DNA binding protein II from *Bacillus stearothermophilus* has been purified as a single species from the nonribosomal cell fraction by a combination of gel filtration and ion exchange chromatography. The protein occurs in solution as a tetramer and is able to bind to 30 S, 50 S, and 70 S ribosomal particles. Circular dichroism studies show that the protein has approximately 45% α-helix. The secondary structure of the *Bacillus* protein is considerably more resistant to the effects of increasing temperature and urea concentration than the homologous protein (NS1 and NS2) from *Escherichia coli*. Proton magnetic resonance experiments show that the protein has a well folded, compact tertiary structure. The DNA binding protein has been crystallized from several precipitants as monochloride needles and tricine plates. The monoclinic form diffracts to at least 3.5 Å and oscillation data from the native crystals have been collected. The protein is able to bind to both single- and double-stranded oligodeoxyribo- nucleotides. Upon binding, several changes occur in the protein NMR spectrum which may be used for further analysis of the mechanistic interaction.

In eukaryotic cells, it has been well established that the genomic DNA is compactly folded into chromatin by an interaction with specific proteins called the histones (1). The existence of a similar packaging mechanism in the prokaryotes has yet to be clearly demonstrated, but chromatin-like structures have been observed by electron microscopy (2, 3). This has prompted the search for histone-like molecules in the prokaryotes and several potential candidates have been reported (4–6).

One protein in particular has emerged as an important DNA binding protein. This small, heat-stable protein has been nonribosomal cell fraction by a combination of gel filtration and ion exchange chromatography. The protein is able to bind to both single- and double-stranded DNA as well as to RNA and, when bound to duplex DNA, DNA synthesis by DNA polymerase protein 11. Both species from measured by gel filtration: the protein binds to both single-

The DNA binding protein from *Bacillus stearothermophilus* is inhibited and in *vivo* transcription is stimulated (6).

These latter observations are consistent with a histone-like role, but more direct evidence is that the protein has the ability to fold up double-stranded DNA into structures resembling chromatin (3, 9).

DNA binding protein II has been isolated from a variety of prokaryotic organisms and the amino acid sequence data demonstrate that the DNA binding proteins II are homologous (3). They are conserved sequences found in the eukaryotic histones (10). Unlike the situation in *E. coli*, only one species of binding protein has been found in each of these organisms.

We report here the isolation and characterization of DNA binding protein II from the thermophilic bacterium *Bacillus stearothermophilus*.

**EXPERIMENTAL PROCEDURES**

**Purification of DNA binding protein II.** 100 ml of *Bacillus stearothermophilus* cells (grown in the presence of rifampicin) were centrifuged at 10,000 × *g* for 15 min at 4°C. The cell debris were removed by centrifugation for 30 min at 15,000 rpm. The supernatant was applied to a Sephadryl S-500 column (16 × 145 cm, volume 6.8 liters) which was equilibrated with 1 M NaCl in “polymer” buffer (15). The column was eluted at a flow rate of 30 ml/h and fractions (290 ml) were collected; 10 μ1 aliquots of each fraction were analysed by SDS gel electrophoresis (12). The appropriate fractions were pooled and dialysed against 40 liters of 0.07 M NaCl in 0.1 M sodium phosphate pH 7.0. The sample was applied to a CM-Sepharose 6B-28 column (5 × 20 cm) which had been equilibrated with the same buffer at pH 7.0 and NaCl concentration of 0.07–0.5 M NaCl in phosphate buffer pH 7.0. The flow rate was 20 ml/h, fractions of 2 ml were collected. The DNA binding protein was eluted at 0.3 M NaCl. The pooled fractions were dialysed against 0.1 M sodium phosphate in 0.01 M sodium phosphate pH 7.0, and applied to a second CM-Sepharose column (14 × 20 cm) which was eluted with a 500 ml gradient of 0.1 M 0.5 M NaCl in phosphate buffer pH 7.0. The flow rate was 20 ml/h, NaCl concentrations of 0.1 to 0.3 M NaCl. Fractions of 2 ml were collected. The eluted protein was stored at 0°C. The purity of the protein was determined by SDS gel electrophoresis on a 12% polyacrylamide gel (12). The protein concentration was determined by absorbance at 280 nm of 1 ml aliquots which had been filtrated through a 0.5 μm Millipore filter. The protein was stored at 0°C in 0.01 M sodium phosphate buffer pH 7.0.

**Determination of optical properties.** Circular dichroism spectra were recorded on a Jasco J-715 spectropolarimeter equipped with a photodiode array detector. The measurements were performed as described in (13).

