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The carboxy-terminal domain of the LexA repressor oligomerises essentially as the entire protein

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The ability of the isolated carboxy-terminal domain of the LexA repressor of *Escherichia coli* to form dimers and tetramers has been investigated by equilibrium ultracentrifugation. This domain, that comprises the amino acids 85–202, is readily purified after self-cleavage of the LexA repressor at alkaline pH. It turns out that the carboxy-terminal domain forms dimers and tetramers essentially as the entire LexA repressor. The corresponding association constants were determined after non-linear least squares fitting of the experimental concentration distribution. A dimer association constant of $K_2 = 3 \times 10^4$ M^{-1} and a tetramer association constant of $K_4 = 2 \times 10^4$ M^{-1} have been determined. Similar measurements on the entire LexA repressor [(1985) Biochemistry 24, 2812–2818] gave values of $K_2 = 2.1 \times 10^4$ M^{-1} and $K_4 = 7.7 \times 10^4$ M^{-1}. Within experimental error the dimer formation constant of the carboxy-terminal domain may be considered to be the same as that of the entire repressor whereas the isolated domain forms tetramers slightly less efficiently. It should be stressed that the potential error in K_4 is higher than that in K_2. The overall conclusion is that the two structural domains of LexA have also well-defined functional roles: the amino-terminal domain interacts with DNA and the carboxy-terminal domain is involved in dimerisation reinforcing in this way the binding of the LexA repressor to operator DNA.

SOS system; LexA repressor; RecA protein; DNA-binding protein; Multi-domain protein; Equilibrium ultracentrifugation

1. INTRODUCTION

The LexA repressor of *Escherichia coli* differs from other repressors in that it controls the expression of a whole set of unlinked genes involved in very different cellular functions like DNA repair, mutagenesis, cell division as well as a set of bacterial toxins and its own gene (for a review see [1]). This regulatory network, the well known 'SOS-system', raises the question how a single repressor may achieve a satisfying regulation of gene products needed at very different basal and constitutive levels.

It turns out that LexA acts as a fairly weak repressor in the case of the excision repair genes *uvr*A and *uvr*B (for a comparison of the strength of the different SOS operators see [2]) and as a 'medium strength' repressor in the case of the genes involved in mutagenesis for example. These differences in LexA affinity are apparently mediated by slight variations within the central part of the different SOS operators.

The overall weakness of the LexA repressor with respect to other repressors is probably linked to its low dimerisation constant of only 2.1×10^4 M^{-1} [3]. This view is supported by our finding that the isolated amino-terminal DNA binding domain of LexA (the first 84 amino acids out of a total of 202) retains substantial DNA binding affinity [2,4,5].

Here we show by equilibrium ultracentrifugation measurements that the isolated C-terminal domain (amino acids 85–202) shows a very similar dimerisation and tetramerisation behaviour to the entire repressor. This finding supports the view of a structural and functional dissection of the LexA

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repressor into an amino-terminal DNA binding and a carboxy-terminal dimerising domain.

2. MATERIALS AND METHODS

2.1. *Protein purification*

The LexA repressor has been purified as described earlier [3]. After self-cleavage of LexA at pH 9 for 4 h the two resulting domains have been purified as described [4]. The concentration of the carboxy-terminal domain was determined using an extinction coefficient at 280 nm of 7300 $M^{-1} \cdot cm^{-1}$ as for the entire repressor [6].

2.2. *Equilibrium sedimentation*

The equilibrium measurements were conducted in a double-sector cell with a column height of 0.4 cm at 27060 rpm (2217 ± 1 μs) during 46 h using a Beckman analytical ultracentrifuge equipped with a home-made optical scanning device [7]. Since the molecular weight of the monomer was known (13.1 kDa), a program of least squares fit was used to achieve the resolution of components in the concentration distribution at equilibrium. The value of the partial specific volume was taken to be 0.740 $cm^3 \cdot g^{-1}$ as determined from the amino acid sequence of the carboxy-terminal domain of the LexA repressor. The measurements were made at 7°C under the same experimental conditions as those used for the entire LexA repressor, that is 10 mM sodium phosphate, pH 7.2, 150 mM NaCl and 0.1 mM EDTA.

