INHIBITION OF DNA-DEPENDENT RNA POLYMERASE BY DERIVATIVES AND ANALOGUES OF THURINGIENSIN

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Structure requirements of the substrate binding site of DNA-dependent RNA polymerase were investigated. For this purpose several analogues and derivatives of thuringiensin modified in the acidic part of the molecule were tested as inhibitors of the enzyme. The substances show a different inhibitory activity depending on the type of modification. The relationship between the structure of the inhibitor and the extent of inhibition is discussed.

It has been shown in our previous papers[1,2] that the inhibition of *Escherichia coli* DNA-dependent RNA polymerase by thuringiensin (exotoxin from *Bacillus thuringiensis*) is due to competition of thuringiensin and ATP for the ATP-specific enzyme binding site. These results indicate that it is the adenine moiety of thuringiensin which provides for the base pairing with the complementary base of the template. This idea was verified in experiments with the thuringiensin altered in the base moiety or by the use of synthetic polydeoxyribonucleotides as templates[3]. Thus, the specific role of the base moiety of thuringiensin seems to be established. On the other hand, the role of the acidic part of thuringiensin molecule is less evident. Some evidence in this respect was obtained by application of thuringiensin derivatives and analogues altered in the acidic part of the molecule.

EXPERIMENTAL

Thuringiensin (I) was isolated from the cultivation medium of *B. thuringiensis* as described earlier[4].

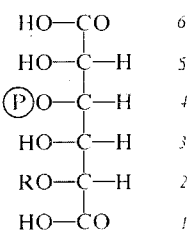
Thuringiensin lactone (II) was prepared by lactonisation of thuringiensin (50 mg) in an acidic medium (5% aqueous trifluoroacetic acid, 30 min at room temperature). The reaction mixture containing approximately equal amounts of the lactone and the unreacted starting material was separated by ion exchange chromatography (Dowex 1X2 formate column, linear gradient elution with 1M to 4·5M formic acid). The yield of the lactone (25%) was affected by hydrolytical cleavage during the isolation of the Aslactone. determined by means of analytical ion exchange chromatography, the thus-obtained lactone *II* contains 10% of thuringiensin (*I*). The identity of the lactone *II* was established mainly by IR spectroscopy in KBr micropellets. The spectrum exhibits a band at 1772 cm^{-1} due to five-membered lactones and a band at 1630 cm^{-1} typical of thuringiensin.

The UV spectrum, phosphorus content, and the adenine/phosphorus ratio remain the same as in the original thuringiensin. No other change of the molecule occurred during the lactonisation process since the resulting lactone may be quantitatively transformed into the original thuringiensin by opening the lactone ring in alkaline medium.

Thuringiensin amide (III) was prepared by opening the lactone ring of compound *II* (30 mg) in liquid ammonia (4 h). After evaporation of the ammonia, the residual amide *III* was purified by ion exchange chromatography. The reaction is almost quantitative. Similarly to the lactone *II*, the thus-obtained amide *III* is contaminated by 10% of thuringiensin as determined by analytical ion exchange chromatography. The IR spectrum of thuringiensin amide exhibits a band at 1650 cm^{-1} typical of amides. In paper electrophoresis at pH 3·8, the mobility of the amide *III* was lower than that of thuringiensin (0·76), in accordance with the lower acidity of the molecule. The other parts of the molecule remain intact as indicated *1*) by unchanged adenine/phosphorus ratio and *2*) by the formation of the original thuringiensin on hydrolysis of the amide *III* in a good yield.

Thuringiensin analogues IV–VI were prepared by the procedure[5] used in the synthesis of thuringiensin from the lactone *VII* except for replacement of this lactone by lactones *VIII*, *IX*, and *X*, resp.[6] Adenine/phosphorus ratio: 1·09 (D-glucaric acid-containing analogue *IV*), 1·10 (D-allonic acid-containing analogue *V*), and 1·13 (D-ribaric acid-containing analogue *VI*).

