

dam methylase from *E. coli*

Circular dichroism investigations of the secondary structure and influence of *S*-adenosylmethionine

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The enzyme *dam* methylase which recognizes and methylates the adenine in the palindromic sequence GATC in DNA was isolated and the secondary structure was determined by CD spectroscopy and various predicting methods from the amino acid sequence. The interaction of *dam* methylase with *S*-adenosylmethionine was studied by CD spectroscopy indicating a decrease of the percentage of α -helix as the amount of *S*-adenosylmethionine bound to the enzyme was increased.

Methylation; α -Helix; β -Sheet; Secondary structure; Predictive method

1. INTRODUCTION

Type II restriction and modification enzymes are perfect candidates for structural studies of the sequence-specific recognition of double-stranded DNA by proteins. The short recognition sequences of 4–6 base pairs facilitate a detailed analysis of the specific interactions. Only two of the several hundred known enzymes have been studied in some detail, *EcoRI* [1] and *DpnII* [2]. This lack of information is due mainly to the existence of these enzymes in very low quantities in bacterial cells. Thus only the cloned enzymes are available for studies. It is known [3] that the endonucleases act as dimers in contrast with most of the methylases which act as monomers, indicating a difference in the mode of action. No information about the

specific interaction of the methylases with the DNA exists, however. *dam* methylase selectively methylates the adenine residues of the sequence GATC of newly synthesized DNA [3]. We chose *dam* methylase for the following reasons: (i) it is a small monomeric enzyme of 30 kDa [4]; (ii) it needs only *S*-adenosylmethionine for its action [4]; (iii) it has several significant biological roles: *dam* methylase discriminates the parent from the daughter strand of DNA during mismatch repair [5], is involved in replication [6], regulates the expression of certain genes, like the *mom* gene [7] and the *dnaA* gene [8]; (iv) it is one of the few cloned methylases so it can be produced in sufficient quantities [9]. It seems to be an appropriate candidate for structural studies of enzyme-DNA interactions.

Here, we present the first part of such a study on the interaction of the enzyme with *S*-adenosylmethionine. We used CD spectroscopy to detect conformational changes during the formation of the complex of *dam* methylase and *S*-adenosylmethionine.

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2. MATERIALS AND METHODS

2.1. *dam* methylase

The enzyme was isolated from an overproducing *E. coli* strain, JC4583/pGG503, obtained from Dr Modrich (Duke University, Durham, NC). A 500 g portion of *E. coli* JC4583 cells, cultured to the middle of the logarithmic phase and stored at -20°C , was treated with lysozyme-sodium deoxycholate in a Waring blender to obtain the cell extract. After centrifugation to remove the cell debris Polymix P was added to a final concentration of 0.03% (v/v). The precipitate was removed by centrifugation and discarded. The supernatant was mixed with 1.5 l Blue-Sepharose and after stirring for 2 h the total was filtered onto a large sintered glass filter. After several washings, the enzyme was eluted with phosphate buffer containing 1 M KCl. After dilution to a conductivity equal to 0.3 M KCl, the active fractions were applied to a phosphocellulose P11 column and the enzyme was eluted with a linear salt gradient (0.2–1 M KCl). The active fractions were rechromatographed first on a Blue-Sepharose column and finally on a tRNA-Sepharose column. The enzyme was obtained in a homogeneous state.

S-Adenosylmethionine (SAM) was from Boehringer Mannheim (FRG). Stock solutions were prepared daily in 0.1 M phosphate buffer (pH 7.4).

2.2. CD spectra

CD measurements were made at 20°C in a 0.1 mm cell in a Jobin-Yvon mark V dichrograph. Before measuring CD spectra the samples were dialyzed at 4°C against 20 mM phosphate buffer (pH 7.4) containing 1 mM EDTA and 0.1 mM dithiothreitol. Samples containing increasing amounts of SAM were prepared by adding 1, 2.5, 4 or 5 μl SAM (5 mg/ml) to separate samples of 100 μl enzyme solution. The spectra of solutions which contained the buffer of the enzyme plus the same amounts of SAM dissolved in buffer were taken as blanks and subtracted from the recorded protein-SAM spectra. 36 spectra were accumulated on an on-line computer (Apple II+) and plotted, manually averaged and readings every 2 nm from 180 to 250 nm were used for decomposition of the spectra. The method of Hennessey and Johnson [10], based upon matrix rank analysis of CD spec-

tra of 15 proteins of known composition, was programmed on an Apple IIe computer. The published input spectra and factors of Hennessey and Johnson [10] were used.

The secondary structure prediction methods of Chou and Fasman [12] and Garnier et al. [13] were used. The amino acid sequence of *dam* methylase was deduced from the DNA sequence of the gene [14]. Both methods were programmed on an Apple IIe computer.

3. RESULTS AND DISCUSSION

The CD spectrum of *dam* methylase is depicted in fig.1 together with the computer-fitted one. The spectrum is characterized by two negative peaks at 208 and 222 nm and a positive one at 192 nm. The crossovers occur at 202 and 180 nm. The 222 nm band is slightly more pronounced than that at 208 nm. The characteristics of the CD spectrum of the enzyme are typical for α/β -type proteins [11]. The Hennessey-Johnson method predicts 43.7% α -helix, 31.3% β -sheet (18.5% antiparallel and 12.8% parallel), 12% turns and 12.6% other structures. These results are in reasonable agreement with the predictions obtained from the amino acid sequence prediction methods (Chou and Fasman [12] and Garnier et al. [13]; see table 1).

