Vol. 90, No. 2, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 27, 1979 Pages 398-404

A POLY d(A-T)-UNWINDING GLYCOPROTEIN FROM ROE-DEER LIVER

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Received March 20, 1979

Summary: A DNA double-helix destabilizing protein was purified from roe-deer liver to near homogeneity. It is a glycoprotein containing fucose, xylose, mannose and at least two undefined shugars. For comparative work an unwinding coefficient was defined and determined for different double-stranded polynucleotides. The present protein revealed a remarkable preference for unwinding A-T deoxybase pairs.

Introduction

Proteins destabilizing the native DNA double-helix were isolated from different sources (1-6) with rather analogous properties. These proteins turned out to melt a variety of double helical polynucleotides including the synthetic deoxyribo and ribo species. There is no preferential binding to distinct base sequences; however, these proteins reveal a significant preference for single stranded over double helix DNA. In contrast to helix-destabilizing proteins from mammals, those from bacteria revealed cooperativity of the interaction with single stranded DNA and catalysed DNA renaturation. In the present work, the isolation and purification to near homogeneity of an unwinding protein from roe-deer liver is reported and some of its properties are described.

Materials and Methods

Amino acid analysis was performed according to standard techniques on a AAA-881 analyser. For determination of a probable saccharide content,

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0006-291X/79/180398-07$01.00/0

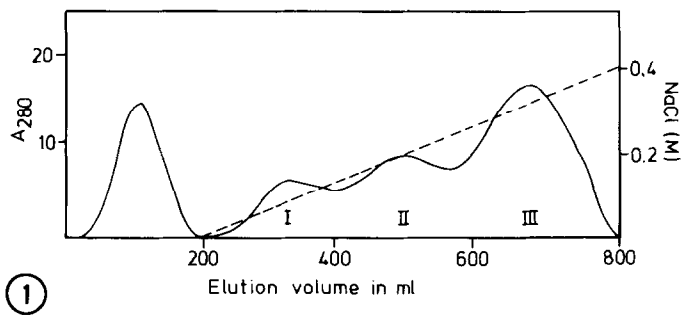
samples were hydrolysed with 1 N H_2SO_4 at 100° C for 4 h. The hydrolyzate was passed through columns of Dowex 50 W x 8, 200-400 mesh (H^+ form) coupled to Dowex 1 x 8, 200-400 mesh (formate form). The neutral shugars, after transformation into the alditol derivatives, were applied on a 300 cm gas chromatography column (OV-225) working at 190°. Concentration of protein was determined according to Lowry et al. (7), shugars using the phenol method (8).

The helix-destabilizing proteins were characterized by their ability to increase the 260 nm absorption of native DNA according to Herrick et al. (9) in 50 mM Tris-HCl, 1 mM EDTA, 1 mM mercaptoethanol, 10 % glycerol, pH 7. The first cuvet contained the protein, the second the DNA (20 µg/ml) and the third contained both components. Readings were made after an incubation time up to 60 min.

Thermal denaturation profiles were determined with a Specord UV-VIS; temperature was measured with a calibrated copper-konstantan thermocouple. The synthetic polynucleotides and Cl. perfringens DNA were purchased from Sigma Chemical Co., calf thymus DNA was prepared according to (10); the shugar standards for gas chromatography were obtained from Merck Darmstadt.

Results and Discussion

The purification of the helix-destabilizing protein: Immediately after slaughtering, the liver was transferred into cold water solution of 0.25 M sucrose of pH 7.0 and washed several times with this solution. The extraction of the helix-destabilizing proteins from bacteria and mammalian tissues is usually performed in 20 mM Tris-HCl of pH 8.8 containing 1 mM EDTA, 1 mM DTT, 5 % glycerol and 50 mM NaCl (2). Improvement of preparation has been supposed from an increase in protein ionisation, i. e. lowering the pH, and a decrease in nucleoprotein solubility, i. e. increase in ionic strength. Hence, different preparations were performed at pH 7 and at NaCl concentrations from 50 to 140 mM without DNase treatment. However, the best method (small amount of contaminants) appeared by using a 50 mM sodium acetate buffer of pH 5, 1 mM mercaptoethanol, 1 mM EDTA, 10 % glycerol and 140 mM NaCl. For homogenisation a Waring blendor at the highest speed was used, all the nuclei appeared to be broken by this treatment. After two centrifugation steps, 5 min at 12 000 g and 2 h at 95 000 g the supernatant was dialysed against three 8 h changes of the above sodium acetate buffer without glycerol, centrifuged for 30 min at 12 000 g and applied to a phosphocellulose



