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Thermodynamic parameters of the binding of the tight-binding I12X86 lac repressor to operator and non-operator DNA

Jean Claude Maurizot and Philippe Grebert

Centre de Biophysique Moleculaire, 1A, Avenue de la Recherche Scientifique, 45071 Orleans Cédex, France

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The thermodynamic parameters ΔH and ΔS corresponding to the binding of the tight-binding double mutant lac repressor I12X86 with operator and non-operator DNA fragments were determined using the nitrocellulose filter binding assay. In both cases the binding processes are entropically driven and accompanied by an unfavorable enthalpy variation. The differences between these parameters and those previously reported for the wild type lac repressor show that the strategy adopted by the mutant to interact with DNA is highly different from that of the wild type repressor and suggest more hydrophobic contacts between the mutant and DNA.

Lac repressor; Lac operator; DNA binding; Thermodynamic parameter

1. INTRODUCTION

The lac operator-repressor interaction has served as a prototype in the investigation of protein-nucleic acid recognition systems (for review see [1,2]). The use of altered repressor molecules with increased affinity for DNA has been a great help in these studies [3–11]. The tight binding I12X86 lac repressor results from two mutations, the X86 mutation (Ser-61 ⟶ Leu) and the I12 mutation (Pro-3 ⟶ Tyr). When compared to the wild-type repressor this mutant shows an increased non-specific affinity for DNA as well as large increased affinities for operator and sequences related to the operator. It has also been shown that the non-specific DNA binding of this modified repressor is isopropyl-β-D-thiogalactoside (IPTG) dependent contrary to the wild-type behaviour [6]. Recently it has been shown that in the non-specific DNA binding the number of ions released from the vicinity of the DNA upon complex formation is less important for this mutant than for the wild-type protein [12].

Knowledge of the enthalpic and entropic contributions to the free energy associated with complex formation, may provide information concerning the forces involved in the higher binding affinities of the mutant repressor as compared to the wild-type protein. In this paper using the classical nitrocellulose filter binding assay, we determine the thermodynamic parameters for the binding of the I12X86 lac repressor to the lac operator and to non-specific DNA.

2. MATERIALS AND METHODS

All chemicals used were reagent grade. Buffer solutions for nitrocellulose filter assays were passed through type HA Millipore filters (0.45 μm pore diameter) prior to use. [α-^{32}P]dATP was purchased from Amersham International. *E. coli* DNA polymerase I was from Boehringer.

The I12X86 protein was purified as described by Rosenberg et al. [13] except that a gradient ranging from 0.12 M to 0.30 M potassium phosphate was applied to the phosphocellulose. This was done to take into account the tighter binding of the double mutant. The phosphocellulose column was followed by gel filtration on an Ultrogel AcA 34 (LKB) column. The purity of the I12X86 repressor was more than 95% as judged by SDS-polyacrylamide gel. Concentrations were determined from ab-

Correspondence address: J.C. Maurizot, Centre de Biophysique Moleculaire, 1A, Avenue de la Recherche Scientifique, 45071 Orleans Cédex, France

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sorption measurements using a molecular extinction coefficient of ϵ_{280} = 90800 l·mol^{-1}·cm^{-1} for the I12X86 repressor tetramer.

The DNA used in the non-specific binding studies was a 210 bp fragment whose preparation has been previously described [12]. A 203 bp DNA fragment [14] corresponding to the entire lac promotor-operator region was used in the specific-binding experiments. The DNA fragments were ^{32}P labelled by nick-translation with [α-^{32}P]dATP as described by Maniatis et al. [15]. Reactions were carried out using 0.5 μg of DNA. Unreacted dATP was removed by filtration through a small Sephadex G50 column. Concentrations of the fragment were determined using a molecular extinction coefficient of ϵ_{260} = 6500 l·mol^{-1}·cm^{-1} per base.

2.1. *Binding measurements*

The experiments were performed as described by Riggs et al. [16] with the modifications described by Winter and Von Hippel [17]. BA-85 nitrocellulose filters (Schleicher and Schull) were used. The binding buffer was 0.01 M Tris-HCl, pH 7.5, 0.1 mM EDTA, 0.1 mM dithioerythritol and 50 μg/ml bovine serum albumin. KCl concentration was adjusted as desired. Prior to use, filters were soaked in filter buffer: 0.01 M Tris-HCl, pH 7.5, 0.1 mM EDTA, 50 mM KCl.

Binding curves were generated by adding increasing concentrations of repressor to a fixed concentration of DNA fragment (2.5×10^{-12} M). The reaction volume was 1 ml. Triplicate samples were filtered, washed with 1 ml of binding buffer having the same KCl concentration as the sample, dried, and counted by liquid scintillation. The filtration rate was 2 ml/min. A twofold increase of the wash volume did not change the observed retention.

