THE SPECIFICITY OF INHIBITION OF DNA-DEPENDENT RNA POLYMERASE BY *BACILLUS THURINGIENSIS* EXOTOXIN

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Received 27 April 1970

1. Introduction

DNA-dependent RNA polymerase of bacterial and mammalian origin is inhibited by the exotoxin produced by *B. thuringiensis* [1, 2]. The exotoxin has been shown to be an adenosine derivative linked in the 5′-position to phosphoallaryl glucose [3]. On the basis of competition studies between exotoxin and the nucleoside triphosphate substrates of DNA-dependent RNA polymerase, a mechanism of inhibition has been proposed [4]. According to this concept, exotoxin is reversibly bound (without being incorporated into the polymer) with its adenosine moiety to the (thymine directed) ATP-specific site on the enzyme-template complex and thus competes exclusively with ATP. The specificity of this interaction has been supported by the finding that, on deamination of the adenosine moiety of the exotoxin to inosine, the inhibitory activity remains unchanged but the inosine-analogue of the exotoxin (denoted as inosine-exotoxin in this paper) competes with GTP instead of ATP. This is in accordance with the fact that ITP, as well as GTP, binds to the (cytosine directed) GTP-specific site of the enzyme-template complex. To test this concept, in the present paper use has been made of synthetic polydeoxyribonucleotides as primers for DNA-dependent RNA polymerase. If the proposed mechanism [4] was correct, inhibition should occur only with an inhibitor containing the base which is coded for by the template.

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2. Experimental

Isolation of *B. thuringiensis* exotoxin [5] and preparation of inosine-exotoxin [4] has been described elsewhere. The polydeoxyribonucleotides used as primers were a gift of Dr. A.Lezius from this Institute (poly d(A–T), poly dT and poly dIdC) and of Dr. T. Jovin from the Max-Planck Insitut für physikalische Chemie, Göttingen (poly d(I–C)). DNA-Dependent RNA polymerase with and without σ-factor was prepared according to Burgess [6, 7]. The solution of the enzyme in 50% glycerol contained 4.8 mg protein/ml and was stored at −20°. The specific activity was 1475 units/mg protein.

Enzyme assay. The reaction mixture contained in a final volume of 0.1 ml : 3 μmoles tris-acetate buffer pH 7.9, 3.0 μmoles Mg acetate, 1.4 μmoles NH_4Cl, 1.0 μmole of each nucleoside triphosphate (the labeled triphosphate containing 0.3 μCi of ^{14}C), 0.1 A_{260} polydeoxyribonucleotide primer and 2.4 mg enzyme protein. The reaction was started by addition of enzyme and the assay mixture incubated at 37°. At appropriate time intervals aliquots were withdrawn and plated on Whatman No. 3MM filters. The filters were washed twice in 5% trichloroacetic acid followed by absolute ethanol and ether, dried and counted in a scintillation counter. The results are expressed in μmoles of the labeled triphosphate incorporated into the polymer per 1 ml of assay mixture.

3. Results and discussion

To test the specificity of inhibition by the exotoxin

Table 1
Inhibition of substrate incorporation by exotoxin and inosine-exotoxin.

Additions to assay mixture	Concn. rel. to labeled substrate	Incorporation (nmole/ml)	(%)	Additions to assay mixture	Concn. rel. to labeled substrate	Incorporation (nmole/ml)	(%)
Poly d(A–T), ^{14}C-UTP + ATP				Poly d(I–C), ^{14}C-CTP + ITP			
–	–	166.5	100.0	–	–	86.5	100.0
Exotoxin	1.0	3.2	1.9	Exotoxin	1.0	80.3	91.5
Exotoxin	0.1	28.5	17.1	Exotoxin	0.1	76.1	86.8
Inosine-exotoxin	1.0	138.0	83.0	Inosine-exotoxin	1.0	4.5	5.2
Inosine-exotoxin	0.1	166.0	100.0	Inosine-exotoxin	0.1	31.5	36.5
Poly dT, ^{14}C-ATP				Poly dIdC, ^{14}C-ITP			
–	–	88.0	100.0	–	–	326.0	100.0
Exotoxin	0.10	59.0	12.1	Exotoxin	0.10	265.0	81.0
Exotoxin	0.02	165.0	34.8	Exotoxin	0.02	376.0	115.0
Exotoxin	0.01	207.0	42.5	Exotoxin	0.01	372.0	114.0
Inosine-exotoxin	0.10	538.0	110.0	Inosine-exotoxin	0.10	21.0	6.6
Inosine-exotoxin	0.02	572.0	117.0	Inosine-exotoxin	0.02	106.0	32.4
Inosine-exotoxin	0.01	595.0	122.0	Inosine-exotoxin	0.01	161.0	49.3

Assay mixture (see experimental part) incubated for 20 min. On top of each column the template and substrate used in the reaction are given.

and its inosine-analogue, four different polydeoxyribonucleotides were used as templates in the RNA polymerase-catalyzed reaction. These were poly d(A–T) and poly d(I–C) for the synthesis of alternating poly r(A–U) and poly r(I–C) and poly dT and poly dIdC for the synthesis of poly rA and poly rI respectively (in the case of the poly dIdC primer only ITP was offered as substrate so that only the dC strand was read). The results obtained with both inhibitors are summarized in table 1. With both templates coding for the incorporation of ATP, exotoxin is strongly inhibitory. In poly rA synthesis, the decrease of incorporation amounts to more than 50% with an inhibitor concentration as little as 1% of that of substrate. The corresponding figures for inosine-exotoxin are essentially similar with templates coding for the incorporation of ITP. As may be expected, the inhibition is lower for both alternating polymers. All results mentioned so far are essentially identical whether the enzyme with or without σ-factor is used.

There is little effect of both inhibitors on the synthesis of polymers not containing the corresponding base. Thus neither exotoxin markedly affects the synthesis of poly r(I–C) or poly rI nor inosine-exotoxin, that of poly r(A–U) or poly rA.

The specificity of inhibition affecting the incorporation of the nucleoside triphosphate of the corresponding base has been found also in the *de novo* synthesis of polyribonucleotides. As models for this reaction, unprimed synthesis of poly rArU [8, 9] and poly r(I–C) [10, 11] under the conditions described in the cited papers were used. In these reactions, exotoxin inhibited ATP polymerisation and inosine-exotoxin the polymerisation of ITP.

In general, the results indicate that exotoxin binds with its adenosine moiety to the enzyme-template complex only in the case when the template induces a binding site for the adenosine residue. On the other hand, inosine-exotoxin binds to this complex only if a cytosine containing template codes for the incorporation of a guanosine or inosine residue. The inhibitors thus act specifically as structural analogues of the respective nucleoside triphosphates in full agreement with the mechanism of inhibition proposed earlier [4]

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