Energy Transfer in Lactose Repressor Protein Modified with *N*-[[(Iodoacetyl)amino]ethyl]-5-naphthylamine-1-sulfonate†

Joseph A. Gardner and Kathleen S. Matthews*
Department of Biochemistry and Cell Biology, Rice University, Houston, Texas 77251
Received September 6, 1990; Revised Manuscript Received December 6, 1990

ABSTRACT: Energy transfer between the two tryptophan residues in the lactose repressor protein and the fluorescent moiety of the cysteine-specific reagent *N*-[[(iodoacetyl)amino]ethyl]-5-naphthylamine-1-sulfonate (1,5-IAEDANS) has been examined. Modification of repressor with this compound did not affect operator or inducer binding. 1,5-IAEDANS reacted primarily with Cys140 in wild-type repressor [Schneider et al. (1984) *Biochemistry 23*, 2221]; in the presence of inducer, modification at Cys107 increased, while reaction at Cys140 remained unchanged. Energy transfer between tryptophans and the AEDANS moiety(ies) in wild-type *lac* repressor occurred with an efficiency of 6.7 ± 1.9% in the absence and 7.8 ± 1.6% in the presence of inducer. The distance between the Trp donor(s) and the acceptor in wild-type repressor was calculated to be in the range ~35 Å under both conditions. The similarity in efficiency despite large differences in the amount of acceptor attached to Cys107 when inducer is bound indicates that the AEDANS group at position 107 does not participate significantly in energy transfer and that the label at position 140 acts as the primary acceptor group. The similarity of energy-transfer efficiency (7.1 ± 3.8%) observed for 1,5-IAEDANS-modified monomeric mutant repressor (Y282D) indicates that the transfer is primarily *intra*-subunit in the native tetramer. Measurements using two mutant repressors (each with a single tryptophan and modified with 1,5-IAEDANS) demonstrated that both tryptophans can serve as donor in the energy-transfer process. The W201Y repressor (containing Trp220) exhibited a transfer efficiency lower than wild type (5.6 ± 2.4%), corresponding to a slightly larger distance between the donor–acceptor pair in this mutant. Energy-transfer efficiency in the W220Y protein (containing Trp201) was closer to that measured for the wild-type repressor (7.5 ± 1.4%). Inducer did not alter the efficiency of transfer observed for these single-tryptophan-containing mutants. We conclude from these results that Trp201 and Trp220 are both involved in *intra*subunit transfer and that Trp201 is slightly closer to Cys140, which has been localized to the NH_2-terminal headpiece/core interface.

The lactose repressor protein from *Escherichia coli* is a prototypic genetic control protein (Jacob & Monod, 1961). Each tetramer (M_r ~150 000; Riggs et al., 1968; Gilbert & Müller-Hill, 1966) binds four inducer molecules and two DNA molecules (Adler et al., 1972; O'Gorman et al., 1980; Culard & Maurizot, 1981; Whitson & Matthews, 1986). Since its isolation (Gilbert & Müller-Hill, 1966), extensive examination of this protein has been performed. Several investigators have attempted to define the physical size of the tetramer using a variety of techniques. Steitz and co-workers combined electron microscopy and powder X-ray diffraction studies of microcrystals to propose tetrameric dimensions of 140 Å × 60 Å × 45 Å (Steitz et al., 1974). Small-angle X-ray studies of the radius of gyration of *lac* repressor tetramer (Pilz et al., 1980; McKay et al., 1982) generated a range of measurements from 125 to 180 Å for the long axis and 60–90 Å for the shorter axis of an elongated molecule with the NH_2-terminal headpieces located at opposite ends. Charlier et al. (1980, 1981) used neutron scattering to estimate long and short axes of 140–160 and 45–50 Å for a tetrameric shape based on a prolate ellipsoid or elongated cylinder. Additionally, some predictions concerning the protein's secondary structure have been published (Chou et al., 1975; Bourgeois et al., 1979). However, despite extensive data on the properties and activities of this protein, minimal information is available regarding the three-dimensional structure of this regulatory element. Recent attempts at crystallization have proved successful (Pace et al., 1990), but until diffraction-grade crystals can be produced and analyzed, alternative methods must be utilized to probe the structure of the repressor.

