DNA was not added. It is obvious that broken down by the enzyme double-stranded DNA was not capable of blocking the active group in act. D.

Fig. 2. Inhibitory effect of actinomycin D on IAA-induced elongation of coleoptile sections. A. Effect of double-stranded DNA; B. Effect of DNA and DNAase; C. Effect of ATP. D. Effect of DNA + ATP. Concentrations used: IAA — 10^{-5} M, DNA — 200 μ g/ml, DNAase — 100 μ g/ml, ATP — 5×10^{-4} M, act. D — 10 μ g/ml 1. IAA, 2. IAA + DNA, 3. IAA + DNA + act. D, 4. IAA + act. D, 5. IAA + DNA + DNAase, 6. IAA + DNA + act. D, 7. IAA + ATP, 8. IAA + ATP + act. D, 9. IAA + DNA + ATP, 10. IAA + DNA + ATP + act. D

Addition of ATP gave a similar effect as double-stranded DNA. This agent reduced effectively the inhibition by act. D of the IAA-induced elongation growth, but not as efficiently as did DNA (Fig. 2c). Noteworthy is the fact that the addition to the basic solution (IAA+act. D) of double-stranded DNA or ATP, respectively, or both together (Fig. 2d) does not completely abolish the inhibitory influence of act. D.

Fig. 3. Effect of pretreatment for various periods of time with 10^{-5} M IAA on the elongation of coleoptile sections in IAA with 10 μ g/ml actinomycin D.

1. IAA, 2. IAA + act. D (from start), 3. 1 h. IAA $\rightarrow$ IAA + act. D, 4. 2 h. IAA $\rightarrow$ IAA + act. D, 5. 3 h. IAA $\rightarrow$ IAA + act. D, 6. 4 h. IAA $\rightarrow$ IAA + act. D.

These experiments and the following ones seem to suggest that the inhibitory action of act. D on IAA-induced elongation is a complex process which cannot be attributed solely to the inhibitory influence of act. D on DNA-dependent RNA synthesis. This supposition is supported by experiments, the results of which are shown in diagram (fig. 3) and table 2. The experiments were run as follows. Coleoptile sections were placed in IAA solution or in the same solution plus act. D. The latter was added to the solution after 1, 2, 3 and 4 h, the increment of the sections was measured (Fig. 3) and the growth rate was calculated at 1-h intervals (Table 2). As seen from the data in diagram (Fig. 3) the addition of act. D to the IAA solution at the start of the experiment causes almost immediate inhibition of elongation. After a certain time the growth of the tissues is weaker, but it is parallel to that induced by auxin. About 4 h after the start, IAA-induced elongation in the presence of act. D is again markedly inhibited. The inhibition is more and more pronounced, and after 7 h it almost reaches maximum, the tissues cease almost completely to grow.

These relations are shown in table 2 in which the growth rate is calculated as the successive increments at 1-h intervals. A similar effect was obtained when to the IAA solution act. D was added at intervals not exceeding 2 h from the start of the experiment. When act. D was, however, added to the IAA solution after 3 or 4 hours of incubation in IAA alone, then for 8 h no inhibition was observed (differences nonsignificant). It appeared again after as long as 24 h of growth but it was incomparably weaker than the effect due to act. D present in the solution from the beginning or added 1–2 h after incubation of the tissues in IAA solution.

Table 2

Inhibitory effect of 10 µg/ml actinomycin D on growth rate of coleoptile sections preincubated in 10^{-5} M IAA for different periods

Preincubation	Time after addition of act. D	Treatment	Growth rate in successive 1-hr intervals from beginning of experiment							
			1	2	3	4	5	6	7	8
			mm/hr							
IAA	—	IAA	0.24	0.26	0.27	0.35	0.29	0.22	0.22	0.19
IAA+act. D	0	IAA+act. D	0.09	0.18	0.19	0.12	0.10	0.09	0.01	0.02
IAA	1	IAA+act. D	0.23	0.07	0.25	0.24	0.11	0.09	0.07	0.08
IAA	2	IAA+act. D	0.25	0.27	0.15	0.28	0.13	0.12	0.08	0.06
IAA	3	IAA+act. D	0.24	0.25	0.28	0.31	0.22	0.21	0.18	0.15
IAA	4	IAA+act. D	0.24	0.26	0.28	0.33	0.21	0.20	0.19	0.16

DISCUSSION

The above described results suggest that inhibition of IAA-induced elongation of *Avena* coleoptile sections due to the action of act. D is a complex process and is not solely caused by the blocking of DNA-dependent RNA synthesis. This assumption is based on the following facts. If we analyse the kinetics of tissue elongation in the presence of IAA and act. D, three steps may be distinguished in the action of the antibiotic: 1. strong inhibition of elongation growth occurring as it seems immediately after placing the tissues in the solution; 2. lack of noticeable inhibition up to 3 h from the beginning of the experiment; and 3. renewed strong inhibition of elongation, which leads to complete growth inhibition about 6 h after placing the tissues in IAA solution with act. D.