The preparation of E. coli DNA-*dependent* RNA *polymerase* and the enzyme assay were performed as reported previously[2]. In order to exclude the possible hydrolytical cleavage of the lactone *II* and the amide *III*, with the formation of thuringiensin (*I*) under conditions of the enzyme assay, both compounds were incubated in the assay mixture in the absence of the enzyme. As shown by analytical ion exchange chromatography, compounds *II* and *III* did not undergo any change under the above conditions.



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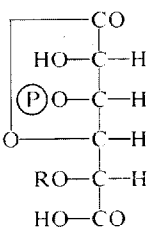

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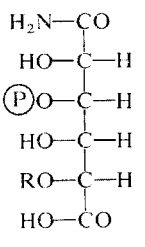

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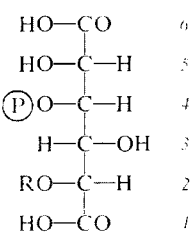

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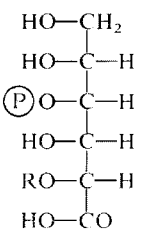

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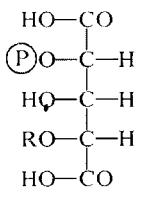
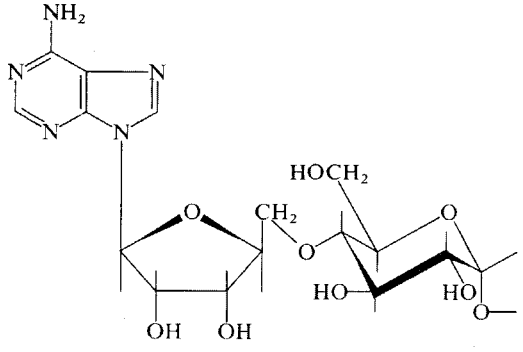
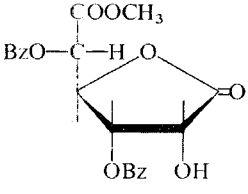


VI

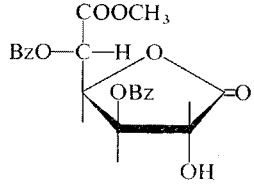
Ⓟ = $PO(OH)_2$



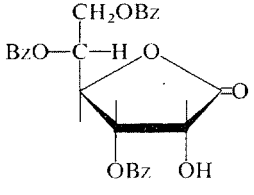
R



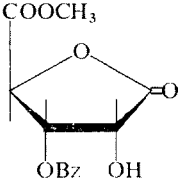
VII



VIII



IX



X

RESULTS AND DISCUSSION

For purposes of the present work, two derivatives and three analogues (altered in the allaric part of the molecule) of thuringiensin were prepared. The lactone *II* is also obtained in the course of isolation of thuringiensin from naturally occurring material. The amide *III* was prepared by opening the lactone ring in liquid ammonia. The D-allaric acid of the original thuringiensin was replaced by D-glucaric acid (in the analogue *IV*), D-allonic acid (in the analogue *V*), and D-ribaric acid (in the analogue *VI*). Since the analogues *IV*–*VI* are modified in the distant part of the molecule, the mutual orientation of most components of the molecule important for binding in the active centre of the enzyme (the heterocyclic base, the ribose residue, the carboxylic function of D-allaric acid at position 6, and the residue of phosphoric acid) remains virtually intact when compared with orientation in the parent thuringiensin molecule. In the case of the D-glucaric acid-containing analogue *IV*, the modification consists in an opposite configuration of the hydroxylic group at position $C_{(3)}$ when compared with the original D-allaric acid-containing thuringiensin. In the case of lactone *II*, the original flexibility of the D-allaric acid component bearing the phosphoric acid residue is somewhat restricted by closure of the five-membered lactone ring.

The derivatives *II* and *III* used to be contaminated with a small amount of compound *I* formed by hydrolysis. The contaminant is difficult to remove but the extent of contamination can be quantitatively determined by analytical ion exchange chromatography. In view of the synthetic procedure, the analogues *IV*–*VI* are homogeneous substances that do not contain any thuringiensin.

