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Large-Scale Purification, Oligomerization Equilibria, and Specific Interaction of the LexA Repressor of *Escherichia coli*†

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ABSTRACT: A rapid large-scale procedure for the purification of the LexA repressor of *Escherichia coli* is described. This procedure allows one to get more than 100 mg of purified protein from 100 g of bacterial paste with a purity of at least 97%. This method is comparable to earlier, far more complicated purification procedures giving clearly smaller yields. It is shown that the LexA protein may be identified spectroscopically by a large A_{235}/A_{280} ratio and very pronounced ripples in the absorption spectrum arising from a high amount of phenylalanine residues with respect to that of the other aromatic amino acids. Polyacrylamide gel electrophoresis has been used to study the specific interaction of LexA with a recA operator fragment. The quaternary structure of LexA has been studied by equilibrium ultracentrifugation and sedimentation velocity measurements. The sedimentation coefficient increases with increasing LexA concentration, indicating that LexA is involved in self-association. This finding has been confirmed by equilibrium ultracentrifugation. The results are best described by a monomer–dimer and a subsequent dimer–tetramer equilibrium, with an association constant of 2.1×10^4 M^{-1} for the dimer and 7.7×10^4 M^{-1} for the tetramer formation. These relatively small association constants determined under near-physiological pH and salt conditions suggest that in vivo LexA should be essentially in the monomeric state. The degree to which LexA decreases the electrophoretic mobility of a 175 base pair fragment harboring the recA operator suggests that the recA operator interacts nevertheless with a LexA dimer. However, our results may be also explained by the binding of a LexA monomer with a simultaneous bending of the DNA fragment.

Exposure of *Escherichia coli* to agents or conditions that either damage DNA or interfere with DNA replication results in the increased expression of genes that are members of the SOS regulatory system [for reviews see Witkin (1976), Little & Mount (1982), and Walker (1984)]. During normal cell growth this set of unlinked genes is repressed by the LexA protein. Upon DNA damage an inducing signal is generated that activates the RecA protein. This activated form of RecA stimulates the specific cleavage of lexA gene product (LexA)[1] at an alanine–glycine bond near the center of the protein, leading to an inactivation of LexA and the derepression of the SOS genes. At elevated pH values the same peptide bond is cleaved in the absence of RecA, suggesting that the inactivation of LexA may proceed via autodigestion (Little, 1984). Under in vivo conditions activated RecA protein is necessary to stimulate this reaction, leading to a complete degradation of LexA within a few minutes (Little, 1983). In contrast to the cleavage of λ repressor (Phizicky & Roberts, 1980; Cohen et al., 1981; Little, 1984) the cleavage of LexA seems to be independent of the repressor concentration (Little, 1984). It has been concluded from these results that in the case of λ repressor only the monomer is susceptible to cleavage whereas the LexA protein can be cleaved as well as if it is a monomer or part of a dimer (Little, 1984).

Here, we show by sedimentation equilibrium and velocity measurements that under near-physiological pH and salt

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[1] Abbreviations: LexA, lexA gene product; IPTG, isopropyl β-D-thiogalactoside; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; ME, 2-mercaptoethanol; DTT, dithiothreitol; CAP, catabolite gene activator protein; bp, base pair; SDS, sodium dodecyl sulfate.

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conditions an appreciable amount of LexA remains in its monomeric state even at fairly elevated protein concentrations. Our results are best explained by the assumption of three species: monomers, dimers, and tetramers.

We present further a very rapid large-scale purification procedure for the LexA repressor using only one-column chromatography. Some results on the specific interactions of the repressor with operator-containing DNA fragments and some spectroscopic properties of the protein are described.

Materials and Methods

Materials. Lysozyme, phenylmethanesulfonyl fluoride, and IPTG were purchased from Sigma. Sodium deoxycholate, EDTA, and DTT were obtained from Merck, polymin P from Bayer Leverkusen, the marker proteins were from Pharmacia Fine Chemicals, and the glycerol was a twice distilled quality from Prolabo. The restriction enzymes used were from Boehringer Mannheim, and the polynucleotide kinase was from Biolabs.

Bacterial Cultures and Cell Lysis for the Purification of LexA. LexA protein was purified from the recA$^-$ strain CSR 603 carrying the plasmid pJL45, which harbors a fusion of the lexA gene with the *lac* UV5 promoter (Little et al., 1981), allowing for overproduction of the LexA repressor. We have shown that, at least for this bacterial host, the cells may be grown in the presence of the inducer IPTG without slowing down the cell division. Bacteria were grown at 37 °C in LB medium complemented with 0.2 mM IPTG and 20 μg/mL tetracycline in 10-L stainless steel vessels under vigorous aeration to near saturation. The cells were harvested by centrifugation in a continuous flow rotor, giving about 20 g of wet bacteria paste/10 L, and stored frozen at −20 °C.