**DNA binding experiments.** DNA binding experiments were performed by the method of P. L. Berg. DNA was prepared by a modification of the procedure of Smith and Berg (12). The protein was dialyzed against 20 ml 20 mM sodium phosphate pH 6.6 to remove any salt. The DNA-protein complex was then incubated at room temperature for 1 h. The DNA was then centrifuged through 5 ml of 5 M sucrose in 20 mM sodium phosphate pH 6.6 for 3 h at 35,000 × *g*.

**Crystallographic data.** The crystallographic data are summarized in Table I. The crystal was grown from the same buffer as used for the DNA binding experiments. The crystal was mounted in a cryo holder. The adsorbed protein was eluted with a small volume of 1 M NaCl in 0.01 M sodium phosphate buffer pH 7.0. The crystal was equilibrated with a solution of 0.3 M sodium phosphate in 0.01 M sodium phosphate buffer pH 7.0 which had been filtered through a 0.22 μm Millipore filter. After filtration through a 0.15 μm Millipore filter. After filtration through a 0.15 μm Millipore filter, the protein was stored in 0.01 M sodium phosphate buffer pH 7.0.

**Crystallization.** Crystallization experiments were carried out by the hanging drop vapour-diffusion method as described in (14).

1“Experimental Procedures” are presented in miniprint as prepared by the authors. The abbreviation used is: SDS, sodium dodecyl sulfate. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 82 M-1901, cite the authors, and include a check or money order for $1.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
RESULTS AND DISCUSSION

Purification—We have observed that DNA binding protein II associates with ribosomal subunits and with 70 S tight couples in appreciable quantities, both in E. coli and in B. stearothermophilus.

The protein has previously been isolated, together with the ribosomal proteins, from 50 S subunits of B. stearothermophilus. A new purification procedure for ribosomes which we have adopted recently allows for a faster purification of the DNA binding protein in large quantities.

This procedure (11) involves the purification of ribosomes by gel filtration in the presence of 1 M NH4Cl, under which condition DNA binding protein II is removed from the ribosome and emerges after the ribosome peak. The protein, being one of the few basic components in the protein mixture, can be easily purified by a two-step ion exchange chromatography.

The analysis of DNA binding protein II from B. stearothermophilus by two-dimensional gel electrophoresis is shown in Fig. 1. The protein appears to consist of only one component. In comparison with the two E. coli proteins (NS1 and NS2), the B. stearothermophilus protein resembles NS2 in electrophoretic behavior (Fig. 1b).

The Association of DNA Binding Protein II with the Ribosome—We have routinely observed the presence of the DNA binding protein in preparations of 30 S and 50 S subunits and in 70 S tight couples. The amount of protein isolated from ribosomal particles was comparable to that of other ribosomal proteins, both for E. coli and for B. stearothermophilus.

70 S, 50 S, and 30 S ribosomal particles depleted of the DNA binding protein II by washing with 1 M NH4Cl were tested for binding. The protein was added to the ribosomes (or subunits) in “Polymix” (11) and the mixture was fractionated on a Bio-Gel A-0.5m column. The excluded ribosomal particles were analyzed for protein content by two-dimensional gel electrophoresis. In all three cases, DNA binding protein II was found to be present.

Solution Properties—The molecular weight of the DNA binding protein was determined using sedimentation equilibrium in the analytical ultracentrifuge. The smallest component detected had a molecular weight of 38,000, which corresponds to that of a tetramer. No evidence for the existence of a dimeric species was found. At higher protein concentrations, components with higher molecular weight were also observed. The Stokes radius of the protein was determined by analytical gel filtration on Sephadex G-50 (superfine). A value of 2.50 nm was found using loading concentrations of 1 mg/ml or

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helix content, estimated at approximately 45%. The E. coli proteins have a similar spectrum with a lower α-helix content of 35% (Fig. 2).

The stability of the secondary structure of the protein as a function of temperature and urea concentration was determined by following the change in ellipticity at 220 nm (Fig. 3). The secondary structure of the protein "melted" at approximately 68 °C, the inflection point in the urea denaturation was around 3.5 M urea. In both cases, the secondary structure could be completely restored by returning the protein to the original condition. In comparison, the E. coli proteins were less stable. "Melting" occurred around 55 °C and the protein

Fig. 4. 270-MHz proton magnetic resonance spectra of DNA binding protein II from B. stearothermophilus. a, original protein at pH 7 and 20 °C; b, in the presence of 6 M urea; c, after removal of urea by dialysis; d, same spectrum as in a, but resolution-enhanced by the convolution difference technique (17).

Fig. 5. The two crystal forms of DNA binding protein from Bacillus stearothermophilus. In each case, the bar represents 0.5 mm. a, the monoclinic needles (space group P2); c, the triclinic plates, 5° precession; h01; d, triclinic plates, 9° precession, h01; e, triclinic plates, 5° precession, h01; f, triclinic plates, 5° precession, h01.