The primary effect of inhibition by act. D of the IAA-induced elongation, thus does not involve growth inhibition by the blocking of RNA synthesis by act. D, since the tissue reaction is almost immediate (Fig. 3, Table 2). It would rather seem that act. D. must influence some other processes associated with the initial phase of tissue growth. After one hour, absence of inhibition is in turn observed, and the tissues

continue to grow. According to the investigations of Masuda and Wada (1966) and Masuda et al. (1967), at this phase of growth of *Avena* tissues, act. D inhibits the synthesis of RNA indispensable for elongation, hence in the next phase of elongation (3 h after the start) the tissues cease to grow. The indispensability of RNA synthesis for the IAA-induced growth is confirmed by the fact that addition of act. D after 4 h of incubation of the tissues in auxin produces a very weak inhibition which seems rather to be associated with a secondary effect of act. D on growth, and not with its influence on RNA synthesis essential for growth.

The fact that IAA-induced elongation growth is dependent on RNA synthesis on native DNA template finds confirmation in experiments with double-stranded DNA. This substance forming an exogenic complex with act. D blocks the active groups in actinomycin (Reich 1964) making the formation of endogenic act. D complex with DNA template impossible. Experiments with DNA-ase confirm these suppositions. The role of ATP as a factor reducing the inhibitory action of act. D in the same phase of elongation of the *Avena* sections as does double-stranded DNA, remains obscure. Perhaps this phenomenon might be explained by the influence of act. D on the inhibition of oxidative phosphorylation. The effect of act. D on ATP synthesis has been demonstrated on the tissues of leukocytes by Laszlo, Miller, McCarty and Hochstein (1966). The problem requires, however, further studies on plant material.

The above reported experiments suggest that the action of auxin on tissue growth is associated at a certain phase of elongation with RNA transcription. The influence of auxin on processes controlling synthesis of RNA essential for elongation growth becomes manifest after about 2 h of incubation of the tissues in IAA solution. If at this phase of growth (up to 2 h from the beginning) RNA synthesis is blocked by the application of act. D, no influence of auxin on elongation is observed. On the other hand, placement of tissues in act. D solution after the phase of growth in which RNA synthesis essential for elongation occurs, does not significantly inhibit IAA activity. The slight inhibition observed may be attributed to a secondary effect of act. D.

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Działanie aktynomycyny D na indukowany auksyną wzrost wycinków koleoptili owsa

Streszczenie

Szereg autorów wykazało, że aktynomycyna D hamuje indukowane auksyną (IAA) wydłużanie koleoptili owsa, hypokotyli soi i słonecznika oraz łodyg grochu wyjaśniając ten fakt zależnością działania IAA od równoczesnej syntezy RNA. Celem pracy było zbadanie, czy hamowanie przez akt. D indukowanego auksyną wydłużania tkanek owsa da się wyjaśnić jedynie wpływem antybiotyku na zależną od DNA syntezę RNA, lub, czy akt. D wywiera ponadto dodatkowy wpływ na wydłużanie, niezależnie od hamowania syntezy RNA. Przeprowadzone badania pozwalają wyciągać następujące wnioski:

Dwuniciowy DNA oraz w mniejszym stopniu ATP redukują hamujące działanie aktynomycyny. D na indukowane auksyną wydłużanie tkanek.

Redukcja hamowania pod wpływem DNA jest istotna w początkowej fazie wydłużania do około 4 godziny wzrostu. Podobne, ale mniej efektywne jest działanie ATP. Obydwie substancje razem nie znoszą całkowicie hamującego działania akt. D

Powyższe dane oraz kinetyczna charakterystyka indukowanego auksyną wydłużania w obecności akt. D sugeruje, że antybiotyk ten hamuje wzrost na skutek blokady syntezy RNA istotnej dla wydłużania, a ponadto powoduje pewne wyraźne komórkowe zmiany, których nie da się wyjaśnić hamowaniem syntezy RNA zależnej od DNA.