About 100 g of bacterial paste was suspended in 500 mL of buffer A (50 mM Tris-HCl, pH 7.6, 5 mM EDTA, 100 mM NaCl, 7 mM ME, and 5% glycerol). Phenylmethanesulfonyl fluoride was added to a final concentration of 1 mM by using a 200 mM stock solution in ethanol, as well as 25 mL of a freshly prepared 5 mg/mL lysozyme solution. The suspension was incubated with stirring for about 15 min. To complete cell disruption and to reduce the viscosity, the suspension was sonicated with an Ultrasons generator keeping the temperature always below 7 °C. After addition of 25 mL of 10% (w/v) sodium deoxycholate and incubation for 15 min under stirring, the lysate was centrifuged for 140 min at 50000g. All following steps of the purification were done at 4 °C unless otherwise noted.

Purification and End Labeling of the DNA Fragments. Plasmid DNA was purified by an adaptation of the procedures developed by Clewell & Helinski (1970) and Katz et al. (1973). The pJL5 plasmid (Little et al., 1981) was digested with *Hin*dIII and *Eco*RI. The two fragments thus obtained were ^{32}P end labeled with polynucleotide kinase. The 175 bp fragment containing the operator/promoter regulatory region of the recA gene was purified by electrophoresis on an 8% polyacrylamide gel under nondenaturing conditions. The DNA was recovered by elution with a low ionic strength buffer. The pJL42 plasmid (Little et al., 1981) grown in a dam *E. coli* strain was digested with *Bcl*I and *Eco*RI, giving two fragments. The 148 bp fragment which contains the lexA operator/promoter regulatory region was labeled and purified as described above. The same procedure was applied to the *Bam*HI–*Sal*I fragment obtained from a double digestion of the pBR322 plasmid. This fragment contains no specific binding site for the LexA repressor.

Study of the Specific Interaction by Electrophoresis. LexA protein was incubated with one of the DNA fragments for 20 min in the following incubation buffer: 100 mM Tris-HCl, pH 7.4, 1,6 mM EDTA, 0.04 mg/mL BSA, 20% glycerol, and 25 mM NaCl. This incubation mixture (25 μL) was loaded on a 5% vertical polyacrylamide gel and electrophoresis begun immediately. The buffer within the gel and the running buffer were both 10 mM Tris–HCl, pH 7.4, and 1 mM EDTA. To maintain a constant pH, a circulation between the two reservoirs was established with the aid of a peristaltic pump. The applied electric field was about 10 V/cm.

Absorption and Fluorescence Measurements. Absorption spectra were recorded on a Cary 118 spectrophotometer and fluorescence spectra on a Jobin Yvon JY3D fluorometer. For the acrylamide quenching studies the excitation wavelength was 300 nm to minimize the inner filter effect from acrylamide absorption. The remaining small absorptive screening was corrected as described earlier (Culard et al., 1982).

Sedimentation Velocity and Equilibrium Measurements. All sedimentation studies were carried out with a Spinco E ultracentrifuge equipped with a Xtal-controlled speed regulator and a digital scanner (Neimark, 1975). The revolution period was determined within ±1 μs accuracy. The optical density, at each radial position in the cell, was an average value of 256 (2^8) individual readings for velocity measurements (±0.005 unit accuracy), or 1024 (2^{10}) readings for equilibrium (±0.003 unit accuracy).

The radial position was determined by counting the clock pulses that a stepping motor needs to move the scanner from the inner to the outer mark of the counterbalance. The average number of steps, depending on the wavelength of the light, was about 4000 from 5.7 to 7.3 cm. The reproducibility of the number of steps was ±2 steps from a scan to another one. The sampling jump was adjustable and was normally of 20 steps between data points.

The light wavelength, given by a Bausch and Lomb grating monochromator illuminated by a 450-W Xe lamp, was 280 nm for all centrifugation experiments. The buffer solution used contained 10 mM sodium phosphate, pH 7.2, 150 mM NaCl, and 0.1 mM EDTA. All experiments were done between 4 and 7 °C.

Sedimentation Velocity. The determinations of the sedimentation coefficients were measured at 45 592 rpm (period of rotation = 1316 ±1 μs) with a double sector synthetic boundary cell. The smoothing of the crude data and the calculation of the derivative curve of the concentration boundary were obtained by the subroutines SE 35 and DET 5 (IBM 360 scientific subroutines package).

The sedimentation coefficients were evaluated by a linear least-squares fit of $\ln r_i f(t_i)$. The values r_i were the 50% points of the integral distribution of concentration, or the values of r_i were determined by the transport method or by the second-order moment of the derivative curve. The $s_{T,S}$ values were converted to the standard conditions $s_{20,w}$.

