Specific binding of chartreusin, an antitumor antibiotic, to DNA

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Chartreusin, an antitumor and antibacterial antibiotic, was found to inhibit negatively superhelical DNA-relaxation catalyzed by prokaryotic topoisomerase I and conversion of the superhelical DNA into unit length linear form catalyzed by single-strand-specific S1 nuclease. The inhibitory effect of the agent was due to the binding to DNA causing the alteration of tertiary structure. To characterize the binding specificity, we investigated the protection of DNA against cleavages by various restriction endonucleases. It was evidenced that the binding of the agent is not at random and correlates to the sequence $\frac{5'CGC\ 3'}{3'GCG\ 5'}$ on DNA stretch.

Chartreusin *Antitumor antibiotic* *Topoisomerase I* *S1 nuclease* *Restriction endonuclease* *Specific binding to DNA*

1. INTRODUCTION

It has been reported [1] that chartreusin [2], an antitumor [3] and antibacterial antibiotic, bound to DNA and inhibited the in vitro reactions of both DNA and RNA polymerases. In [4] it was demonstrated that chartreusin caused a single-strand scission into DNA in the presence of reducing agents such as dithiothreitol, sodium borohydride and ascorbic acid, and the cleavage was stimulated by ferrous ion.

Here, we clarified the specificity of binding of chartreusin to DNA and the consequent inhibition of the actions of topoisomerase I and S1 nuclease on the superhelical DNA.

2. MATERIALS AND METHODS

2.1. *DNAs*

Colicin E1 (ColE1) DNA was prepared from *Escherichia coli* strain JC411 (ColE1) thy$^-$ according to the combined procedure in [5,6]. *Bacillus subtilis* phage M2 DNA was a generous gift from Dr Matsumoto, Sophia University.

2.2. *Enzymes and reactions*

The *Haemophilus gallinarum* topoisomerase I was prepared as in [7]. The reaction mixture (20 μl) containing 20 mM Tris–HCl (pH 7.5), 5 mM $MgCl_2$, 0.3 μg superhelical ColE1 DNA and the indicated amount of the topoisomerase I was incubated at 37°C for 1 h. The reaction was stopped by addition of an equal volume of phenol. One unit of topoisomerase I activity is defined as the amount of the enzyme that converts 0.3 μg superhelical DNA into a completely relaxed form under the above conditions. S1 nuclease provided by Sankyo Chemicals was further purified as in [8]. The S1 reaction was performed in the buffer (20 μl) containing 30 mM sodium acetate buffer (pH 5.0), 50 mM NaCl, 1 mM $ZnSO_4$, 0.3 μg superhelical ColE1 DNA and the indicated amount of S1 nuclease at 37°C for 30 min. The reaction was stopped by addition of an equal volume of phenol after neutralization with 1/10th vol. 1 M Tris–HCl (pH 8.5). One unit of S1 activity is

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defined as the amount of enzyme that converts 50% of 0.3 μg single-stranded ColE1 DNA to acid-soluble form under the above conditions. Restriction endonucleases except *Hga*I [9] were purchased from Bethesda Res. Labs. and used according to the supplier's instruction. *Hga*I was purified and used as in [10]. The reactions were stopped by addition of an equal volume of phenol.

2.3. *Agarose gel electrophoresis*

One percent agarose tube gels (0.6 × 20 cm) and horizontal slab gels (15 × 20 × 0.3 cm) were prepared; 1/10th vol. 0.05% bromophenol blue–90% glycerol was added into the reaction mixtures extracted with phenol, and the resulting DNA samples were loaded onto the agarose gels. The electrophoreses were done in Tris–acetate buffer (50 mM Tris–HCl, 20 mM sodium acetate, 18 mM NaCl and 2 mM EDTA, pH 8.2) at 50–120 V for 3–10 h. Visualization and photography of the DNA bands were done as in [11].

2.4. *Chartreusin*

In the course of our screening program for DNA-binding substances utilizing the reaction system of topoisomerase I from *Haemophilus gallinarum* [12], chartreusin has been found as an inhibitor of the enzyme reaction from culture filtrate of a strain of *Streptomyces* (fig.1). The antibiotic was isolated by solvent extraction followed by preparative thin-layer chromatography, and finally purified by high-pressure liquid chromatography (Nucleosil $5C_{18}$, 75% aq. methanol). The IR, UV and visible spectra were in good accord with [2].