Binding data were fitted by using the binding polynomial procedure as developed by Clore et al. [18]. The parameter θ which is calculated corresponds to the fraction of molecules to which at least one protein is bound. It is defined by $\theta = (Z - 1)/Z$ where Z is the binding polynomial as defined by Wyman [19]. The binding polynomial was calculated taking into account the efficiency of protein-DNA retention as indicated by Woodbury and Von Hippel [20]. The efficiency parameter can be considered as the probability that a repressor molecule complexed to the DNA is held on the filter. The quantity $\zeta = (1 - \epsilon)$ is then the probability that this complex is not retained. The probability of retaining a DNA fragment binding i repressor molecules is $(1 - \zeta^i)$. As pointed out by Woodbury and Von Hippel [20] one must notice that while the effectiveness of individual protein molecules in retaining the DNA on the filter is constant, the overall efficiency of DNA retention $(1 - \zeta^i)$ increases drastically with the number of repressor bound on the DNA fragment. The best fits for our data were obtained with efficiency parameters between 0.85 and 0.95. In the specific-binding experiments the non-specific binding was taken into account. The equilibrium constants are expressed in M^{-1} of fragment.

3. RESULTS AND DISCUSSION

The interaction between the I12X86 lac repressor and the 203 bp operator DNA fragment and that with the 210 bp non-operator DNA fragment were studied by the classical nitrocellulose filter binding method and analyzed as described in section 2. Fig.1 shows the binding isotherms of the mutant repressor on both fragments at various temperatures. In both the specific and the non-specific case the affinity increases when the temperature increases.

The apparent van 't Hoff enthalpy (ΔH) of complex formation was determined from the temperature dependencies of the equilibrium constants (fig.2). Such determinations assume that ΔH is independent of temperature, and consequently that there is no change in the heat capacity of the system. The good linearity of the van 't Hoff plots strongly suggests that it is indeed the case at least in the temperature domain where our experiments were performed (4–30°C). The value found for the specific binding is $\Delta H = +21 \pm 3$ kcal/mol whereas it is $\Delta H = +14 \pm 5$ kcal/mol for the non-specific binding to the 210 bp DNA fragment. The corresponding entropy variations are $\Delta S = +125 \pm 15$ cal/mol·deg for the specific complexation and $\Delta S = +80 \pm 15$ cal/mol·deg for the non-specific complexation.

In table 1 the thermodynamic parameters determined in this study are shown, together with those previously reported for the binding of the wild-type lac repressor to operator and non-operator DNA [21,22].

Our results indicate that both the specific and the non-specific reaction are entropically driven and accompanied by an unfavorable enthalpy variation since the values of H are positive.

When comparing the parameters corresponding to the binding of the I12X86 lac repressor to those of the wild-type protein, one notices an increase in the entropic term, whereas the enthalpic term which is unfavorable in the wild-type repressor is more unfavorable in the mutant. Generally it is considered that the entropic contribution to complex formation is related either to a rearrangement of the water molecules following the electrostatic interaction leading to cation ejection from the vicinity of the DNA, or to hydrophobic interactions. We have shown, using the variation of the equilibrium constant as a function of the salt concentration, that the number of counter ions ejected upon specific complex formation is similar for the I12X86 and wild-type lac repressor (unpublished results). One may therefore think that the supplementary energy for the mutant versus the wild