3. RESULTS AND DISCUSSION

Fig.1 shows the distribution of protein along the

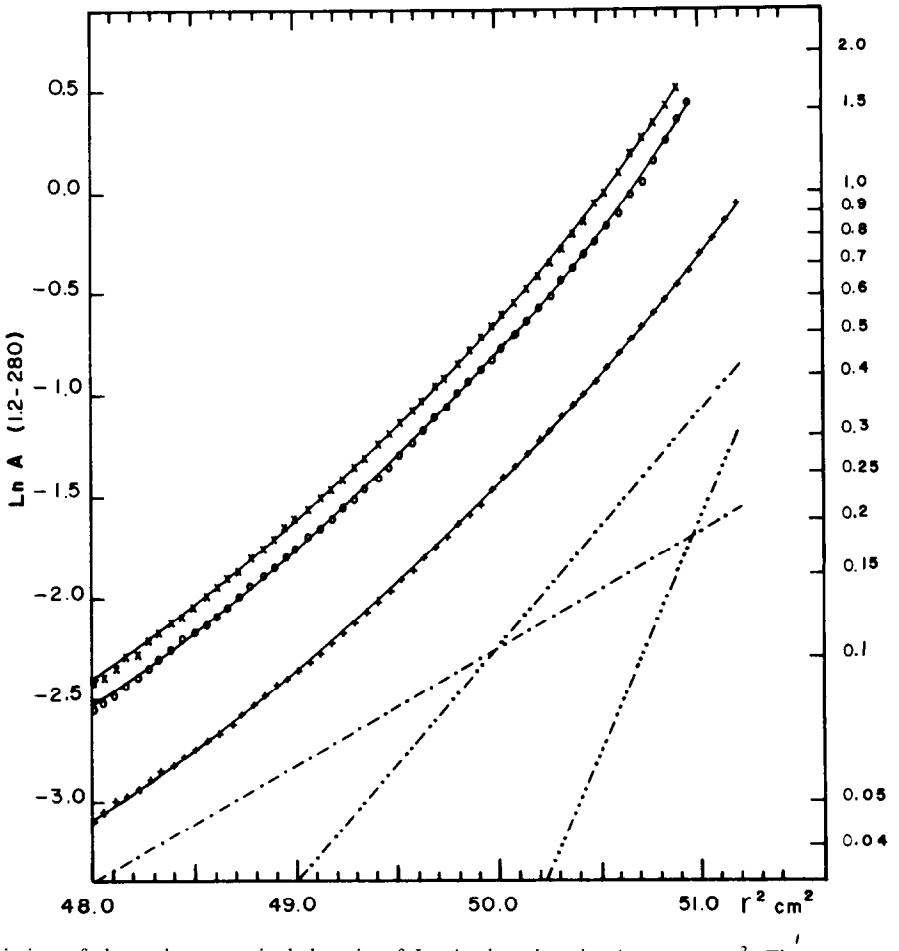

Fig.1. Self-association of the carboxy-terminal domain of LexA plotted as ln $A_{(1.2-280)}$ vs r^2. The measurements were done at 27060 rpm, T = 281 K. Initial loading concentrations were (+) 0.408 mg/ml (cell 1), (○) 0.684 mg/ml (cell 2), (×) 1.03 mg/ml (cell 3). The solid curves through the experimental points are the total concentration distributions calculated from the results of the least-squares fits. The straight lines are the concentration distributions of the individual components in cell 1: monomer (—•—), dimer (—••—), tetramer (—••••—).

cell as measured from the absorption A at 280 nm as a function of the distance r from the rotor axis. The precision of successive readings within the same cell is fairly high with errors not exceeding the size of the symbols used in the figures. Such $\ln A$ versus r^2 plots are linear for monodisperse systems. In the case of the carboxy-terminal domain of LexA these plots are curved showing that the system undergoes oligomerisation.

It is not immediately obvious from such a distribution which are the limiting species of oligomerisation, that is the smallest and the biggest one. A useful way to answer this question is the so-called 'two-species plot' [8], which consists of a plot of respectively M_w vs $1/M_n$ or M_z vs $1/M_w$. For a definition of the different average molecular weights see the legend of fig.2.

The two-species plot (fig.2) shows that all the data points are comprised within the triangle defined by the monomer, the dimer and the tetramer as was the case for the entire LexA repressor [3]. This finding suggests that the smallest species is the monomer and the biggest one the tetramer. In contrary to phage lambda repressor [9], LexA and its