3. RESULTS

Fig.1 shows the inhibitory effect of various concentrations of chartreusin on ColE1 DNA-relaxation catalyzed by *Haemophilus* topoisomerase I. The reaction was inhibited by 2.4 μM of the drug (track 4). Over 2.4–39.0 μM, the scission of DNA strand by chartreusin was not observed on its direct treatment with ColE1 DNA. As reported in [4], the single-strand cleavage was confirmed on the treatment of DNA with 39 μM chartreusin in the presence of both 1 mM dithiothreitol and 10 μM ferrous sulfate. Little effect, however, was observed by the addition of either dithiothreitol or

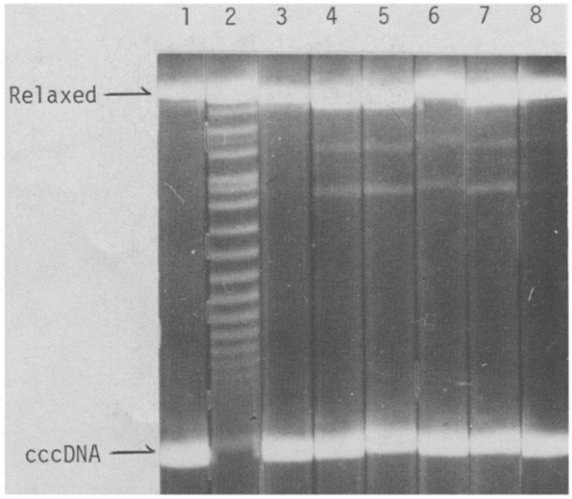

Fig.1. Inhibitory effect of chartreusin on superhelical DNA-relaxation catalyzed by topoisomerase I from *Haemophilus gallinarum*: (1) ColE1 DNA alone; (2) with enzyme (0.2 unit); (3) with 39 μM agent; (4–8) with both enzyme and agent – (4) 2.4 μM, (5) 4.8 μM, (6) 9.7 μM, (7) 19.5 μM, (8) 39 μM.

ferrous ion. To solve the problem whether the inhibitory effect of chartreusin on topoisomerase I reaction was caused by its binding to enzyme or DNA, we investigated the change of inhibition pattern by adding double-stranded linear DNA to the reaction mixture. It is known that double-stranded linear M2 DNA almost does not affect the enzyme activity [7]. The result showed that DNA-relaxation is recovered by coexistence of the linear DNA, indicating that the inhibition is actually due to the binding to DNA (fig.2). This was supported by the fact that chartreusin also inhibited the DNA-relaxation reaction using topoisomerase I prepared from *Streptomyces achromogenes* [13] which is a distinct protein from the enzyme purified from *Haemophilus gallinarum* (not shown).

S1, a single-strand-specific nuclease can cleave both strands of a negatively superhelical DNA at the unpaired regions to generate unit length linear duplex molecule with intact single-strand [14,15]. The reaction proceeds by two steps; first, cleavage occurs in either one of two strands and then, the nicked, circular DNA is cleaved on the opposite strand at or near the nicks to yield a linear molecule. We investigated the effect of chartreusin on this S1 action. The enzyme reactions were car-

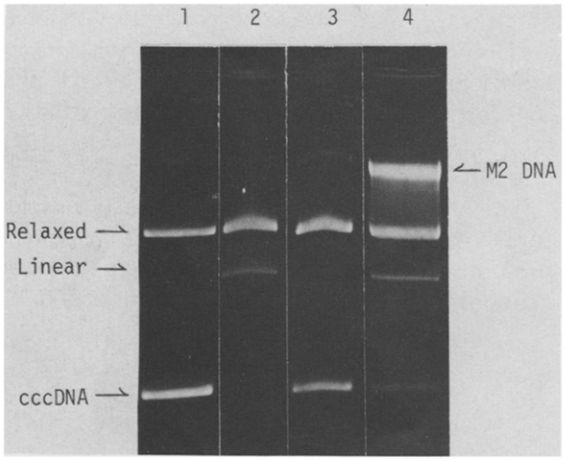

Fig.2. Effect of M2 DNA on agarose electrophoretic patterns of superhelical DNA-relaxation: (1) ColE1 DNA alone; (2) with enzyme (1 unit); (3) with both enzyme and 19.5 μM agent; (4) with enzyme (1 unit), 19.5 μM agent and M2 DNA.