Energy-transfer measurements using intrinsic tryptophan fluorescence can provide information regarding distance relationships within a molecule (Cantor & Schimmel, 1980; Lakowicz, 1983). Several specific conditions are required for this method to be useful, including introduction of a fluorophore with an absorbance spectrum suitable for energy transfer from intrinsic tryptophan(s) at a single, defined location within the protein and in an orientation to allow energy transfer. Our laboratory has attempted to measure distances within the core region of the *lac* repressor by specifically modifying cysteine residues with the reagent *N*-[[(iodoacetyl)amino]ethyl]-5-naphthylamine-1-sulfonate (1,5-IAEDANS).[1] The AEDANS moiety exhibits an excitation spectrum that allows energy transfer from tryptophan residues given proper orientation and distance. This reagent reacts specifically with two of the three cysteines present in the *lac* repressor: Cys140 is readily labeled under native conditions with lower reactivity observed for Cys107 (Schneider et al., 1984). Reaction with iodoacetamide derivatives at Cys107 can be modulated by the inclusion of inducer in the reaction mixture (Gardner & Matthews, 1987a). Cys281 is not appreciably labeled in wild-type repressor,

† This work was supported by grants from the National Institutes of Health (GM22441) and the Robert A. Welch Foundation (C-576).
* To whom correspondence should be addressed.

[1] Abbreviations: 1,5-IAEDANS, *N*-[[(iodoacetyl)amino]ethyl]-5-naphthylamine-1-sulfonate; Tris, tris(hydroxymethyl)aminomethane; HPLC, high-performance liquid chromatography; IPTG, isopropyl β-D-thiogalactopyranoside.

0006-2960/91/0430-2707$02.50/0

presumably due to its location at a subunit interface (Matthews, 1987). In contrast, T-41 mutant monomer protein (Schmitz et al., 1976) exhibits extensive reaction at Cys281 as well as reaction at Cys107 and Cys140 with several cysteine-specific reagents (Daly et al., 1986).

lac repressor has two trytophans at positions 201 and 220 (Beyreuther et al., 1973; Farabaugh, 1978; Beyreuther, 1978). Mutant repressors with single tryptophans have been described previously (Sommer et al., 1976), but the suppressor strains carrying these mutant *lac* genes were unstable and produced the mutant proteins in very low yield. We have utilized site-specific mutagenesis techniques (Kunkel et al., 1987) to produce two missense mutant repressor molecules each containing a single tryptophan (Gardner & Matthews, 1990). Tyrosine was selected as the substitute residue for each of the tryptophans based on analogy to the amber suppression mutants (Sommer et al., 1976) and because the hydrophobic properties of Tyr may substitute at least in part for the apolar character of the Trp. These mutant repressors, along with wild-type repressor, were utilized in order to attempt distance measurements within the lactose repressor protein. The results of this study have been presented in preliminary form (Gardner & Matthews, 1987b).

Materials and Methods

Sources. Reagents and materials were obtained from the following sources: 1,5-IAEDANS, tris(hydroxymethyl)-aminomethane, trypsin, chymotrypsin, and NH_4HCO_3 from Sigma Chemical; ultrapure urea from International Biotechnologies Inc.; HPLC/spectra-grade acetonitrile from American Burdick & Jackson. Wild-type and single-Trp *Escherichia coli* lactose repressor proteins were isolated as described previously (O'Gorman et al., 1980; Gardner & Matthews, 1990). Mutant *Escherichia coli* Y282D lactose repressor protein was isolated as described by Daly and Matthews (1986).

Production of Mutant Repressors. Single-tryptophan mutant *lac* repressors were produced by site-specific mutagenesis and characterized as described (Gardner & Matthews, 1990). T-41 monomeric mutant has been characterized previously (Schmitz et al., 1976; Daly & Matthews, 1986); DNA sequencing of the coding region amplified by polymerase chain reaction has demonstrated that this mutant is Y282D (Chen and Matthews, unpublished results). Production of the Y282D mutant by site-specific methods provided material for the experiments described in this report; this protein exhibited properties similar to the T-41 monomeric mutant (Chen and Matthews, unpublished results).

Labeling of Protein. Wild-type, Y282D monomer, and W201Y and W220Y mutant repressors were each reacted with 1,5-IAEDANS following the same methodology. The labeling procedure followed that outlined by Schneider et al. (1984) with the following modifications. Repressors were dialyzed into labeling buffer (0.2 M Tris-HCl, pH 8.0, 0.5 M KCl, and 0.1 mM EDTA) purged with nitrogen to remove oxygen. Repressor controls (wild type and mutants) were taken through each step of the reaction except dimethylformamide (DMF) was added without 1,5-IAEDANS. Wild-type, W201Y, and W220Y mutant repressors were modified both in the presence and in the absence of 10^{-3} M isopropyl β-D-thiogalactopyranoside (IPTG); Y282D mutant repressor was modified in the absence of IPTG. A stock solution of 1,5-IAEDANS was prepared in DMF to a final concentration of 40–50 μg/mL. From this stock, a 50-fold molar excess of 1,5-IAEDANS over repressor monomer was added to the protein and reacted for 2 h at 4 °C in the dark. The reaction was stopped by addition of a 1000-fold molar excess of dithiothreitol (DTT). The excess reagent was separated from the protein by dialyzing against at least five changes of dialyzing buffer (0.2 M Tris-HCl, pH 7.5, and 0.1 mM DTT) at 4 °C in the dark. Modification was also carried out by denaturing the protein with 8 M urea before addition of the reagent in order to label all the cysteine residues. Unmodified and modified repressors were assayed for the ability to bind ^{32}P-labeled 40 base pair operator by the nitrocellulose filter binding method (Riggs et al., 1968) and ^{14}C-labeled IPTG by the $(NH_4)_2SO_4$ precipitation method (Bourgeois, 1971) and equilibrium dialysis (Friedman et al., 1977). Analysis of the K_d for operator DNA utilized least-squares fit of binding constants to experimentally determined bound counts after subtracting background counts (Ha et al., 1989).

