The Interaction of L-Arabinose and D-Fucose with AraC Protein*

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Summary

The interaction of the inducer L-arabinose and an anti-inducer D-fucose with araC protein is studied using spectrofluorimetric methods. AraC protein has a fluorescence spectrum typical of tryptophan-containing proteins. The fluorescence is quenched by L-arabinose suggesting that L-arabinose causes at least one conformational change in araC protein. The quenching of fluorescence does not occur in the presence of D-fucose. These observations are consistent with the model for positive control of the L-arabinose operon BAD by araC protein and suggest that the conformational change induced by L-arabinose reflects the transition to araC activator. The interaction of D-fucose with araC protein prevents the conformational change which yields activator, thus, accounting for the anti-inducer properties of this analogue of L-arabinose.

Materials and Methods

Bacterial and Phage Strains—Bacterial strains used in this study containing lesions in the ara region of the chromosome were originally isolated in Escherichia coli B/r in the laboratory of Ellis Englesberg. SB7515, the source of araC protein for these experiments, was constructed by P. Cleary and is an E. coli B/r strain which is singly lysogenic for a heat-inducible, lysis-inhibited defective ara transducing phage, Xh8Odara+ cI857 (7). SB7500, a strain unable to actively transport L-arabinose, was isolated by the method of Issacson and Englesberg on inhibitory media (mineral L-arabinose glycerol media containing reduced amounts of K+ ions) (10). A series of L-broth cultures is inoculated with approximately 200 cells from an overnight L-broth culture of an F-araAthr+leu- strain and incubated for 24 hours at 37°C. A loopful from each culture in streaked once across a plate of inhibitory media and the plates are incubated for 48 hours at 37°C. Five to ten resistant mutants are picked from each streak and purified by restreaking on homologous media. A mutant (SB5500) was found which on the basis of complementation tests was araD'A'B'C-. L-arabinose permease activity as measured by the procedure of Novotny and Englesberg (11) is completely absent in SB5500.

Proteins—The purification of electrophoretically pure araC protein from SB7515 has been described (7). The procedure involves polyethylene glycol-dextran phase partition, followed by chromatography on 4-(4-aminophenyl)butanamide-phenyl-β-D-6-deoxygalactopyranoside Sepharose and salmon sperm DNA cellulose. Purified lac repressor was generously provided by Arthur Riggs, City of Hope National Medical Center, Duarte, California.

Preparation of Cell-free Extracts for Assay of L-Ribulokinase—A 5-m1 culture of SB5500 is inoculated into 100 ml of CM (1% casein hydrolysate (Difco), 1% KH2PO4-K2HPO4 (pH 7.0), 0.01% MgSO47H2O, and 0.1% (NH4)2SO4 with different L-arabinose concentrations. The cultures are incubated at 37°C with shaking for 15 hours and then chilled in an ice bath. The bacteria are harvested by centrifugation, resuspended in 1.5 ml of a solution containing 1 mmole of EDTA and 1 mmole of glutathione, pH 7.6, and disrupted with the microtip of a Branson Sonifier. Cell debris is removed by one 15-min centrifugation at 27,000 × g. L-Ribulokinase is assayed as described by Englesberg et al. (2).

Fluorescence Methodology—The spectrofluorimeter used is an uncorrected Hitachi MPF-2A equipped with a cell holder thermostatically controlled at 25°C. Spectra are measured at an emission wavelength of 350 nm and an excitation wavelength of 280 nm. The emission spectrum typical of tryptophan-containing proteins. The fluorescence is partially quenched by L-arabinose suggesting that L-arabinose causes at least one conformational change in araC protein. This conformational change is prevented by D-fucose, an analogue of L-arabinose and an anti-inducer of the L-arabinose operon. Binding constants obtained from the spectrofluorometric studies are in good agreement with those obtained by other methods.

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allowing a calculation of the apparent $K_m$ of the interaction. The data shown in Fig. 2 are plotted as suggested by Scatchard (14) where $\bar{v}$ is the fractional quenching of fluorescence and $L$ is the free L-arabinose concentration. The value for the maximum quenching is obtained from a plot of $1/L$ versus $1/quenching$. The concentration of free L-arabinose can be assumed to be equal to the total concentration of L-arabinose since the concentration of araC protein monomers in the cuvette is approximately $4 \times 10^{-7}$ M and that of L-arabinose at least $10^{-4}$ M where binding begins to take place. If one assumes that the amount of binding of L-arabinose to araC protein is directly proportional to the quenching observed, then the data shown in Fig. 3 suggest that L-arabinose binds to independent sites with an apparent $K_m$ of $3 \times 10^{-4}$ M.

