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The selective inhibitory effect of netropsin on relaxation of sequence specificity of restriction endonuclease *Sgr*AI recognizing the octanucleotide sequence 5′-CR↓CCGGYG-3′

F.Laue, W.Ankenbauer, G.G.Schmitz and C.Kessler
Boehringer Mannheim GmbH, Biochemical Research Center, Nonnenwald 2, D-8122 Penzberg, FRG

Submitted April 24, 1990

DNA-binding agents like ethidium bromide, proflavin, actinomycin D, distamycin A as well as netropsin are known to inhibit the cleavage activity of restriction endonucleases in a dose-dependent manner (Goppelt *et al.*, 1981; Nilsson *et al.*, 1982; Österlund *et al.*, 1982). Some of these agents display a preference for particular bases or sequences. Actinomycin D binds preferentially to CG-containing sequences, whereas distamycin A and netropsin prefer AT-rich regions.

The restriction endonuclease *Sgr*AI, recognizing the octanucleotide sequence 5′-CR↓CCGGYG-3′ (Tautz *et al.*, 1990) shows relaxation of sequence specificity if a 10-fold excess of enzyme is used. We tested whether the DNA-binding oligopeptide netropsin can be used to selectively suppress the relaxed specificity of *Sgr*AI without affecting the activity of *Sgr*AI itself.

Using 0.2 mM netropsin relaxation of sequence specificity can be prevented even in the presence of a 160-fold excess of enzyme (Fig. 1). The cleavage activity of *Sgr*AI in the presence of 0.2 mM metropsin is almost not affected; under these conditions the enzyme is more than 95% active as compared to the activity in the absence of netropsin.

Analogous selective suppression of sequence relaxation using a DNA binding agent was also observed with the restriction enzyme *Eco*RI. Its star activity can be selectively suppressed using 10 μM actinomycin D (data not shown). This shows the more general applicability of DNA binding agents with sequence preference as selective suppressors of sequence relaxation of restriction endonucleases.

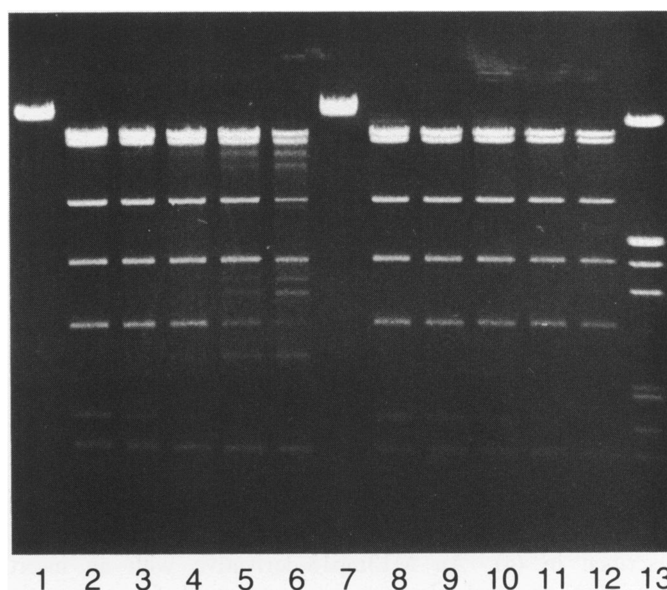

Figure 1. Effect of netropsin in *Sgr*AI activity. *Sgr*AI was isolated from *Streptomyces griseus* (DSM 561) grown at 26°C by multi-step chromatography on Sephadex G25, DEAE Sephacel, Cellulose-Phosphate P11, Heparin-Sepharose CL-6B and Fractogel TSK AF Orange resulting in a highly purified enzyme with a specific activity of 13800 units/mg. One unit *Sgr*AI is defined as the amount of enzyme cleaving 1 μg λ-cI857Sam7 DNA in 1 h at 37°C in buffer A [33 mM Tris·acetate (pH 7.9, 37°C)/10 mM Mg acetate/66 M K acetate/0.5 mM DTT]. Incubation of 1 μg λ-cI857Sam7 DNA with more than 10 units *Sgr*AI in 1 h or more than 3 units *Sgr*AI for 16 h in buffer A yielded a further degradation of the final λ[*Sgr*AI]-fragments (16677, 14850, 7071, 4198, 2775, 1616 and 1321 bp) into well-defined minor degradation products. The minor degradation products are correlated with *Sgr*AI* activity because they can also be generated with low amounts of enzyme, by changing Mg^{2+} to Mn^{2+} ions, or by increasing the glycerol concentration (data not shown). Lanes 2−6, fragment patterns obtained by cleavage with 2.5, 5, 7.5, 10 or 20 units *Sgr*AI for 16 h in buffer A; lanes 8−12, fragment patterns obtained by cleavage with 2.5, 5, 7.5, 10 or 20 units *Sgr*AI for 16 h in buffer A complemented with 0.2 mM netropsin. Lanes 1, 7, undigested λ-cI857Sam7 DNA; lane 13, λ[*Eco*RI+*Hin*dIII]-fragments used as M_r-markers.

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