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PRELIMINARY COMMUNICATION

REVERSAL OF THE ANTIZYME INHIBITION OF ORNITHINE DECARBOXYLASE BY NUCLEIC ACIDS

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Polyamines, the end products of the ornithine decarboxylase (ODC) (EC 4.1.1.7) reaction, induce the synthesis of a non-competitive protein inhibitor of ODC in a variety of cultured cancer and normal mammalian cells as well as in animals and in Escherichia coli (1,2). Three such proteins from E. coli, the acidic antizyme (pI = 3.8; mol. wt = 49,500) and two basic antizymes (pI = 9.5), antizyme 1 (mol. wt = 11,000) and antizyme 2 (mol. wt = 9,000), have been purified recently (3,4). Antizyme 1 has an amino acid composition (5) similar to that of the E. coli histone-like protein HU (6) and to the eukaryotic histone H2B (7); antizyme 2 is characterized by an unusually high arginine content (5). The two basic antizymes inhibit both the biosynthetic ornithine decarboxylase as well as the biosynthetic arginine decarboxylase of E. coli (5,8). The acidic antizyme remains to be tested against arginine decarboxylase. These results re-emphasize the possibility that polyamine synthesis in both E. coli and in eukaryotic cells may be under the control of antizymes. We have indicated previously that E. coli DNA reverses the inhibition of ODC by antizyme (5).

In the course of purifying E. coli ODC, we encountered a "Factor" (3) that shares some properties with the anti-antizyme found by Hayashi and his collaborators in rat liver extracts (9). This E. coli "Factor" elutes immediately after the ODC peak during gel filtration chromatography, and has no ODC activity; when added to an inactive ODC-antizyme complex, it liberates active ODC from the complex (3). Upon investigating the nature of this E. coli "Factor", we found that the RNA present in this region reversed the inhibition of ODC by antizyme. We extended this finding and we present our results on the interaction of the ODC-antizyme complex with nucleic acids. Our findings apply to the E. coli "Factor"; we have not investigated the nature of the rat liver anti-antizyme (9).

MATERIALS AND METHODS

E. coli DNA was purchased from Worthington. Ribo- and deoxyribonucleotide homopolymers were obtained from P. L. Biochemicals. The isolation of E. coli "Factor" from E. coli has been described (4). It was phenol extracted and precipitated by ethanol; it was then treated with proteinase K, phenol extracted and reprecipitated with ethanol. The molecular weight of the RNA in this region is approximately 40,000-60,000, using t-RNA and a Hind III digest of lambda DNA as standards. We wish to thank Drs. Dean Rupp and K. Brooks Low for the pBR322 DNA and for the pBR322 plasmid DNAs carrying the uvrA, uvrB and uvrC genes of E. coli.

A 40µl reaction mixture containing ODC and antizyme in assay buffer (50 mM Tris-HCl, pH 8.3, 0.1 mM EDTA, 0.05 mM pyridoxal phosphate, and 2.5 mM dithiothreitol) was incubated at 37° for 2-5 min to form an ODC-antizyme complex. The nucleic acids and radioactive ornithine were added to the reaction mixture to a final volume of 50 µl. The assay of ODC and antizyme has been described (1,2). The purification of E. coli ODC (2) and of the antizymes (2-4) has been reported.

RESULTS AND DISCUSSION

Figure 1 shows that both antizyme 1 and 2, as well as the acidic antizyme of E. coli, inhibited E. coli ODC and that the addition of E. coli DNA reversed this inhibition. The reversal may be more effective for the acidic antizyme than for antizymes 1 and 2. More detailed investigation will be necessary to establish the relative effects of these antizymes.

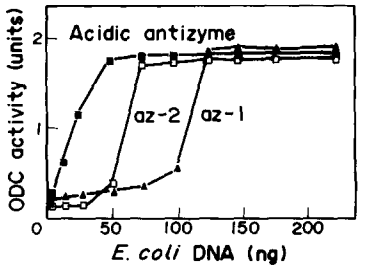

Fig. 1. Reversal by E. coli DNA of the inhibition of ODC by the acidic antizyme and by antizymes 1 and 2 of E. coli. To 1.8 units of E. coli ODC were added 1.6 units of acidic E. coli antizyme or of antizyme 1 or 2, and the resultant complexes were titrated with E. coli DNA. Key: acidic antizyme (■—■); antizyme 1 (▲—▲); antizyme 2 (□—□).

The E. coli "Factor" that elutes after the ornithine decarboxylase peak is devoid of ODC activity (4) but, as previously shown (4) and detailed in Fig. 2, it reverses the inhibition of ornithine decarboxylase by antizymes 1 and 2 and by the acidic antizyme. It can now be seen that, after phenol extraction followed by proteinase K treatment and another phenol extraction, the ethanol precipitable material in the E. coli "Factor" region was DNAase and trypsin resistant while it was sensitive to RNAase T1. Its UV spectrum was determined and found to be typical of nucleic acids. Acrylamide gel electrophoresis indicated that the active component was an ethidium bromide fluorescent band, which banded reasonably tightly at the 40,000-60,000 mol. wt region and which disappeared following RNAase T1 treatment.