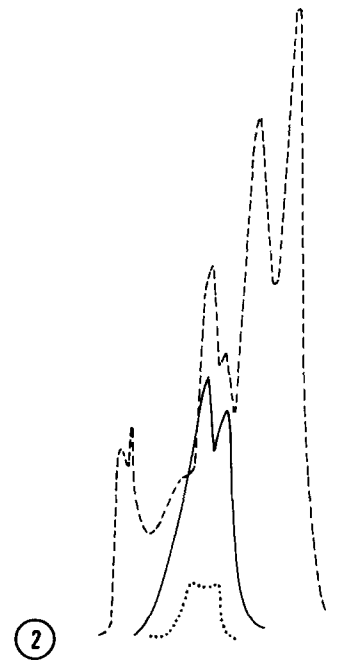


Figure 1. Chromatography of proteins extracted from roe-deer liver on phosphocellulose. The column, 2 x 23 cm, was equilibrated and run with a 50 mM sodium acetate-acetic acid buffer of pH 5 and the NaCl gradient indicated.

Figure 2. SDS polyacrylamide gel electrophoresis of the active protein fraction from Fig. 1. (peak III - - - - -) and from the CM-cellulose chromatography (peak eluted at 0.3 M NaCl ————). The gels were stained with Coomassie blue and the latter gel also with fuchsin to indicate shugar residues (. . . . .). The gels were prepared and run as described by Fairbanks et al. (12). Protein standard used for molecular weight calibration were, bovine serum albumin, pepsin, bovine trypsin, myoglobin and lysozyme.

column as described in Fig. 1. The active fraction appeared in a peak (III) eluted at about 0.3 M NaCl. This fraction was further purified on a CM-cellulose column equilibrated with 50 mM sodium acetate buffer of pH 4.5; the column was eluted by a stepwise increase in the NaCl concentration (steps of 0.1 M) in this buffer and DNA unwinding activity was found exclusively in the fraction eluted at 0.3 M NaCl.

The results from SDS PAGE of the pooled active fractions of both columns are presented in Fig. 2. The fraction from the CM-cellulose column reveals two bands only with estimated molecular weights of 24 000 and

Table 1

Amino Acid composition of the unwinding protein

	Residues per molecule		Residues per molecule
Ala	20	Phe	9.3
Asp	24.3	Pro	15.3
Arg	10.4	Ser	13.6
Cys	1.6	Thr	12.4
Glu	29.8	Tyr	7.4
Gly	21.4	Val	18
Ile	11.4	(Asp + Glu)	54.1
Leu	19	(Lys + Arg + His)	26.5
Met	4		

The protein fraction obtained after CM-cellulose chromatography was hydrolyzed for 24 h in 6 N HCl at 110° C and analysed under standard conditions. Results are from double estimations; the molecular weight of the protein was assumed to be 26 000 (cf. Fig. 2).

27 000 d. Both of these contains shugar components with a shugar to protein weight ratio of one to four. Analysis by gas chromatography indicated fucose, xylose and mannose, and two compounds which could not be assigned. The amino acid composition of this fraction is presented in Table 1. There are large amounts of acidic residues (Asn and Gln not determined) and also aromatic residues (Trp not determined). The ratio of acidic to basic residues is about 2 and is similar to other unwinding proteins (11).

The DNA-unwinding property of the isolated glycoprotein was investigated with poly(dA-dT)·poly(dA-dT); Fig. 3 shows the influence of the protein-DNA weight ratio upon the hyperchromicity. Maximum increase in absorption is observed above a weight ratio of 7. Fig. 4 shows the effect of the NaCl concentration upon the hyperchromicity obtained at a protein-DNA weight ratio of 8. DNA-unwinding is totally abolished above a NaCl concentration of 0.5 M,