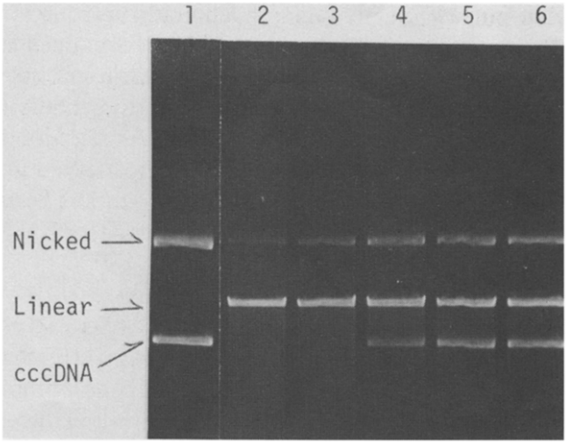

Fig.3. Inhibitory effect of chartreusin on S1 nuclease action on superhelical DNA: (1) ColE1 DNA alone; (2) with enzyme (5 units); (3–6) with both enzyme and agent – (3) 0.6 μM, (4) 2.4 μM, (5) 9.7 μM, (6) 39 μM.

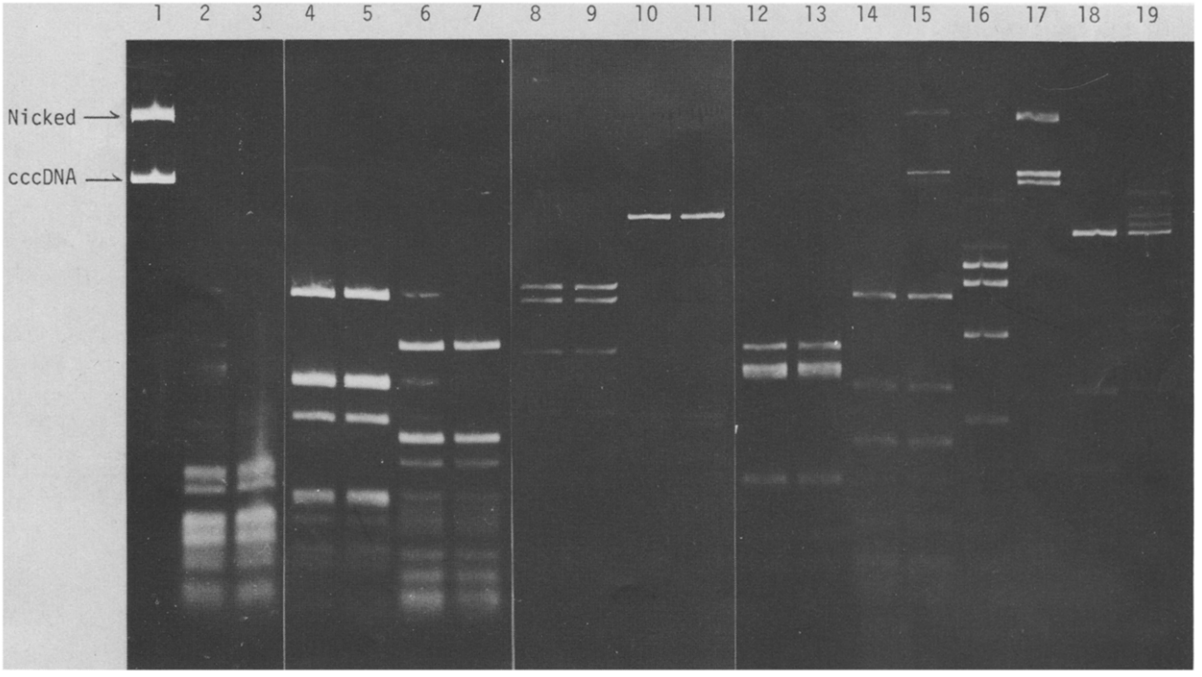

Fig.4. Effect of chartreusin on restriction endonuclease digestion patterns: (1) ColE1 DNA alone; with enzyme, even numbers; with both enzyme and 19.5 μM agent, odd numbers:

(2,3) *Alu*I ($\overset{\downarrow}{\text{AGCT}}$ / $\text{TCGA}\uparrow$); (4,5) *Hin*fI ($\overset{\downarrow}{\text{GANTC}}$ / $\text{CTNAG}\uparrow$); (6,7) *Hap*II ($\overset{\downarrow}{\text{CCGG}}$ / $\text{GGCC}\uparrow$); (8,9) *Dde*I ($\overset{\downarrow}{\text{CTNAG}}$ / $\text{GANTC}\uparrow$); (10,11) *Bst*NI ($\overset{\downarrow}{\text{CCA(T)GG}}$ / $\text{GGT(A)CC}\uparrow$); (12,13) *Hae*III ($\overset{\downarrow}{\text{GGCC}}$ / $\text{CCGG}\uparrow$); (14,15) *Hha*I ($\overset{\downarrow}{\text{GCGC}}$ / $\text{CGCG}\uparrow$); (16,17) *Hae*II ($\overset{\downarrow}{\text{PuGCGCPy}}$ / $\text{PyCGCGPu}\uparrow$); (18,19) *Hga*I ($\overset{\downarrow}{\text{GACGC(N)}_5}$ / $\text{CTGCG(N)}_{10}\uparrow$).

ried out without reducing agents and ferrous ion. The conversion efficiency to a linear form decreased in proportion to the concentration of chartreusin (fig.3). To test whether the drug binds to the enzyme protein thus inhibiting its activity or not, S1 was incubated with the drug, dialyzed and then the enzyme activity was checked. The result revealed that the antibiotic does not affect the S1 activity.