Mapping of Modified Residues in lac Repressor. The modified proteins were heat-precipitated, and the protein was centrifuged at 7000 rpm for 10 min, followed by resuspension in 1 mL of 0.05 M NH_4HCO_3 (pH 7.0). Additions of trypsin and chymotrypsin [1% (w/w)] were made, and the solution was incubated with shaking at 37 °C. After 3 h, similar aliquots of the two proteases [1% (w/w)] were added, and digestion was continued at 37 °C for another 3 h. This digested protein was frozen and lyophilized to dryness in a Savant vacuum concentrator. Separation of the peptides was accomplished on a Gilson Model 302 HPLC system fitted with a 4.6 × 250 mm Brownlee RP-300 Aquapore Octyl reverse-phase column. The column was equilibrated with HPLC buffer (0.05 M NH_4HCO_3, pH 7.0) prior to injection of the sample. The lyophilized peptides (1–2 mg) were solubilized in 20 μL of HPLC buffer, and the insoluble material was removed by centrifugation in an Eppendorf centrifuge for 5 min. The soluble peptides were injected onto the HPLC column and eluted with a linear gradient of 0–30% acetonitrile in 30 min. The elution was monitored at 365 nm with an LKB 2238 Uvicord SII UV monitor and an LKB 2210 two-channel chart recorder. The column was then washed with 60% acetonitrile/40% HPLC buffer for 5 min and reequilibrated in HPLC buffer for the next run.

Energy Transfer in the Repressor. To assess the presence of energy transfer, samples of wild-type protein and modified protein were provided to Dr. Catherine Royer, University of Illinois, for lifetime measurements. The lifetime values obtained confirmed that energy transfer was occurring (tryptophan lifetime was decreased in the presence of 1,5-IAEDANS) with an estimated efficiency of ~7–8%. For routine determination of transfer efficiency, the excitation and emission spectra of the proteins were recorded on an SLM series 400 polarization fluorometer. The tryptophans were excited at 285 nm, and emission was scanned from 300 to 600 nm; the AEDANS groups were excited at 345 nm, and emission was scanned from 350 to 600 nm. The absorbance spectra of the proteins were recorded on a Cary 18 double-beam spectrophotometer. Absorbance scans covered the range 240–500 nm. The following equation (Tu et al., 1978) was used to determine the efficiency of energy transfer from the sensitized fluorescence:

$$E = \frac{F^{475}_{DA285} f_{A345} I_{345} A_{DA345}}{F^{475}_{A345} f_{D285} I_{285} A_{DA285}} \quad (1)$$

This value is the ratio of the actual fluorescence emission of the acceptor AEDANS in the presence of donor Trp at 475 nm when exciting at 285 nm to the fluoresence emission of AEDANS at 475 nm when exciting at 345 nm (measured under identical conditions and instrument settings). To nor-

malize these fluorescence values to quanta absorbed at the two wavelengths, the following values are utilized: the fractional absorbances of AEDANS at 345 nm and Trp at 285 nm (f_{A345} and f_{D285}, respectively), the intensities of exciting light at 285 (I_{285}) and 345 (I_{345}) nm (measured by using rhodamine as a quantum counter in a front-face fluorescence cuvette), and the total absorbances of the donor/acceptor pair at 285 (A_{DA285}) and 345 (A_{DA345}) nm. An alternate method of determining energy-transfer efficiency is to monitor the decrease in donor quantum yield in the presence of acceptor. These measurements yielded results qualitatively similar to the efficiencies determined by sensitized fluorescence, but the variability was significantly greater. Therefore, only the values derived from sensitized fluorescence measurements were used to calculate distance relationships.