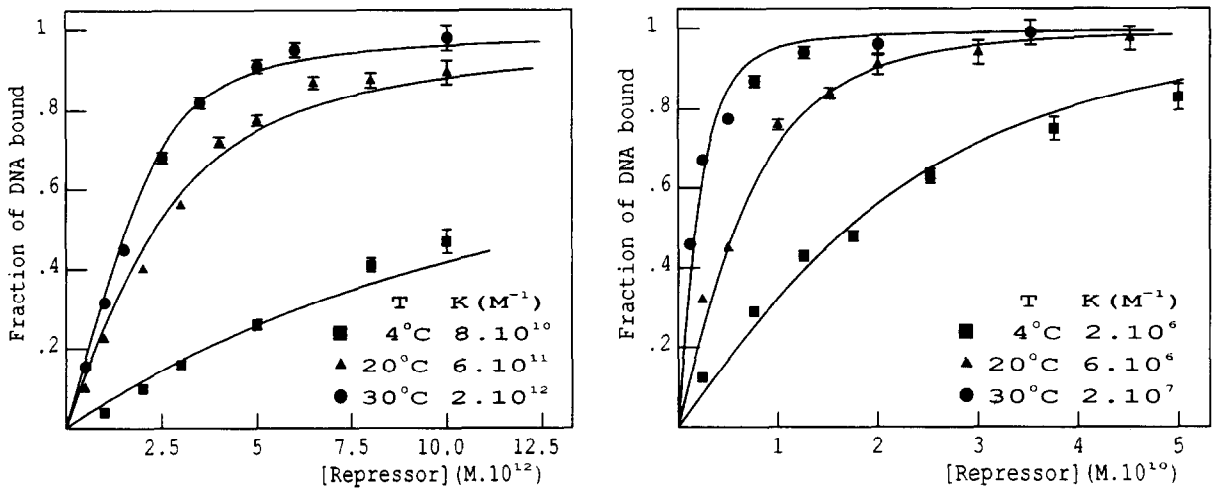

Fig.1. Binding isotherms for the interaction of the I12X86 lac repressor with the specific 203 bp DNA fragment (left) and non-specific 210 bp DNA fragment (right). Experimental conditions are described in section 2. The solid curves are the theoretical binding isotherms generated using the equilibrium constants indicated in the figure.

type is related to the presence of supplementary hydrophobic interactions.

Among the two mutations present in the I12X86, the Pro-3⟶Tyr should not by itself allow supplementary hydrophobic contact between the repressor and the nucleic acid. On the contrary in the second mutation, the leucine residue which replaces a serine at position 61 is much more hydrophobic. This mutation occurs in a region of the repressor which is thought to be the transmitter region, the region which links the inducer binding site to the operator binding site [1]. That position is close to a residue which is exposed to the solvent, the lysine 59 which can be easily attacked by trypsin, and at the beginning of a long hydrophobic region as calculated using the method described by Kyte and Doolittle [23]. Therefore, one possibility is that the leucine at position 61 makes a supplementary contact with the DNA or more probably that it changes the orientation of the headpiece and thus allows it to make more hydrophobic contacts with the lac operator.

Concerning the specific binding, our results show that the thermodynamic parameters are drastically different from those previously obtained with the wild-type lac repressor. The values obtained for ΔH and ΔS are more reminiscent of the specific binding of the wild-type repressor, show-

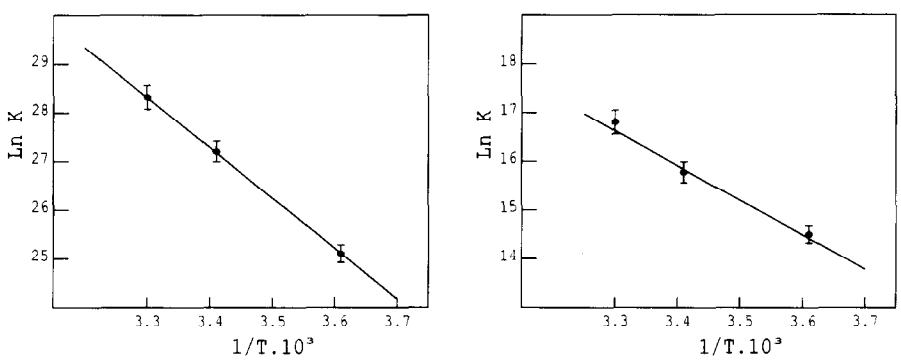

Fig.2. Van 't Hoff plots for the interaction of the I12X86 lac repressor with the specific 203 bp DNA fragment (left) and the non-specific 210 bp DNA fragment (right).

Table 1

Thermodynamic parameters for the binding of the mutant I12X86 and wild type lac repressor

	Wild type repressor	I12X86 repressor
Specific binding	$\Delta H = +14 \pm 5$ kcal/mol $\Delta S = +100 \pm 20$ cal/mol·deg	$\Delta H = +21 \pm 3$ kcal/mol $\Delta S = +125 \pm 15$ cal/mol·deg
Non-specific binding	$\Delta H = -6$ kcal/mol $\Delta S = +4.4$ cal/mol·deg	$\Delta H = +14 \pm 3$ kcal/mol $\Delta S = +80 \pm 15$ cal/mol·deg

Data for the wild type are from references 21 and 22

ing a large entropic favorable contribution and a positive enthalpic term. We have previously shown that the number of ion released from the vicinity of the DNA upon complex formation is less important for the mutant than for the wild type [12]. As for the specific binding it is therefore probable that the increase in binding energy is related to supplementary hydrophobic contacts. Recently the cloning and characterisation of several tight-binding mutants has been described by Betz [24]. It is interesting to notice that all the mutations characterized were concerned with hydrophobic amino acids of the headpiece. In any case our results show that the strategy adopted by the mutant to interact non-specifically with non-operator DNA is highly different from that of the wild-type repressor.

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