Equilibrium Sedimentation. The equilibrium measurements were conducted in a double sector cell with a column height of 0.3 cm at 15 198 rpm (3948 ±1 μs) during 38 h. The data were processed following Roark & Yphantis (1969).

Since the molecular weight of the monomer was known, a program of least squares fit was used to achieve the resolution of components in the concentration distribution at equilibrium.

The value of partial specific volume was taken to be 0.740 as determined from the amino acid sequence of the protein.

Results

Purification of LexA. Polymin P Precipitation. Earlier purification procedures used either coprecipitation of LexA with the DNA at elevated polymin P concentrations followed

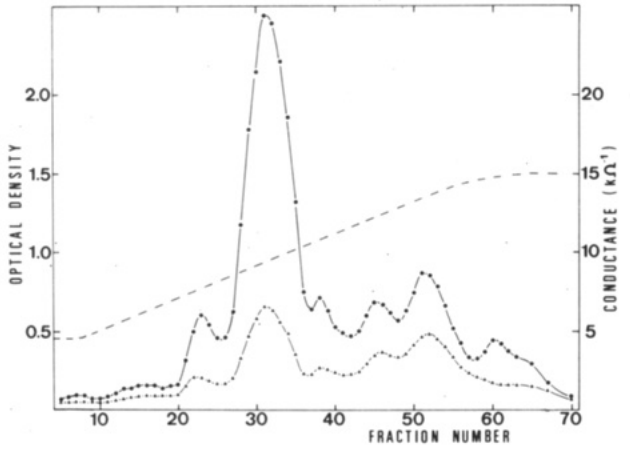

FIGURE 1: Elution profile of the phosphocellulose column as revealed by absorption at 280 (▲) and 235 nm (●). The linear NaCl gradient (---) varies between 80 and 500 mM NaCl. The volume per fraction was about 15 mL, and the conductance was measured at 4 °C.

by salt elution of the protein (Little, 1981; Brent & Ptashne, 1981) or the precipitation of the DNA alone (Horii et al., 1981a,b). We find that LexA is not easily precipitable by polymin P up to at least 0.7% (data not shown). The optical density at 260 nm of the supernatant after centrifugation has been measured for different polymin P concentrations. Up to 0.3% polymin P optical density decreases rapidly, but only slightly for more important concentrations, indicating that essentially all the DNA has been precipitated at about 0.3%. A coprecipitation of LexA seems thus not to be justified.

A 2% polymin P solution (pH 7.6) was added to the cleared lysate (see Materials and Methods) to give a final concentration of 0.35% and stirred for 15 min. Upon addition of polymin P a white flocky precipitate appears immediately. The mixture was centrifuged for 20 min at 20000*g*. The optical density at 260 nm was respectively 170 and 57 before and after polymin P precipitation. To the resulting supernatant was added solid ammonium sulfate to give 35% saturation at 0 °C, and the precipitate was removed by centrifugation. To this supernatant ammonium sulfate was added to 70% saturation. After centrifugation the resulting pellet was dissolved with 100 mL of buffer B (20 mM KH_2PO_4/K_2HPO_4, 2 mM EDTA, 80 mM NaCl, 5 mM ME, and 5% glycerol, adjusted exactly to pH 7.2) and dialyzed overnight against the same buffer.

Phosphocellulose Chromatography. Careful preparation of the phosphocellulose is essential to the success of this purification method. Dry Whatman P11 phosphocellulose was activated as described by Greene et al. (1978). Each column (100 mL bed volume) was used only once and washed with several volumes of buffer B prior to loading. After the application of the sample, the column was extensively washed with buffer B. The bound proteins were eluted with a linear NaCl gradient of 1000 mL from 80 to 500 mM NaCl. Figure 1 shows the resulting absorption profile determined at 235 and 280 nm. The main peak eluting at 280 mM NaCl corresponds to the LexA protein, as revealed by SDS–polyacrylamide gel electrophoresis and some other structural and functional criteria (see below). The large A_{235}/A_{280} ratio as compared to that of most of the proteins is due to the small number of aromatic residues in the protein (one tryptophan and one tyrosine residue) and constitutes a useful tool for the identification of the protein. Fractions with an optical density of at least 1.5 at 235 nm are pooled and either precipitated with ammonium sulfate or directly dialyzed against a storage buffer containing 10 mM NaH_2PO_4/Na_2HPO_4, pH 7.2, 150 mM NaCl, 0.1 mM EDTA, 0.1 mM DTT, and 50% glycerol.