Fig. 6. Precession photographs of the two crystal forms of the DNA binding protein. a, monoclinic needles, 9° precession, h01; b, monoclinic needles, 15° precession, h01; c, triclinic plates, 9° precession, h01; d, triclinic plates, 9° precession, h01.
The resolution of the spectra was enhanced by the convolution difference technique.

The best crystals are obtained from protein has a well defined tertiary structure (Fig. 4, grown at room temperature using a variety weight) of 1.86 Å/dalton, whereas a dimer gives a value of 3.73 Å/dalton and a mean of 2.4 Å/dalton.

Two crystal forms predominate. The first grows as mono-crystals up to 2 mm in length, but only 0.15 mm in the clinic needles. The second crystal form rarely appears spontaneously. Enigmatically, the crystals grow after attempted seeding experiments with microscopic fragments of the monoclinic needles described above. A monoclinic crystal is mechanically ground up in 35% α-methyl-2,4-pentandiol and a minimal volume of this solution transferred to a hanging drop, using growth conditions identical with those for the monoclinic needles. The second form grows, however, as thin triclinic platelets (Fig 5b) with dimensions up to 0.5 × 0.5 × 0.15 mm³. Precession photographs (Fig. 6, c and d) show the space group to be P1 with cell dimensions a = 36 Å, b = 80 Å, c = 37 Å, α = 85°, β = 62°, γ = 79°. Assumption of one tetramer per unit cell gives a Vₙ of 2.39.

Nucleotide Binding—Mixtures of binding protein and oligonucleotides were fractionated by gel filtration on Sephadex G-50 and the association of the nucleotide with the protein was followed using the absorbance at 260 nm. A substantial part of the oligonucleotide added is bound to the protein.

Deoxyribonucleotides in the range of di- to octanucleotide bind quite well, both in the single- and in the double-stranded form. Ribonucleotides appear to be bound very weakly or not at all. The binding becomes weaker at increasing salt concentrations and is rather low above 0.1 M NaCl.

The circular dichroic spectrum of the protein does not change after binding of oligonucleotides, the spectrum of the complex equals the sum of the two component spectra. However, in the proton magnetic resonance spectra of complexes, changes can be observed, e.g. in the methylene resonances of arginine (3.2 ppm) and in the 0.7-0.9-ppm region (Fig. 7).

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REFERENCES
1. McGhee, J. D., and Felsenfeld, G. (1980) Annu. Rev. Biochem. 49, 1115-1156
2. Griffith, J.D. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 563-567
3. Rouviere-Yaniv, J., Yaniv, M., and Germond, J. (1979) Cell 17, 265-274
4. Varshavsky, A. J., Nedospasov, S. A., Bakayev, V. V., Bakayeva, T. G., and Georgiev, G. P. (1977) Biochim Biophys Acta 474, 2725-2745
5. Busby, S., Kolb, A., and Buc, H. (1979) Eur. J. Biochem. 99, 105-111
6. Geider, K., and Hofmann-Berling, H. (1981) Annu. Rev. Biochem. 50, 233-260
7. Mende, I., Timm, B., and Subramanian, A. R. (1988) FEBS Lett. 260, 395-398
8. Laine, B., Kmiecik, D., Sautiere, P., Biserte, G., and Cohen-Solal, M. (1990) Eur. J. Biochem. 193, 447-461
9. Zentgraf, H., Berhold, V., and Geider, K. (1977) Biochim Biophys Acta 474, 629-636
10. Kimura, M., and Wilson, K. S. (1983) J. Biol. Chem. 258, 4007-4011
11. Jelenc, P. C. (1980) Anal. Biochem. 105, 369-374
12. Laemmli, U. K., and Favre, M. (1973) J. Mol. Biol. 80, 575-599
13. Morrison, C. A., Bradbury, E. M., Littlechild, J., and Dijk, J. (1977) FEBS Lett. 73, 348-352
14. Appelt, K., Dijk, J., Reinhardt, R., Sanhueza, S., White, S. W., Wilson, K. S., and Yonath, A. (1981) J. Biol. Chem. 256, 11787-11790
15. Matthews, B. W. (1968) J. Mol. Biol. 33, 491-497
16. Geyl, D., Bock, A., and Isom, K. (1981) Mol. Gen. Genet. 181, 309-312
17. Campbell, I. D., Dobson, C. M., Williams, R. J. P., and Xavier, A. V. (1979) J. Magnetic Res 11, 172-181
On the DNA binding protein II from Bacillus stearothermophilus. I. Purification, studies in solution, and crystallization.
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