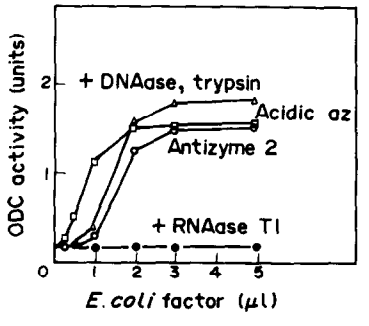

Fig. 2. Reversal by the E. coli "Factor" of the inhibition of E. coli ODC by antizyme 2 and by the acidic antizyme. To 1.8 units of E. coli ODC were added 1.6 units of antizyme 2 or of acidic antizyme. Titration with native "Factor" of: 'ODC-acidic antizyme' complex (acidic az) (□—□); 'ODC-antizyme 2' complex (antizyme 2) (●—●). Titration with DNAase and trypsin-treated "Factor" (+DNAase, Trypsin) (△—△) of the 'ODC-antizyme 2' complex. Titration with RNAase T1-treated "Factor" of the 'ODC-acidic antizyme' complex and of the 'ODC-antizyme 2' complex (RNAase T1) (○—○).

We used the relief of the antizyme inhibition of ODC activity as an assay of the relative effectiveness of various nucleic acid sequences. In Fig. 3 the pBR322 plasmid DNAs carrying the uvrA, uvrB, and uvrC genes of E. coli (10) are compared to the pBR322 plasmid DNA alone and to E. coli DNA. The pBR322 plasmid DNA and the pBR322-uvrA and pBR322-uvrB plasmid DNAs were more effective than E. coli DNA; however, the pBR322-uvrC

plasmid DNA was much more effective than the other plasmid DNAs in relieving the inhibition of E. coli ODC activity by antizyme 2. We also compared the ability of the supercoiled form and of the linearized form of the pBR322-uvrC plasmid DNAs to reverse the inhibition of ODC by treating this DNA with the restriction enzyme Bam H1 which cleaves it at one site only (10). The linearized form retained all of the activity of the original supercoiled DNA (data obtained but not shown); consequently, the supercoiled structure is not essential for the reactivation of ODC in the ODC-antizyme complex.

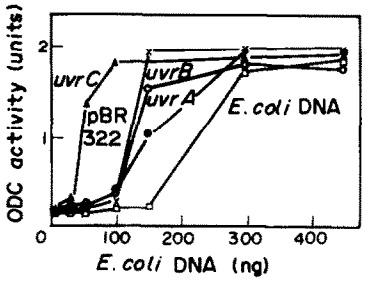

Fig. 3. Reversal by various DNAs of the inhibition of E. coli ODC by antizyme 2. To 1.8 units of E. coli ODC were added 1.6 units of antizyme 2, and the resultant complex was titrated with E. coli DNA (□—□); pBR322 plasmid DNA (pBR322) (×—×); pBR322-uvrA DNA (uvrA) (●—●); pBR322-uvrB DNA (uvr B) (○—○); pBR322-uvrC DNA (uvrC) (▲—▲).

We also compared the relative effectiveness of single-stranded deoxynucleotide and ribonucleotide homopolymers in relieving the inhibition of E. coli ODC by antizyme 1 and antizyme 2. We found that poly (dA) was much more effective than the other deoxynucleotide homopolymers, i.e. poly (dC), poly (dT), poly (dU), poly (dI) and poly (dG),in relieving the inhibition of ODC activity by antizymes 1 and 2. The ribonucleotide homopolymers poly (rA), poly (rC), poly (rU) and poly (rG) were equivalently effective in relieving the inhibition of ODC activity by both antizymes 1 and 2. The base specificity was not as marked among the ribonucleic acid homopolymers as it was among the deoxynucleic acid homopolymers. Because of space limitations, these results cannot be presented in further detail and will be presented elsewhere.

Because both the acidic antizyme (pI = 3.5) and the nucleic acids are negatively charged at the assay pH, these interactions cannot be ascribed to charge effects alone. The nucleic acids are not equivalent in their abilities to relieve the inhibition of ODC by antizymes: the plasmid pBR322-uvrC DNA was more effective in inhibiting antizyme activity than the other related plasmid DNAs, and poly (dA) was more effective than the other deoxynucleotide homopolymers. These results indicate that the antizymes may have higher affinity for some nucleotide sequences than for others.

Many DNA or RNA binding proteins have been described (11-13). Although most of these proteins bind with greater specificity to certain regions of DNA, they share in common with the antizymes the property of binding to a variety of DNA and RNA molecules (11-13). Our research is currently directed towards identifying the regions of DNA to which the antizymes bind with higher specificity. The main property that differentiates the antizymes from many of the double or single-stranded DNA binding proteins and from the RNA binding proteins is that the antizymes also bind and inhibit ornithine decarboxylase; subsequently, upon binding to DNA, their inhibition of ODC is reversed.

In summary, the inhibition of E. coli ornithine decarboxylase by the basic and by the acidic antizymes was relieved upon addition of E. coli DNA to the ornithine decarboxylase-antizyme complex. A similar relief of inhibition of the ornithine decarboxylase-antizyme complex was exerted by the RNA derived from the E. coli "Factor" that we described earlier. The differences in the degree of relief of inhibition exerted by the various segments of E. coli DNA suggest that there is a specificity of interaction between the nucleic acids and the antizymes.

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