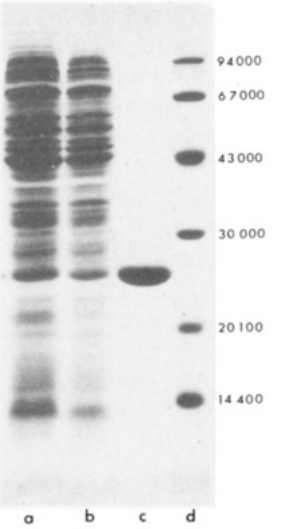

FIGURE 2: SDS–polyacrylamide gel electrophoresis (13%) of crude protein extracts before polymin P precipitation (lane a) and after polymin P precipitation (lane b), of the purified LexA protein (lane c), and of several marker proteins (lane d) with molecular weights as indicated.

Lane c in Figure 2 shows that the purity of LexA may be estimated from electrophoresis gels to be of at least 97%. Scanning of lane a in Figure 2 with a microdensitometer shows that LexA corresponds to about 4% of the total protein content in crude extracts after removal of the cell membranes. We have observed that the storage buffer used here gives less rise to aggregation of the protein at high concentrations than the Tris buffer used by Horii et al. (1981a). Dialysis against storage buffer results in an approximately threefold concentration of the sample. Final protein concentration is determined from an absorption spectrum, corrected if necessary for light scattering, and an extinction coefficient of $\epsilon^{280} = 7300$ M^{-1} cm^{-1} per LexA monomer. This value, which is in good agreement with that expected for a protein containing only one tryptophan and one tyrosine residue, has been determined by using the spectroscopic method described by Scopes (1974) which makes use of the absorption ratio A_{280}/A_{205}. As expected we find a very low value of $A_{280}/A_{205} = 0.0115$ for the LexA protein (Schnarr & Daune, 1984). The final concentration of the protein is 4–5 mg/mL. The total yield is about 120 mg in the central fraction and another 40 mg in the less pure fraction from the edges of the peak. This yield is fairly superior to those obtained by the more complicated purification procedures cited above, and a similar degree of purity is reached.

Absorption and Fluorescence Spectroscopy. Figure 3 shows the absorption spectrum of the LexA protein with a maximum at 279 nm. An interesting feature of this spectrum is the relatively pronounced ripples between 250 and 270 nm. They originate from the phenylalanine side chains of the protein, which are masked for most of the proteins by a stronger absorption of the tryptophan and tyrosine side chains. None of the other proteins eluted from the phosphocellulose column shows such a pronounced fine structure. This property can be easily used to identify the LexA-containing peak. We wondered if this fine structure might be used further to quantify the number of phenylalanine residues within the protein. To test this assumption, we measured the height of the most pronounced ripple centered at about 258 nm, after a large-scale expansion of both absorbance and wavelengths. This value was compared with a calibration curve obtained from absorption spectra of mixtures of tryptophan, tyrosine,

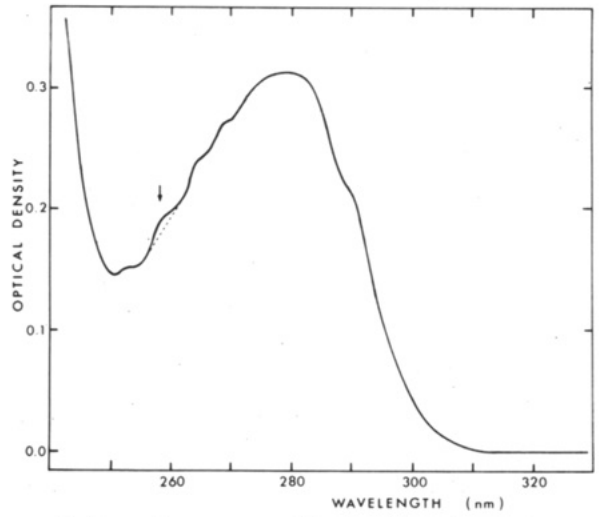

FIGURE 3: Absorption spectrum of LexA at 4.8 mg/mL in the storage buffer in a 2-mm cell at 20 °C. No correction for light scattering has been necessary. The shoulder indicated by an arrow has been used for the estimation of the phenylalanine content.

and a variable amount of phenylalanine (1:1:X). In this way we find 5.5 phenylalanine residues per LexA monomer which is in good agreement with the value of 6 phenylalanine residues given by the sequence of the LexA gene (Markham et al., 1981; Horii et al., 1981b).

Fluorescence spectroscopy is a potentially useful method in the study of LexA properties and interactions, since LexA contains a single tryptophan residue in position 201 that may serve as an intrinsic fluorescent probe. As expected the fluorescence spectrum is dominated by the tryptophan emission at all excitation wavelengths. No clear-cut evidence for a tyrosine emission around 305 nm could be found. The maximum of the spectrum is at 350 ± 2 nm and thus very close to the value observed for tryptophan in water, which is generally taken as an argument for a good accessibility of the chromophore within a protein.