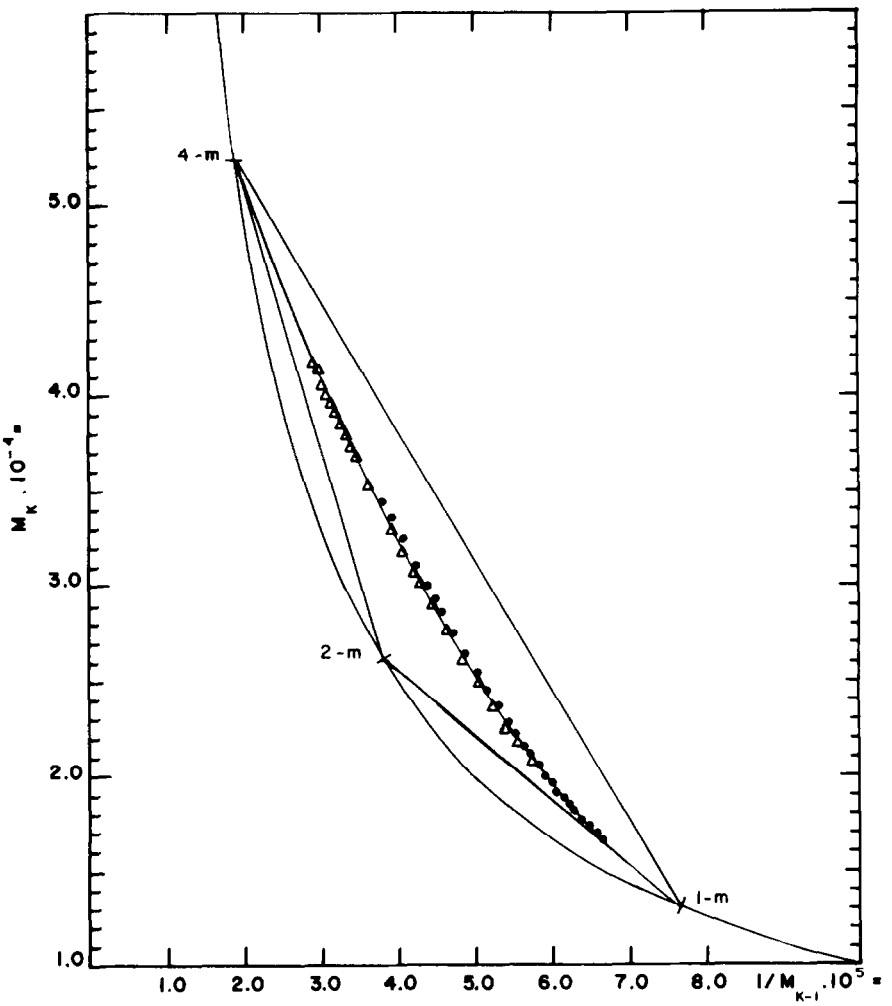

Fig.2. Two-species plot for the carboxy-terminal domain of LexA as determined from the equilibrium experiment shown in fig.1. (●) M_w versus $1/M_n$; (Δ) M_z versus $1/M_w$. The solid curve is drawn with the values of the average molecular weight calculated from the least squares fit. The different average molecular weights are defined as follows: $M_n = \Sigma\, C_j/(\Sigma\, C_j/M_j)$; $M_w = \Sigma\, C_jM_j/\Sigma\, C_j$; $M_z = \Sigma\, C_jM_j^2/(\Sigma\, C_jM_j)$. The hyperbola corresponds to $M_K(1/M_{-1}) = 1$.

carboxy-terminal domain apparently do not form higher molecular weight aggregates.

Using the monomer and the tetramer as limiting species we have fitted the experimental protein distribution in the three measuring cells (fig.1) by a sum of exponentials using a least-squares fitting procedure (for details of this procedure see [3]). As in the case of LexA the existence of a trimer is not compatible with the experimental data, since if it is included into the fitting procedure its concentration always turns out to be negative. The system may thus be described by two coupled equilibria: a monomer-dimer and a subsequent dimer-tetramer equilibrium. Once the observed protein distribution has been decomposed into its three components (monomer, dimer, tetramer) the dimer formation constant K_2 and the tetramer formation constant K_4 may be determined from the following equation [3]:

$$(C_T - C_1)/C_1^2 = 2K_2 + 4K_2^2 \cdot K_4 \cdot C_1^2$$

where C_1 and C_T are respectively the free monomer and the total protein concentration. From a plot of $(C_T - C_1)/C_1^2$ vs C_1^2 the equilibrium constants K_2 and K_4 are readily obtained.

This procedure gives slightly different results of K_2 and K_4 from one measuring cell to the other. The calculated average association constants for the three different loading concentrations are $K_2 = 3 \times 10^4\ M^{-1}$ ($\pm$ 20%) for dimer formation and $K_4 = 2 \times 10^4\ M^{-1}$ ($\pm$ 50%) for tetramer formation.