To determine the distance between the donor/acceptor pair requires an evaluation of the overlap integral (J_v) of the donor emission spectrum and the acceptor excitation spectrum from the formula (Fairclough & Cantor, 1978):

$$J_v = \frac{\sum F_D(\lambda)\epsilon_A(\lambda)\lambda^4\Delta\lambda}{\sum F_D(\lambda)\Delta\lambda} \text{ cm}^3\text{ M}^{-1} \quad (2)$$

where $F_D(\lambda)$ represents the fluorescence emission of the Trp donor group at wavelength λ and $\epsilon_A(\lambda)$ is the value of the absorption coefficient of the acceptor AEDANS group at wavelength λ. By combining this value with the orientation factor ($\kappa^2 = {}^2/_3$), the quantum yield of the donor(s) (Q_D), and the refractive index of the buffer (n = 1.3352, measured on a Bausch & Lomb refractometer), one can calculate R_0, the distance at which 50% energy transfer occurs (Förster, 1965; Stryer & Haugland, 1967):

$$R_0 = (9.79 \times 10^3)(J_v\kappa^2 Q_D n^{-4})^{1/6} \text{ Å} \quad (3)$$

To calculate the actual distance between the pair of fluorophores under investigation, R_0 is combined with the value calculated previously for efficiency of energy transfer (E) to generate the apparent R:

$$R = R_0[(E^{-1} - 1)^{1/6}] \text{ Å} \quad (4)$$

Results

Labeling and Mapping of Proteins. All species of repressor (wild type and mutants) reacted readily with 1,5-IAEDANS. Labeled wild-type, Y282D monomer, and the single-Trp mutant repressors were denatured and completely digested with trypsin and chymotrypsin. Peptides liberated by this digestion were examined by HPLC. As shown by HPLC analysis, cysteine residues were the primary target of modification for this reagent (Figure 1). The order of elution of modified cysteine-containing peptides from *lac* repressor has been shown to be 107 > 281 > 140 (Gardner & Matthews, 1987a). This identification was confirmed by isolation and purification of AEDANS-labeled *lac* peptides absorbing at 365 nm. These peptides were sequenced on an Applied Biosystems peptide sequencer and shown to contain the expected residues in the correct sequence for peptides containing each of the cysteines. Cys140 was modified to ≥80% in wild-type repressor with much lower modification of Cys107 (Figure 1A). When IPTG was included in the reaction mixture, reaction at Cys107 was increased significantly while reaction at Cys140 was not changed (Figure 1B). Single-Trp mutant repressors reacted with 1,5-IAEDANS in a manner similar to wild-type protein; Cys140 was the primary labeled residue in the absence of IPTG (Figure 1C,E). In some reactions, label at Cys107 was minimal. Inclusion of IPTG in the reaction mixture increased modification of Cys107 (Figure 1D,F). Y282D repressor showed an opposite effect at Cys107: when IPTG was added to the reaction mixture, modification at Cys107 was decreased, while reaction at Cys140 remained about the same (data not shown). Modification of Cys281 was not observed in wild-type protein or the single-Trp mutants but was evidenced in the Y282D monomer as expected from previous studies (Daly & Matthews, 1986). Reacted proteins exhibited only small alterations in inducer and operator binding when compared with the equivalent unreacted species (Table I). The maintenance of functional activity suggests minimal alterations in structure as a consequence of this modification.

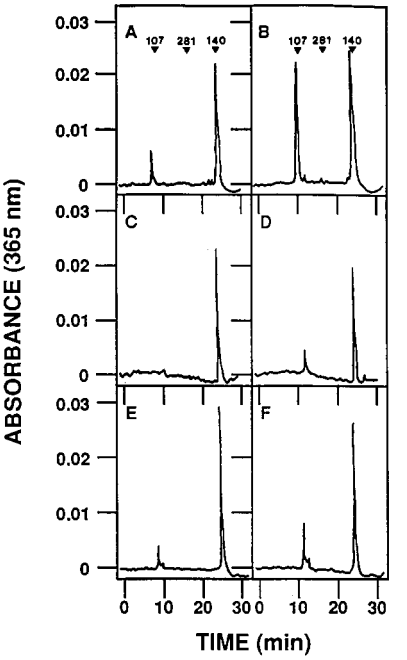

FIGURE 1: HPLC analysis of AEDANS-labeled repressor peptides. Wild-type, W201Y, and W220Y mutant repressors were modified, digested, and analyzed by HPLC as detailed under Materials and Methods. (A) Wild-type repressor modified without IPTG. (B) Wild-type repressor modified in the presence of 10^{-3} M IPTG. (C) W201Y mutant repressor modified without IPTG. (D) W201Y mutant repressor modified in the presence of 10^{-3} M IPTG. (E) W220Y mutant repressor modified without IPTG. (F) W220Y mutant repressor modified in the presence of 10^{-3} M IPTG.