An acrylamide-quenching study of the LexA protein does not fully support this view. The Stern–Volmer plots are linear within experimental error, fitting the simple relation $F_0/F = 1 + K[Q]$, with F_0 and F being respectively the fluorescence intensity in the absence and presence of the quenching agent, [Q] the acrylamide concentration, and K the Stern–Volmer constant. A fully exposed tryptophan should exhibit a nonlinear, upward curved Stern–Volmer plot due to static quenching at elevated acrylamide concentrations (Eftink & Ghiron, 1976, 1977). Under the solvent conditions used for the ultracentrifugation experiments at a protein concentration of 0.2 mg/mL, we find a Stern–Volmer constant of 4.5 M^{-1} which is related to the rate constant for quenching, k_q, by the relationship $K = k_q\tau$ where τ is the fluorescence lifetime. Our preliminary results indicate that under these conditions the fluorescence lifetime is 3.75 ± 0.2 ns (G. Duportail and M. Schnarr, unpublished results), giving a k_q value of 1.2×10^9 M^{-1} s^{-1}. This value is at least 3 times smaller than that expected for a fully exposed tryptophan side chain (Eftink & Ghiron, 1976). We observe moreover a blue shift of about 7 nm of the fluorescence spectrum upon addition of acrylamide. Such a behavior is quite common for proteins containing several tryptophan residues but rather unexpected for a protein with a unique tryptophan (Eftink & Ghiron, 1976). An attractive explanation for this finding is that the single tryptophan may exist in two different conformations with different degrees of accessibility and different maxima of their emission spectra. The more exposed conformation would be quenched for lower acrylamide concentrations resulting in a blue shift of the overall spectrum. At the same time this hypothesis may explain why the overall accessibility is lower than that of a fully exposed tryptophan side chain.

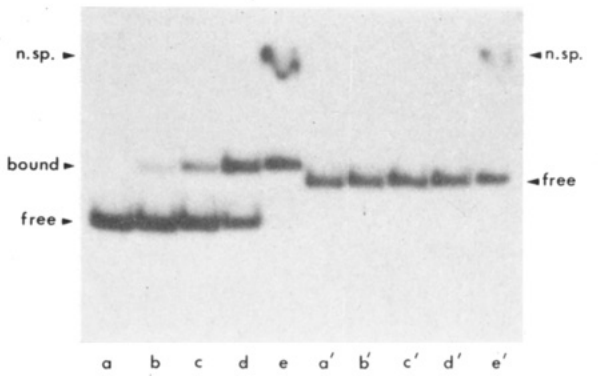

FIGURE 4: Titration of the 175 bp recA operator fragment (lanes a–e) and the 275 bp nonspecific fragment (lanes a′–e′) with LexA. LexA monomer concentrations are 0 (a, a′), 10^{-9} (b, b′), 10^{-8} (c, c′), 10^{-7} (d, d′) and 10^{-6} M (e, e′). The denotation n.sp. corresponds to the nonspecific complex. The concentration of the DNA fragments is $(4 \pm 2) \times 10^{-9}$ M.

Specific Interaction of LexA with Operator DNA. To monitor the specific interaction of LexA with an operator containing DNA fragment, we have used polyacrylamide gel electrophoresis as initially developed by Garner & Revzin (1981) and Fried & Crothers (1981). Figure 4 shows that LexA forms a well-defined complex with a 175 base pair DNA fragment harboring the recA promotor/operator region at fairly small protein concentrations. Under identical conditions a 275 bp fragment without operator sequence shows no complex formation up to a LexA concentration of at least 10^{-7} M. At 10^{-6} M LexA concentration, the recA fragment shows an additional band, probably due to a cooperative nonspecific binding, since an isolated nonspecific binding should lead to a ladder of bands as in the case of *lac* repressor (Fried & Crothers, 1981). We have shown earlier by circular dichroism that LexA binds cooperatively to poly[(A-T)] (Schnarr & Daune, 1984). A similar nonspecific complex of weaker intensity is observed in the case of the nonoperator fragment.

The specific complex between LexA and the 175 base pair operator fragment migrates in the gel like a fragment of 340 base pairs as determined from a calibration curve with DNA fragments of known length. This corresponds to a N'/N value of 1.94, with N and N' being respectively the initial and the apparent DNA length of the complex.

We have undertaken similar experiments with a 148 base pair fragment harboring the promoter/operator regulatory region of the LexA gene that contains two SOS boxes (Little et al., 1981; Brent & Ptashne, 1981). The major band for the specific complex migrates in the gel like a 450 base pair fragment, corresponding to a N'/N value of 3.04, thus definitely slower than the specific complex with the recA operator fragment. A second less intense band is observed with $N'/N = 1.86$, a value comparable to that found for the recA operator fragment.

Sedimentation Velocity. Figure 5 shows a plot of the $s_{20,w}$ values vs. the intitial concentration of the solution. The increase of $s_{20,w}$ with the concentration is indicative of a self-associating system. The derivative curves of the boundary for the individual scans (data not shown) present a slight dissymmetry of the peak which suggests the presence in the solution of heavier components than the monomer.