The inhibitory effects of compounds *II*–*VI* and the parent thuringiensin are shown on Fig. 1. In the case of derivatives *II* and *III* (Fig. 1*a*), the contamination with a small amount of thuringiensin must be taken into account. Inhibition due to the content of thuringiensin alone is designated by a dashed line. With respect to this circumstance, the inhibitory effect of derivatives *II* and *III* is approximately equal to that of analogues *IV* and *VI* (Fig. 1*b*). The D-allonic acid-containing analogue *V* shows a different behaviour since it does not inhibit the DNA dependent RNA polymerase under the concentration conditions examined (Fig. 1*b*).

With the knowledge on inhibitory effects of thuringiensin derivatives (*II* and *III*) and analogues (*IV*–*VI*), the structural requirements of the ATP binding site of DNA-dependent RNA polymerase can be defined with more precision. As reported earlier from this laboratory[1,2], the base moiety of thuringiensin is decisive for its attachment to the respective ribonucleoside triphosphate binding site on the enzyme molecule. Furthermore, the residue of phosphoric acid is absolutely indispensable for the binding since all the inhibitory activity is lost by dephosphorylation of thuringiensin.

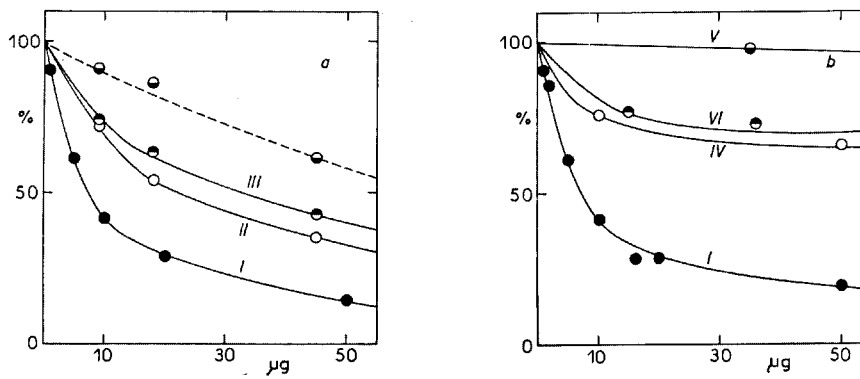


FIG. 1

Inhibition of DNA-Dependent RNA Polymerase by Derivatives and Analogues of Thuringiensin (*I*)

The reaction mixture (final volume, 0·25 ml) contained the salts, DNA, and the enzyme as described earlier[2]. Varied amounts of thuringiensin, its derivatives and analogues were added to the reaction mixture prior to the enzyme. The activity of the enzyme is expressed in %; the amount of the inhibitor is given in μg. *a*, effect of thuringiensin (*I*) and the derivatives *II* and *III*; the effect of the thuringiensin content in derivatives *II* and *III* is designated by the dashed line; *b*, effect of thuringiensin (*I*) and the analogues *IV*–*VI*.

As shown by experiments with the D-allonic acid-containing analogue *V*, the inhibitor binding requires the presence of a carbonyl group at the end which is more distant from the ethereal bond. The role of the less distant carboxylic function of D-allaric acid has not been so far examined because of the difficult accessibility of thuringiensin analogues modified at this place.

On the basis of the above results, the structural requirements of the ATP binding site appear considerably strict. Even a small deviation from the steric structure of thuringiensin such as reversal of the configuration of one hydroxylic function in D-allaric acid decreases the inhibitory activity to approximately one third. It is somewhat surprising that the inhibitory activity is not lost (except for the analogue *V*) by such modifications of the inhibitor molecule as shortening of the acidic component by one carbon atom (*cf.* the analogue *VI*) or introduction of the rigid lactone ring (*cf.* the analogue *II*) and that the inhibitory activity is decreased by compounds *II*, *III*, *IV*, and *VI* to a similar extent. However, the present results unambiguously demonstrate a very precise adaptation of the steric arrangement of the thuringiensin molecule to specific requirements of the ATP binding site on the DNA-dependent RNA polymerase.

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