To define the specificity of its binding to DNA, the influence of chartreusin on DNA cleavage by various restriction endonucleases was investigated. The enzyme reactions were carried out without reducing agents and ferrous ion. The following endonucleases were used: *Alu*I, *Hin*fI, *Hap*II, *Dde*I, *Bst*NI, *Hae*III, *Hha*I, *Hae*II and *Hga*I. An inhibitory effect of chartreusin (19.5 μM) on the DNA cleavage was observed in 3 endonucleases, *Hha*I, *Hae*II and *Hga*I (track 15,17,19, fig.4). The inhibition was partial at this concentration. Higher concentration of the drug could not be used for the experiment because of low solubility. The recognition and the cleavage sites of all the enzymes used in the experiment are shown in the figure legends. The recognition sites of *Hha*I, *Hae*II and *Hga*I involve the common sequence $\begin{smallmatrix}5'CGC\,3'\\3'GCG\,5'\end{smallmatrix}$ suggesting the specific binding of chartreusin to DNA.

4. DISCUSSION

Here, it was shown that chartreusin binds site-specifically to DNA without any strand scission in the absence of a reducing agent and ferrous ion. It was suggested that the sequence $\begin{smallmatrix}5'CGC\,3'\\3'GCG\,5'\end{smallmatrix}$ is the target for chartreusin. The binding of the drug to superhelical DNA seems very likely to cause unwinding of double-helix, resulting in decrease of superhelical turns. The formation of unpaired regions, owing to negative superhelicity, is not so significant in partially relaxed DNA as compared with native DNA. Therefore, S1 nuclease can not work effectively on the chartreusin-bound DNA. Shishido [†1] has observed that 60–70% relaxed DNA molecules exhibit complete resistance to S1-cleavage. With the same concentration of the antibiotic, the extent of inhibition in the topoisomerase I reaction is higher than that in the S1 reaction. This suggests that chartreusin prohibits the swivel of the DNA strand which is required for DNA-relaxation.

The mechanism of the binding of the antibiotic to DNA is not clear, although the aglycone is presumed to be intercalated as shown in the case of actinomycin and anthracycline antibiotics.

REFERENCES

[1] Li, L.H., Clark, T.D., Murch, L.L., Wooden, J.M., Pschigoda, L.M. and Krueger, W.C. (1978) Cancer Res. 38, 3012–3018.
[2] Leach, B.E., Calhoun, K.M., Johnson, L.E., Teeters, C.M. and Jackson, W.G. (1953) J. Am. Chem. Soc. 75, 4011–4012.
[3] Mcgovren, J.P., Neil, G.L., Crampton, S.L., Robinson, M.I. and Douros, J.D. (1977) Cancer Res. 37, 1666–1672.
[4] Yagi, M., Nishimura, T., Suzuki, H. and Tanaka, H. (1981) Biochem. Biophys. Res. Commun. 98, 642–647.
[5] Clewell, D.B. and Helinski, D.R. (1969) Proc. Natl. Acad. Sci. USA 62, 1159–1166.
[6] Blair, D.G., Sherratt, D.J., Clewell, D.B. and Helinski, D.R. (1971) Proc. Natl. Acad. Sci. USA 69, 2518–2522.
[7] Shishido, K. and Ando, T. (1979) Biochim. Biophys. Acta 563, 261–265.
[8] Shishido, K. (1979) Agric. Biol. Chem. 43, 1093–1102.
[9] Takanami, M. (1974) in: Methods in Molecular Biology (Wickner, R.B. ed) vol.7, pp.113–133, Marcel Dekker, New York.
[10] Shishido, K. and Berg, P. (1976) J. Virol. 18, 793–798.
[11] Shishido, K. (1980) FEBS Lett. 111, 333–336.
[12] Shishido, K., Tachibana, T. and Ando, T. (1980) Agric. Biol. Chem. 44, 1207–1209.
[13] Watabe, H., Hayase, E. and Ando, T. (1980) Agric. Biol. Chem. 44, 3021–3023.
[14] Beard, P., Morrow, J.F. and Berg, P. (1973) J. Virol. 12, 1303–1313.
[15] Shishido, K. and Ando, T. (1975) Agric. Biol. Chem. 39, 673–681.