The extrapolation of the data to zero concentration gives $s_{20,wc=0} = 2.5$ S, a value slightly lower than that calculated for

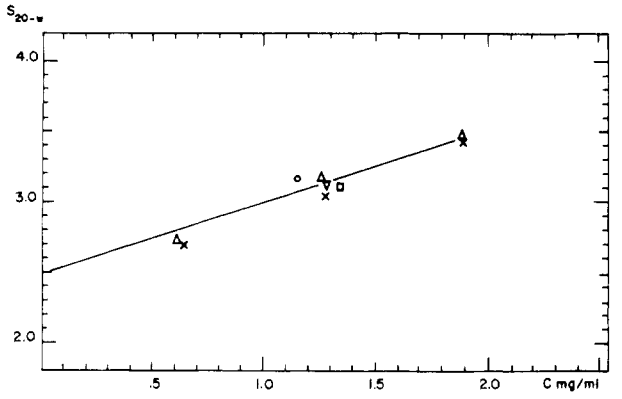

FIGURE 5: Variation of sedimentation coefficients $s_{20,w}$ with concentration for LexA, 45 592 rpm, 10 mM sodium phosphate, 0.15 mM NaCl, and 0.1 mM EDTA, pH 7.2, λ = 280 nm. (△) T = 7.7 °C; (×) T = 6.5 °C; (□) T = 20.5 °C; (▽) T = 4.1 °C; (○) T = 8.7 °C, with the ionic strength increased to 1 M NaCl.

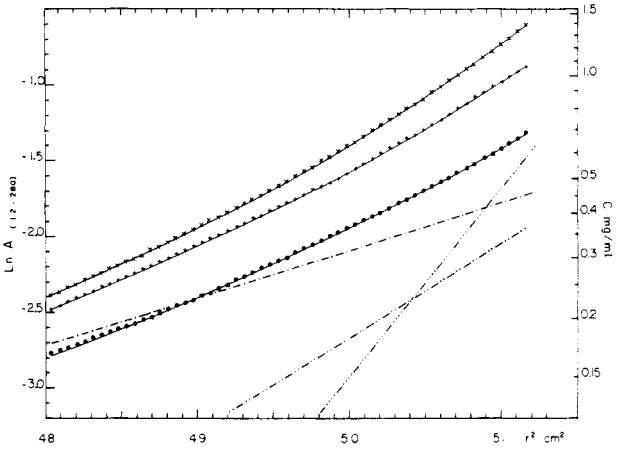

FIGURE 6: Self-association of LexA plotted as ln $A_{(1.2-280)}$ vs. r^2, 15 198 rpm, T = 280.8 K. Initial loading concentrations: (●) 0.300 mg/mL (cell 1); (+) 0.555 mg/mL (cell 2); (×) 0.815 mg/mL (cell 3). The solid lines through the experimental points are the total concentration distributions calculated from the results of the least-squares fit. The straight lines are the concentration distributions of the individual components in cell 3: monomer (- - -); dimer (- · - · -); tetramer (- · · · -).

a molecule of M_r 22 300 and a partial specific volume of 0.740 mL/g. If a spherical shape is assumed, the increase in frictional coefficient may be attributed to hydration which can be evaluated to about 0.13 g of H_2O/g of protein. Within experimental error, the sedimentation coefficient seems to be independent of the temperature between 4 and 20 °C and of the ionic strength between 0.15 and 1.0 M (see individual points on Figure 5).

Equilibrium Sedimentation. The classical plot of ln c vs. r^2 shows an upward curvature reflecting also the self-interaction of the solute. Figure 6 shows such a plot for three different initial concentrations of LexA.

The data are further analyzed with the Roark & Yphantis method (1969) to get an initial indication of the limiting species, the smallest and the biggest one, involved in the different equilibria. Figure 7 shows the representation of the data according to the so-called "two-species plot", which consists of a plot of M_w vs. $1/M_n$ or M_z vs. $1/M_w$, respectively (for a definition of the different average molecular weights see the legend of Figure 7). The experimental points for the two types of representations show a very satisfying overlap, and all experimental values indicate the monomeric species to be the smallest one and the tetrameric species to be the biggest one. This is important, initial information for fitting