Table I: Ligand Binding Constants (M) of Wild-Type and Mutant Repressors Modified with 1,5-IAEDANS[a]

	40 base pair operator	IPTG inducer
wild type		
unmodified	$(5.6 \pm 0.1) \times 10^{-11}$	$(1.4 \pm 0.6) \times 10^{-6}$
modified	$(1.1 \pm 0.2) \times 10^{-10}$	$(1.6 \pm 0.7) \times 10^{-6}$
Y282D		
unmodified	NA[b]	$(4.1 \pm 0.4) \times 10^{-6}$
modified	NA	$(5.1 \pm 1.1) \times 10^{-6}$
W201Y		
unmodified	$(2.8 \pm 0.4) \times 10^{-10}$	$(1.6 \pm 0.8) \times 10^{-6}$
modified	$(2.0 \pm 1.7) \times 10^{-10}$	$(1.3 \pm 0.5) \times 10^{-6}$
W220Y		
unmodified	$(2.5 \pm 0.9) \times 10^{-10}$	$(4.0 \pm 2.4) \times 10^{-5}$
modified	$(3.1 \pm 1.1) \times 10^{-10}$	$(3.2 \pm 1.1) \times 10^{-5}$

[a] Unmodified repressors and repressors modified with 1,5-IAEDANS were assayed for binding to synthetic 40 base pair *lac* operator and to the inducer molecule IPTG as described under Materials and Methods. [b] NA = not applicable (Y282D monomer does not bind operator).

Energy Transfer in the lac Repressor. When excited at 285 nm, tryptophan residues emit with an emission maximum near 345 nm. The repressor proteins examined in this study exhibited an emission maximum in this range, with the exception of the W220Y mutant, which had a maximum at 335 nm.

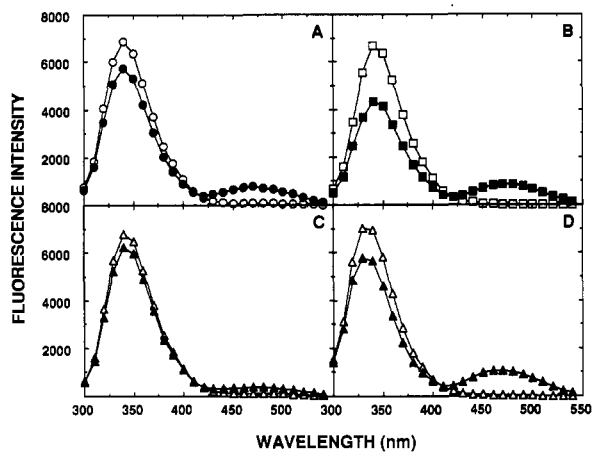

FIGURE 2: Fluorescence spectra of unmodified and modified repressors. Wild-type and mutant proteins were modified with 1,5-IAEDANS as described under Materials and Methods. Unmodified (open symbols) and modified (filled symbols) proteins [(~1–2) × 10^{-6} M] were excited at 285 nm, and emission was scanned from 300 to 550 nm. (A) Wild-type repressor. (B) Y282D monomer repressor. (C) W201Y mutant repressor. (D) W220Y mutant repressor. Reduced emission from tryptophan at 345 nm (335 nm for W220Y protein) was accompanied by increased emission from the AEDANS moiety at 470 nm in all cases.

Additionally, this mutant exhibited no fluorescence shift when bound to IPTG (Sommer et al., 1976; Gardner & Matthews, 1990). In repressors reacted with 1,5-IAEDANS, tryptophan emission at 345 was reduced compared with an equal concentration of unreacted repressor (Figure 2). Some of this energy was transferred to the AEDANS moiety which emitted at ~475 nm. Repressors not reacted with 1,5-IAEDANS exhibited only background emission in the range 400–600 nm and had a larger intensity at their emission maximum than did reacted repressors. Lifetime measurements on the wild-type repressor indicated a decreased lifetime of the tryptophan fluorescence, as expected when energy transfer occurs.

Each species of repressor examined exhibited energy transfer from the tryptophan moiety(ies) to the AEDANS group(s), although the transfer efficiency varied slightly between wild-type and mutant proteins (Y282D, W201Y, and W220Y) (Table II). Wild-type repressor demonstrated an efficiency of transfer of 6.7 ± 1.9%. The mutant repressors each containing single-Trp residues differed in their efficiencies, with W220Y displaying a value similar to wild type (7.5 ± 1.4%) and W201Y exhibiting a slightly lower efficiency (5.6 ± 2.4%). Y282D mutant repressor transferred energy with an efficiency range of 7.1 ± 3.8%, similar to the values for the W220Y mutant and wild-type repressor. This monomeric repressor exhibited an efficiency value similar to that determined for wild-type repressor, a result which suggests that energy transfer is occurring primarily within the subunits in the wild-type tetrameric protein, given that this is the only route of transfer available in the Y282D monomer mutant. It should be noted that the large degree of reaction at Cys281 observed in Y282D repressor did not result in a notable effect on the energy-transfer efficiency; from these data, it is apparent that energy transfer in the native protein does not involve label attached to this residue.