**Fucose Inhibits Interaction with L-Arabinose**—As mentioned above, n-fucose has only a slight effect on the fluorescence emission spectrum of araC protein. The greatest decrease in fluorescence observed upon addition of n-fucose with three different preparations of araC protein was 6%. This is in great contrast to the 45% quenching observed in the presence of saturating amounts of L-arabinose. If one adds n-fucose before the addition of L-arabinose, the quenching of fluorescence normally seen upon the addition of L-arabinose is not observed. In addition, if araC protein fluorescence has been quenched by the addition of L-arabinose, the quenching can be reversed by the addition of n-fucose. The data presented in Fig. 3 are obtained in the following way: varying amounts of n-fucose are added to araC protein in Buffer A containing 0.1 M L-arabinose causing an increase in fluorescence intensity proportional to the amount of n-fucose added. The data are plotted by the same method used in the experiment shown in Fig. 2. $\bar{v}$ is the fractional increase in fluorescence and $L$ is the free n-fucose concentration which may be equated to the total concentration because again $1/K$ is much greater than the ligand concentration. If we assume that the quenching observed reflects the binding of L-arabinose, then the n-fucose must bind to independent sites with an apparent $K_r$ of $6 \times 10^{-4}$ M.

**Other Estimates of Binding Constants**—The strength of binding of L-arabinose and n-fucose to araC protein has been estimated by indirect methods both in vivo (15, 16) and in vitro (17, 18) and the values obtained are on the order of $10^{-4}$ to $10^{-6}$ M. How-
for Fig. 6894 was measured as a function of the D-fucose concentration. Conditions of the experiment and treatment of the data are the same as for Fig. 2.

The fluorescence of a solution of araC protein and 0.1 M L-arabinose was measured as a function of the D-fucose concentration. Conditions of the experiment and treatment of the data are the same as for Fig. 2.

However, in vivo measurements have revealed possible strain differences (16) so we measured the induction of the L-arabinose operon with known internal concentrations of L-arabinose in a strain with an araC region isogenic with the strain used as the source of araC protein for the fluorescence experiments. Assuming that induction is directly proportional to the binding of L-arabinose to araC protein, an apparent Kₐ of 5 × 10⁻³ M is calculated (Fig. 4). This is in excellent agreement with the value of 3 × 10⁻³ M obtained above by the spectrofluorimetric method.

**DISCUSSION**

The interconversion of activator and repressor mediated by L-arabinose is the key feature of the model for regulation of the L-arabinose operon proposed by Englesberg et al. (2, 3). L-Arabinose is considered to be both the inducer and effector because L-ribulokinase is inducible in araD⁻ mutants and no evidence has been found for any alteration in the accumulated L-arabinose in these strains (1). Further support for a direct interaction between inducer and araC protein has been obtained from studies using D-fucose, a non-metabolizable analogue of L-arabinose which prevents growth of *Escherichia coli* B/r on a mineral salts L-arabinose medium by inhibiting induction of the operon (2). Mutations conferring D-fucose resistance map in araC (the araC³ allele) and result in constitutive expression of the L-arabinose operon (2). Many araC³ alleles also permit D-fucose to serve as a gratuitous inducer of the araBAD operon and suggest that the fucose-resistant araC³ allele produces a product with modified inducer specificity (19). Similar araC³ alleles have also been found in *Escherichia coli* K12 (18). These mutants provide strong evidence for a direct interaction between the inducer and araC protein in wild-type cells. *In vitro* studies with permidopil suggest that while D-fucose does not significantly alter repressor function, it may block the L-arabinose-mediated conversion of repressor to activator (19). *In vitro* studies with cell-free systems (17, 18) further support this proposed interaction of D-fucose with araC protein and rule out the possibility that the only effect is at the level of transport.

The results presented above demonstrate that L-arabinose interacts directly with araC protein and may induce a conformational change. Furthermore, the anti-inducer D-fucose can prevent the conformational change induced by L-arabinose. These observations are consistent with the model for control of the L-arabinose operon by araC protein and suggest that the observed conformational change reflects the transition from araC repressor (P₁) to araC activator (P₂). The interaction of D-fucose with araC protein does not result in the conformational change which yields araC activator, thus accounting for the anti-inducer properties of this analogue of L-arabinose. Finally, the interaction of L-arabinose with araC protein occurs only at high L-arabinose concentrations (> 10⁻³ M).

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