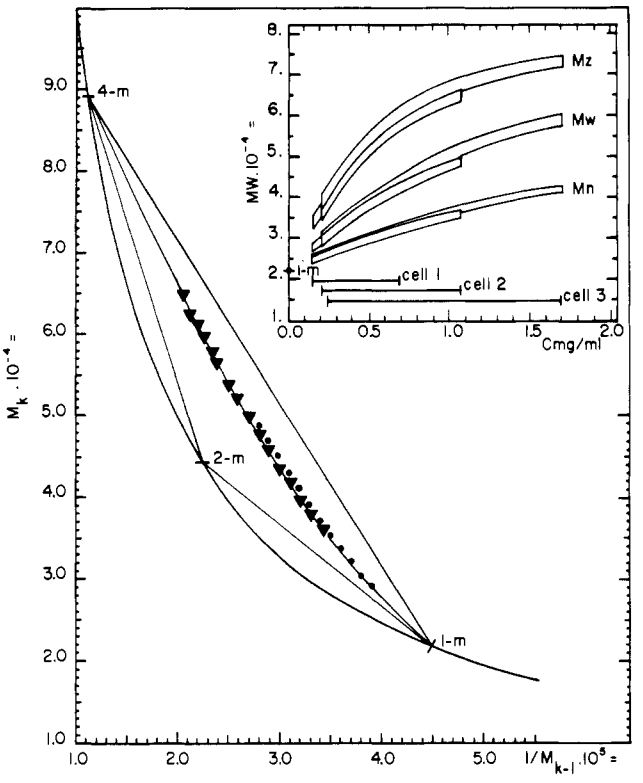

FIGURE 7: Two-species plot for LexA. Data presented are for the equilibrium experiment of Figure 6. (●) M_w vs. $1/M_n$; (▼) M_z vs. $1/M_w$. The solid curve is drawn with the values of average molecular weight calculated from the least-squares fit [$M_n = \sum c_j/(\sum c_j/M_j)$, $M_w = \sum c_j M_j/\sum c_j$, $M_z = \sum c_j M_j^2/(\sum c_j M_j)$]. The hyperbola is $M_k(1/M_{k-1}) = 1$. The insert shows the z, w, and n average molecular weight vs. concentration determined from the Roark and Yphantis program for three different loading concentrations. The width of the boxes indicates the range of accuracy given by this point-weight average molecular weight method. Boxes are almost superimposed giving evidence of the unique fitting of the system in equilibrium. The three horizontal lines, at the bottom of the insert, give the span of concentrations for each cell.

the concentration distributions shown in Figure 6.

Since the molecular weight of the monomeric unit is known, the concentration distributions of components in the solution are resolved by a least-squares fit program according to

$$c_r = \sum_j (c_m)_j \exp[\sigma_j (r^2 - r_m^2)/2]$$

where $\sigma_j = M_j(1 - \bar{v}_j\rho)\omega^2/(RT)$ and M_j = molecular weight of the j species, $\bar{v}$ = partial specific volume, ρ = solvent density, ω = angular velocity, R = gas constant, T = absolute temperature, and $(c_m)_j$ = concentration of the j species at the meniscus. In this particular case

$$M_j = M_1 j \qquad 1 \leq j \leq 4$$

In this analysis, we assume that all $\bar{v}_j$ values are the same and are taken as 0.740 mL/g and that all the n-mers have the same extinction coefficient.

The data analysis shows that (a) the presence of a trimer is incompatible with the experimental data and the system may be reduced to 1–2–4, and (b) the concentration of each component (monomer, dimer, and tetramer) is estimated for each point in the cell, and it is possible to recalculate the different molecular weight averages in these points. In Figure 7 are reported, in comparison with the two species plot calculated by the Roark and Yphantis method from the crude experimental data, the results of the fit. The agreement is really

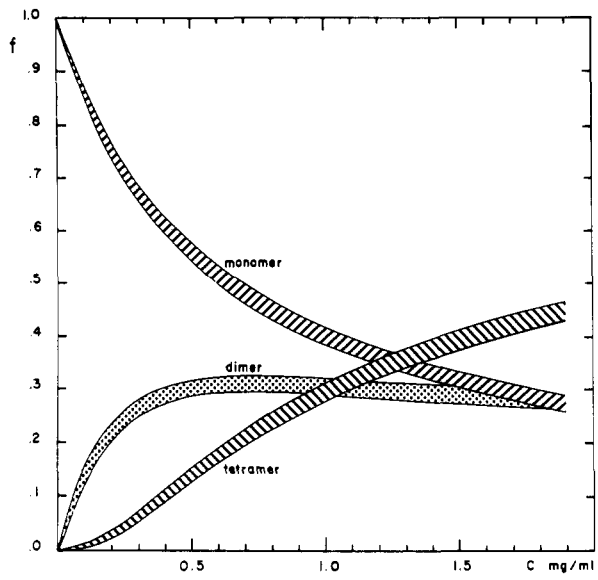

FIGURE 8: Plot of the fractions of monomer, dimer, and tetramer of LexA vs. total concentration. The values of equilibrium constants are $2.0 \times 10^4 \leq K_2 \leq 2.4 \times 10^4$ and $6.5 \times 10^4 \leq K_4 \leq 7.7 \times 10^4$.