The two single-Trp mutants exhibited slightly different energy-transfer efficiencies. Trp220 (W201Y mutant) transferred with 5.6 ± 2.4% efficiency, and Trp201 (W220Y) transferred with 7.5 ± 1.4% efficiency. In both cases, the major amount of the AEDANS acceptor group was located at Cys140; reaction at Cys107 was very much less than reaction at Cys140 in the single-Trp mutant repressors. Reaction

Table II: Energy-Transfer Measurements for 1,5-IAEDANS-Modified Repressor Species

	J_v	% energy transfer	R_0 (Å)	R (Å)
WT	(4.3 ± 0.01) × 10^{-15}	6.7 ± 1.9 (n = 6)	22.8	~35 (range: 33.8–37.5)
+IPTG	(4.3 ± 0.01) × 10^{-15}	7.8 ± 1.6 (n = 6)	22.8	~35 (range: 33.2–35.8)
W201Y	(4.3 ± 0.01) × 10^{-15}	5.6 ± 2.4 (n = 6)	22.7	~37 (range: 34.1–40.1)
+IPTG	(4.3 ± 0.01) × 10^{-15}	4.7 ± 0.5 (n = 5)	22.7	~37 (range: 36.8–38.2)
W220Y	(4.3 ± 0.03) × 10^{-15}	7.5 ± 1.4 (n = 6)	23.1	~35 (range: 34.1–36.9)
+IPTG	(4.3 ± 0.03) × 10^{-15}	6.6 ± 0.8 (n = 3)	23.1	~35 (range: 35.2–36.5)
Y282D	(4.2 ± 0.01) × 10^{-15}	7.1 ± 3.8 (n = 6)	22.7	~35 (range: 32.3–39.7)

at Cys281 was not detected in either case, as expected based on the results with wild-type protein and evidence that this residue is involved in a subunit interface in the tetrameric protein (Sams et al., 1985; Daly & Matthews, 1986).

Inclusion of IPTG in the reaction had only a small effect on energy-transfer efficiency in wild-type (7.8 ± 1.6%), W201Y (4.7 ± 0.5%), and W220Y (6.6 ± 0.8%) repressors. The addition of IPTG increased modification of Cys107 in wild-type repressor and the single-tryptophan mutants (Figure 1). The slight increase in the range of energy-transfer efficiency when wild-type repressor was modified in the presence of IPTG and the corresponding small decreases for the mutants do not correlate with increased label at Cys107. While we cannot completely rule out a small amount of transfer between tryptophan(s) and the group at Cys107, the majority of transfer appears to occur between the tryptophans and the AEDANS moiety attached to Cys140.

The energy-transfer efficiency values for all the protein species examined were used to calculate distance ranges between the donor group (Trp) and the acceptor (AEDANS). The overlap integrals for wild-type, Y282D, and single-Trp mutants were calculated on the basis of the spectra for each individual protein species multiple times and were similar, all near 4.3 × 10^{-15} cm^3 M^{-1}, despite the differences in the fluorescence emission maximum for the inducer-bound repressor species and the W220Y mutant. An important consideration in calculating molecular distances from energy-transfer data is the treatment of the orientation factor κ^2. This factor describes the degree of freedom of rotation of the donor and acceptor groups. Dale and Eisinger (1974) have provided evidence that κ^2 cannot be exactly determined but may only be limited to a range of values. These values will be dependent on the orientation(s) of the transition dipoles of both donor and acceptor moieties at the moment of transfer and can range from 0 to 4 (Eftink, 1991). When both donor and acceptor are free to rotate and the transition dipoles can assume any orientation (at the ends of a rigid α-helix, for example), κ^2 is $^2/_3$, the average of all the values for κ^2 for all orientations. If time-resolved anisotropy measurements with a single class of fluorophore in a uniform environment can be performed on the donor and acceptor, κ^2 may be limited to minimum and maximum values (Dale & Eisinger, 1974; Eftink, 1991).