Within experimental error we find thus the same dimerisation constant for the carboxy-terminal domain as that determined earlier for LexA, that is $2.1 \times 10^4\ M^{-1}$ [3]. The tetramerisation constant of the carboxy-terminal domain appears somewhat smaller than that of the entire repressor ($K_4 = 2 \times 10^4\ M^{-1}$ instead of $7.7 \times 10^4\ M^{-1}$). Taking into account the rather high error for this constant (errors in K_2 are propagated to K_4) the difference between the two protein species in tetramerisation appears not very significant.

We may thus argue that the isolated carboxy-terminal domain of LexA forms dimers and tetramers essentially with the same binding constants as the entire protein.

This finding immediately suggests that essentially no dimerisation energy should arise from interactions between the amino-terminal domains of LexA. Unfortunately this domain harbors neither tyrosine nor tryptophan residues and is thus not suitable for measurements with our optical scanning ultracentrifuge. There is nevertheless indirect evidence from NMR measurements at high concentrations of amino-terminal domain (about 6 mM) that this domain has no pronounced tendency to aggregate, because well-resolved two-dimensional data sets could be collected (our unpublished results). This is apparently not possible in the case of the isolated DNA binding domain of the phage lambda repressor [10] that has been shown to dimerise with an association constant of about $3 \times 10^3\ M^{-1}$ [11].

The LexA repressor and the phage lambda repressor share significant homology within their carboxy-terminal domains [12]. This homology may be functionally linked to the dimerisation of each of these domains and/or to the fact that both domains probably contain the catalytic center for the (self-) cleavage reaction. In the case of LexA the amino acids serine-119 and lysine-156 seem to be crucially involved in this cleavage reaction [13]. Despite this partial homology of the two repressor species the dimerisation of LexA occurs with an about 3000-fold smaller association constant ($2.1 \times 10^4\ M^{-1}$ instead of $5.9 \times 10^7\ M^{-1}$ [14] in the case of the phage lambda repressor). This difference in dimerisation probably accounts for the finding that the self-cleavage reaction of phage lambda repressor is strongly inhibited by increasing repressor concentration, whereas the self-cleavage of LexA shows no concentration dependence in the range from 10^{-9} to 10^{-5} M [15]. We may estimate that at 10^{-5} M of LexA always about 75% of the protein should be in the monomeric state [3] and it is thus not surprising that no concentration dependence of the cleavage reaction has been observed. If the LexA dimer is unable to undergo self-cleavage, as seems to be the case for the phage lambda repressor, a concentration dependent inhibition would be expected to occur in the range from 10^{-5} to 10^{-3} M of LexA.

Fig.3 shows the distribution of each species as a function of the concentration of the carboxy-terminal domain. Up to 0.35 mg/ml, the monomer is the dominating species. Using a molecular mass of 13.1 kDa this corresponds to a concentration of 27 μM, which is far above the concentration expected to occur in vivo.

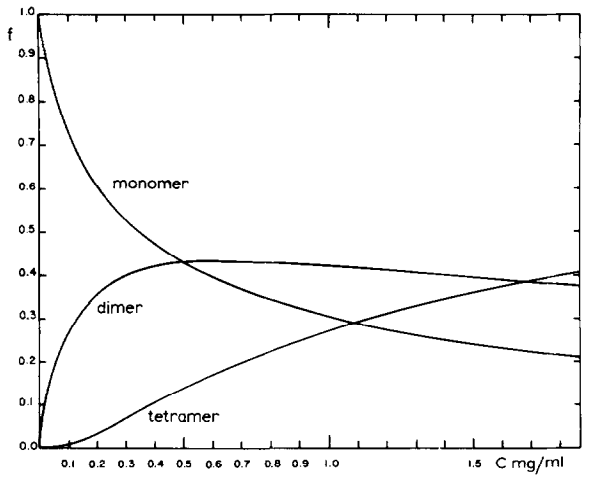

Fig.3. Plot of the fractions of monomer, dimer and tetramer of the carboxy-terminal domain of LexA vs the total concentration. The values of the equilibrium constants are respectively $K_2 = 3 \times 10^4\ M^{-1}$ and $K_4 = 2 \times 10^4\ M^{-1}$.

4. CONCLUSION

The specific self-cleavage reaction of LexA allows one to isolate two unique structural and functional domains without loss of smaller peptide fragments in between. Such a favorable situation is normally not encountered if structural domains are isolated following cleavage with proteolytic enzymes. The two structural domains have apparently also well-defined functional roles: the amino-terminal domain interacts with DNA, and the carboxy-terminal domain is mainly involved in dimerisation reinforcing in this way the binding of the entire LexA repressor to operator DNA. The oligomerisation data reported here constitute so far the only measurable 'activity' of the isolated carboxy-terminal domain and provide indirect evidence that the domain does not undergo unfolding or a major structural reorganisation upon cleavage.

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