good and allows us to describe the behavior of LexA in solution in terms of two successive equilibria:

$$M \overset{K_2}{\rightleftharpoons} D \overset{K_4}{\rightleftharpoons} T$$

Equilibrium Constants. Since the concentration of each component and the total concentration is known for each radial position in the cell, the equilibrium constants of the system are evaluated following the scheme in which all molar concentrations are expressed in molar concentration of the monomeric unit:

$$c_T = c_1 + 2c_2 + 4c_4$$

Introducing the equilibrium constants

$$c_T = c_1 + 2K_2c_1^2 + 4K_2^2K_4c_1^4$$

and rearranging c_1 give

$$(c_T - c_1)/c_1^2 = 2K_2 + 4K_2^2K_4c_1^2$$

From a plot of the left side of the equation vs. c_1^2, K_2 and K_4 values are readily obtained.

The mean values with different loading concentrations are $K_2 = 2.1 \times 10^4$ M^{-1} (±8%) and $K_4 = 7.7 \times 10^4$ M^{-1} (±20%). Figure 8 shows the distribution of each component calculated with the aid of the equilibrium constants given above and expressed as the fraction of the total concentration.

The curves are drawn as domains reflecting the accuracy with which the equilibrium constants are evaluated. Below a total concentration of 0.5 mg/mL, the monomer is the predominant species and the proportion of the dimer increases sharply. Above this concentration, the tetramer is formed via the dimer, the proportion of which decreases very slowly after the maximum is reached at about 0.6 mg/mL.

Discussion

The essential finding from the equilibrium ultracentrifugation studies is the rather moderate capacity of LexA to form dimers or higher aggregates at least under the experimental conditions used in these experiments. We recall that most of the well-known DNA binding proteins either are stable tetramers like *lac* repressor, are stable dimers like the CAP protein, or are at least involved in a monomer–dimer equilibrium with a rather large association constant as in the case of the λ repressor where a K_A of 5.9×10^7 M^{-1} has been determined by partition chromatography methods (Johnson et al., 1980). The association constant for the monomer–dimer equilibrium of LexA is only 2.1×10^4 M^{-1}. We are not aware of a determination of the number of LexA molecules in a bacterial cell, but nevertheless, we may predict that in vivo a significant fraction of the LexA molecules should be in a monomeric state.

On the other hand, the operator sequences determined so far (Walker, 1984) all show a more or less pronounced twofold palindromic symmetry. This leads to the rather reasonable assumption that the *interacting* species should be a LexA dimer. Our results tend to give additional evidence to this assumption. The N'/N value determined for the interaction of LexA with a recA operator containing DNA fragment is nearly identical with that found for the interaction of the CAP protein with a 200 bp fragment harboring the galactose operon regulatory region ($N'/N = 2.085$) (Kolb et al., 1983). In this fragment the CAP binding site is situated at one of the ends of the fragment. This minimizes the influence of DNA bending on the electrophoretic mobility. Under these conditions the molecular weight of the fixed protein should be the essential factor for the decelerated migration as in the case of *lac* repressor (Fried & Crothers, 1981). When it is taken into account that a CAP monomer has about the same molecular weight as LexA, the very similar N'/N values of the two proteins suggest that LexA *interacts* as a dimer, too. Nevertheless, we cannot completely exclude that the determined N'/N value might arise from the binding of a LexA monomer *plus* a bending of the DNA, slowing down the migration further. In view of the fact that another repressor, the *lac* repressor, introduces no measurable bending of the DNA (Wu & Crothers, 1984), we favor the interpretation of LexA dimer binding. Obviously this interpretation leads to the question whether in vivo the dimer is formed on the DNA only, or if the probably very small amount of free dimers is the active species.

Our findings on the interaction of LexA with its own regulatory region, containing two SOS boxes, confirm essentially the results of Brent & Ptashne (1981) and Little et al. (1981) that both SOS boxes are occupied by LexA. The major band corresponds to a N'/N value of 3.04, showing that the mobility of the lexA operator fragment is more strongly affected than that of the recA operator fragment. Nevertheless, we find a minor band with $N'/N = 1.86$ corresponding probably to the occupation of a single SOS box only.

It has been shown that both the base-catalyzed and the RecA-catalyzed cleavages of LexA are independent of LexA concentration (Little, 1984, and Burbee and Roberts cited therein). It has been concluded that LexA can be cleaved about equally well whether it is a monomer or part of a dimer. Our results show that at the maximum concentration of LexA studied by Little (1984) about 40% of the LexA molecules are always in the monomeric state although under rather different solvent conditions.

We will undertake additional sedimentation velocity measurements to see if pH changes, low ionic strength, and divalent cations will influence the oligomerization equilibria of the protein. If the tendency of LexA to remain in a monomeric state would be even reinforced by these factors, the conclusion that LexA dimers are cleavable would have to be revised.

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