We have opted to use the averaged value of $^2/_3$ for κ^2 for two reasons. First, neither the tryptophan residues (Royer et al., 1990) nor the covalently attached AEDANS groups in modified repressor exhibit single fluorescence emission lifetimes. This complex system (even in the single-Trp mutants) cannot be analyzed adequately for the purpose of limiting κ^2 to maximum and minimum values as outlined by Dale and

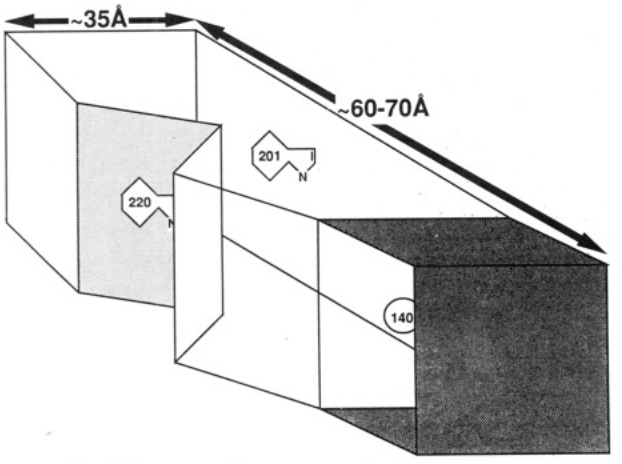

FIGURE 3: Diagrammatic representation of repressor monomer. Molecular dimensions are adapted from several determinations of monomer size (Steitz et al., 1974; Pilz et al., 1980, 1982; Charlier et al., 1980, 1981; McKay et al., 1982). The dark gray area represents the NH_2-terminal headpiece; the light gray area represents the inducer binding site located approximately in the center of the core by analogy to the periplasmic sugar binding proteins (Müller-Hill, 1983; Sams et al., 1984; Quiocho, 1986). Trp201 is buried within the subunit unavailable to solvent in the native tetramer. Trp220 is positioned in the inducer binding site, allowing interaction with the sugar. Cys140 has been postulated to be in the NH_2-terminal headpiece/core interface (Schneider et al., 1984). We have positioned residues 201 and 220 approximately equidistant from Cys140 in the monomer, as indicated by energy-transfer measurements.

Eisinger (Dale & Eisinger, 1974; Eftink, 1991) using time-resolved anisotropy measurements for wild-type repressor and the single-Trp mutant proteins (Royer et al., 1990). Second, these anisotropy measurements demonstrate rapid movement in the AEDANS moiety, suggesting that random orientation may prevail during the energy-transfer process (the lifetime of the tryptophan excited state). In such cases of rapid motion by one or both groups involved in energy transfer, the averaged value of $^2/_3$ for the orientation factor κ^2 is usually applied.

When the random orientation factor value of $^2/_3$ was used, wild-type, W220Y, and Y282D repressors exhibited similar molecular distance ranges calculated from energy-transfer efficiency values (Table II). The distance calculated for wild-type protein was ~35 Å, with a range based on the efficiency error of 33.2–37.5 Å. The error in the Y282D efficiencies expanded this range to 32.3–39.7 Å for the monomeric mutant. The W201Y average distance of ~37 Å (range 34.1–40.1 Å) was slightly longer than the corresponding W220Y and wild-type values. These calculated distance values are well within the postulated dimensions for the *lac* repressor monomer (Steitz et al., 1974; Pilz et al., 1980, 1982; Charlier et al., 1980, 1981; McKay et al., 1982).

Discussion

Cysteine residues in wild-type *lac* repressor protein were modified with 1,5-IAEDANS in the presence and absence of the inducer IPTG. When inducer was included in the reaction mixture, modification at Cys140 remained unchanged, but Cys107 was modified to a greater extent. Reaction at Cys281 was not detectable in either case for tetrameric repressors. Energy-transfer measurements were performed on both species of repressor, and the calculated values for transfer efficiency and distance between donor and acceptor were compared. The efficiency of transfer in the two cases was very close, and the distance calculated was similar. This result leads us to conclude that although some energy transfer may occur between the tryptophans and the acceptor at Cys107, it is not a significant component of the total energy-transfer process.

Of greater interest were similar experiments performed on a mutant repressor protein which does not form tetramers. Although Cys281 is modified to a large extent in the monomeric protein, the introduction of label at this site does not lead to an increase in the efficiency of energy transfer. This moiety may be too far distant from the tryptophan donors located at positions 201 and 220, or the orientation of these donors may be unfavorable for energy transfer to this group. As with wild type, we conclude that the main acceptor group is the AEDANS attached to Cys140. The similarity in wild-type and monomer transfer efficiencies is indicative that *intra*subunit transfer is the primary mode for both proteins.