To study any conformational changes upon the formation of the *dam* methylase-SAM complex the

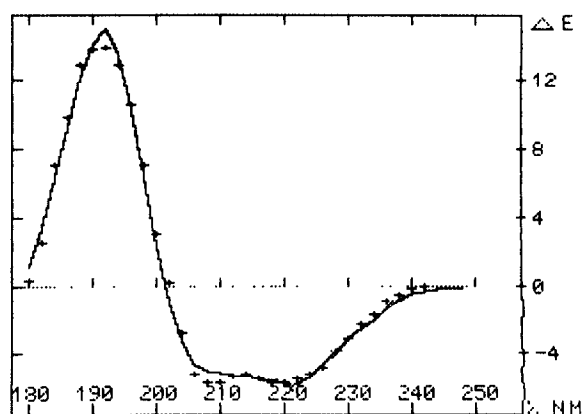


Fig.1. CD spectrum of *dam* methylase fitted by the method of Hennessey and Johnson [10]. Spectrum 1 of fig.2 was used. (+) Experimental points, (---) fitted curve.

Table 1

Analysis of CD spectra of *dam* methylase by the method of Hennessey and Johnson [10] compared with the predictions from Chou and Fasman [12] and Garnier et al. [13]

SAM/ <i>dam</i>	% α -helix	% β -sheet	% turn	% other
0	43.7	31.3	12.3	12.6 [10]
	35.3	25.5	18.0	21.2 [13]
	44.6	14.1	20.5	20.9 [12]
5	28.9	24.0	18.0	26.8 [10]
12.5	14.6	30.8	12.8	34.5 [10]
20	11.6	31.5	20.0	36.5 [10]
25	11.1	30.9	20.3	37.2 [10]

CD spectra of the enzyme with increasing amounts of SAM were recorded. These spectra are depicted in fig.2 and are characterized again by the two negative peaks at 208 and 222 nm and the positive one at 192 nm. As the amount of the substrate is increased the spectrum of the protein is clearly changed. The negative band at 222 nm disappears and the negative band at 208 nm increases. The positive band at 192 nm decreases and at high SAM/enzyme ratios becomes negative. The crossovers are shifted from 202 to 195 nm and from 180 to 188 nm. These new characteristics of the spectrum of the protein indicate a transition from an α/β -type protein to an $\alpha + \beta$ -type protein [11].

The prediction for secondary structure according to Hennessey and Johnson indicates a decrease in α -helix from 44 to 12% and an increase in antiparallel β -sheet from 18 to 25%, as well as an increase in aperiodic structures from 13 to 37% (table 1). Inverse experiments, i.e. titrating highly concentrated SAM solutions with increasing amounts of enzyme, did not indicate any extrinsic CD contributions by SAM. It is improbable that stacking of the adenosine moiety of SAM within the enzyme will cause such large changes, although they cannot be excluded. In this case, however, exciton bands in the 260 nm region would be expected which should be at least an order of magnitude larger than the spectra observed [15].

Surprisingly, even at an SAM/*dam* methylase ratio = 25 the enzyme does not appear to be

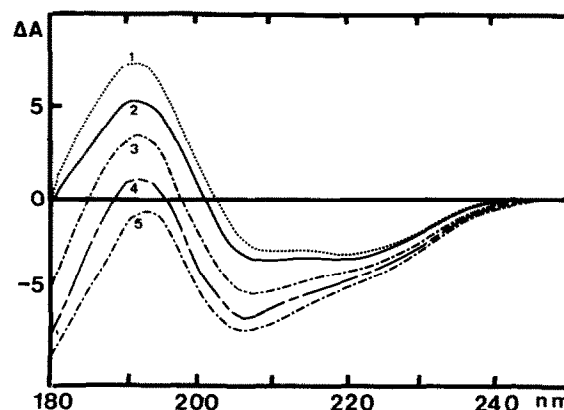


Fig.2. CD spectra of *dam* methylase in the presence of increasing amounts of SAM. SAM/*dam*: 0 (1), 5 (2), 12.5 (3), 20 (4), 25 (5). Concentration of *dam* methylase was 20 μ M. Spectra were recorded in 0.1 mm cuvettes.

saturated: the CD changes of the enzyme are virtually linearly related to SAM concentration. In this context it is noteworthy that the $K_m = 15 \mu$ M of *dam* methylase for SAM is rather high [9]. This implies that the SAM/*dam* methylase ratio must be over 100 in the in vitro experiments. We can suggest that the *dam* methylase-SAM interactions are not specific. Since SAM carries a net positive charge, conformational changes upon these interactions possibly open up the enzyme's structure and lead to the specific site recognition of the DNA substrate. We believe that a full explanation of the role of SAM will come probably from studies of the enzyme-DNA complexes and enzyme-SAM-DNA complexes. Also DNase I footprints may give information, if SAM is essential for specific site recognition on the DNA.

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