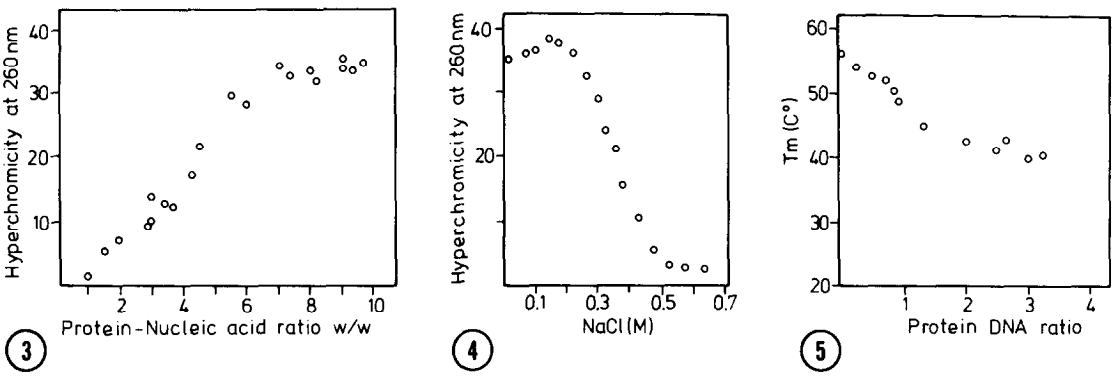


Figure 3. Denaturation of poly(dA-dT)poly(dA-dT) by roe-deer unwinding protein. Experiments, described in the text, were performed in 5 mM Tris-HCl of pH 7, 1 mM EDTA, 1 mM mercaptoethanol and 10 % glycerol at room temperature. Readings after 45 to 60 min after addition of nucleic acid (20 µg in 1.8 ml). Hyperchromicity is the increase in absorption divided by the absorption without addition of protein (corrected for protein absorption) times 100).

Figure 4. Effect of NaCl upon hyperchromicity induced by the unwinding protein. Protein to poly(dA-dT)·poly(dA-dT) ratio was 8; for further explanation cf. legend of Fig. 3.

Figure 5. Melting temperature of unwinding protein complexes with Cl. perfringens DNA with increasing protein-DNA ratios. DNA concentration and buffer were the same as described in the legend of Fig. 3.

half the activity is present at 0.35 M NaCl. Urea was found not to change hyperchromicity up to a concentration of 3.5 M. Hence, these results point to a significant ionic contribution of the protein-DNA interaction and the DNA unwinding process.

Roe-deer liver unwinding protein is thermolabile, the DNA denaturing properties are abolished after heating the protein to 65^{o} and more. Furthermore, the denatured protein causes an increase in the T_m of poly(dA-dT)·poly(dA-dT), whereas the native protein, as other unwinding proteins, decreases the T_m of double-stranded polynucleotides. Fig. 5 shows the melting temperature of DNA from Cl. perfringens as a function of the protein-DNA weight ratio. Maximum decrease is 17^{o} at a weight ratio of about 3.

The efficiency of the present unwinding protein towards different double-stranded polynucleotides is indicated in Table 2. For comparison

Table 2

Unwinding efficiency of the roe-deer liver glycoprotein towards different double-stranded deoxyribopolynucleotides

Polynucleotide	protein-DNA ratio for 50 % hyperchromicity	time(min) after obtaining 50 % of maximum hyperchromicity	unwinding coefficient
poly(dA-dT)poly(dA-dT)	4.5	20	90
poly d(AT)	1.0	210	210
calf thymus DNA	9.0	180	1620
Cl. perfringens DNA	2.25	80	180

Details are given in the text.

an unwinding coefficient was determined in the following way. At the first stage from the dependence of hyperchromicity upon the protein-polynucleotide ratio the respective value was determined which is correlated to a 50 % denaturation (first column), then at this ratio the change of hyperchromicity with time was observed and the time for a 50 % denaturation was noted (second column). The unwinding coefficient is a product of both values and indicates high efficiency at small numbers (third column). Poly(dA-dT)· poly(dA-dT) shows highest efficiency and the lowest was found with calf thymus DNA. The double-stranded polynucleotides poly(dG)poly(dC), poly(A)poly(U) and poly(I)poly(C) were also tested. However, complexes up to a protein-polynucleotide weight ratio of 7 or 8 hyperchromicity was not observed also at very long incubation times. On the other hand, these complexes revealed a melting point increase of about 2^{o} indicating helix stabilization.

In conclusion the helix unwinding protein from roe-deer liver is different from similar proteins described previously in that it contains a shugar component and a remarkable preference to denature A-T deoxybase pairs. The protein is purified to near homogeneity, it is likely that the two bands appearing in SDS PAGE are only different in their shugar component.

Acknowledgements

This work was supported by grant R.III 13.3.3 from the Polish Ministry of Education. We are grateful to Prof. K. G. Wagner for helpful discussions, to Mrs. K. Szczepanik for her technical assistance, to Mrs. R. Jähne for expert typing.

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