Energy-transfer efficiencies measured in the single-Trp mutant repressors provide information to assist in interpreting the complex transfer arrangements in the wild-type structure. As Table II shows, Trp201 (W220Y mutant) transfers with an efficiency comparable to wild-type protein, while Trp220 (W201Y mutant) displays a slightly lower efficiency. The differences in transfer efficiency between the repressor proteins examined are small, and the distances calculated from these values were quite similar in all cases, suggesting that the range calculated is likely reflective of the distance between donor tryptophan(s) and Cys140 in the monomer. A comparison of these distances to the various estimates of monomer dimensions (Steitz et al., 1974; Pilz et al., 1980, 1982; Charlier et al., 1980, 1981; McKay et al., 1982) reveals that they are easily contained within the core domain of the protein. Additionally, the distance ranges derived from energy transfer calculated for the repressor monomer correlate well with the known distances measured for several carbohydrate binding proteins which are postulated to be structurally similar to the *lac* repressor core (Müller-Hill, 1983; Sams et al., 1984; Gardner & Matthews, 1990). Galactose binding protein and arabinose binding protein are monomeric sugar binding periplasmic proteins that have molecular dimensions of ~70 Å for the long axis and ~35 Å for the short axis of a solid ellipsoid (N. Vyas, personal communication). Figure 3 provides a diagrammatic representation of the lactose repressor monomer derived from the distances measured in this study and the molecular dimensions determined by a variety of methods (Steitz et al., 1974; Pilz et al., 1980, 1982; Charlier et al., 1980, 1981; McKay et al., 1982). The primary acceptor AEDANS at Cys140 is located at the NH_2-terminal headpiece/core interface of the monomer as postulated by Schneider et al. (1984). Both tryptophan residues are located approximately equidistant from position 140; Trp220 resides relatively exposed to the solvent in the inducer binding site, while Trp201 is buried within the monomer (Sommer et al., 1976; Gardner & Matthews, 1990). The monomer is approximately 60–70 Å in length and 35 Å in diameter (Steitz et al., 1974; Pilz et al., 1980, 1982; Charlier et al., 1980, 1981; McKay et al., 1982). The distance between Cys140 and Trp201/220 of ~35 Å places these residues in the midregion of the monomer core domain, consistent with the placement of the tryptophans by analogy to the periplasmic sugar binding proteins (Gardner & Matthews, 1990). Confirmation of the distance measurements and precise placement of these residues await the solved X-ray crystal structure of the lactose repressor (Pace et al., 1990).

Registry No. IPTG, 367-93-1; Cys, 52-90-4; Trp, 73-22-3.

References

Adler, K., Beyreuther, K., Fanning, E., Geisler, N., Gronenborn, B., Klemm, A., Müller-Hill, B., Pfahl, M., & Schmitz,

A. (1972) *Nature 237*, 322–327.
Beyreuther, K. (1978) *Nature 274*, 767.
Beyreuther, K., Adler, K., Geisler, N., & Klemm, A. (1973) *Proc. Natl. Acad. Sci U.S.A. 70*, 3576–3580.
Bourgeois, S. (1971) *Methods Enzymol. 21D*, 491–500.
Bourgeois, S., Jernigan, R. L., Szu, S. C., Kabat, E. A., & Wu, T. T. (1979) *Biopolymers 18*, 2625–2643.
Cantor, C. R., & Schimmel, P. R. (1980) *Biophysical Chemistry, Part II: Techniques for the Study of Biological Structure and Function*, pp 409–480, W. H. Freeman and Company, San Francisco.
Charlier, M., Maurizot, J.-C., & Zaccai, G. (1980) *Nature (London) 286*, 423–425.
Charlier, M., Maurizot, J.-C., & Zaccai, G. (1981) *J. Mol. Biol. 153*, 177–182.
Chou, P. Y., Adler, A. J., & Fasman, G. D. (1975) *J. Mol. Biol. 96*, 29–45.
Culard, F., & Maurizot, J.-C. (1981) *Nucleic Acids Res. 9*, 5175–5184.
Dale, R. E., & Eisinger, J. (1974) *Biopolymers 13*, 1573–1605.
Daly, T. J., & Matthews, K. S. (1986) *Biochemistry 25*, 5474–5478.
Daly, T. J., Olson, J. S., & Matthews, K. S. (1986) *Biochemistry 25*, 5468–5474.
Eftink, M. R. (1991) *Methods Biochem. Anal.* (in press).
Fairclough, R. H., & Cantor, C. R. (1978) *Methods Enzymol. 48*, 347–379.
Farabaugh, P. J. (1978) *Nature 274*, 765–769.
Förster, T. (1965) in *Modern Quantum Chemistry, Istanbul Lectures Part III* (Sinanoglu, O., Ed.) pp 93–137, Academic Press, New York.
Friedman, B. E., Olson, J. S., & Matthews, K. S. (1977) *J. Mol. Biol. 111*, 27–39.
Gardner, J. A., & Matthews, K. S. (1987a) *Anal. Biochem. 167*, 140–144.
Gardner, J. A., & Matthews, K. S. (1987b) *Fed. Proc. 46*, 2153.
Gardner, J. A., & Matthews, K. S. (1990) *J. Biol. Chem.* (in press).
Gilbert, W., & Müller-Hill, B. (1966) *Proc. Natl. Acad. Sci. U.S.A. 56*, 1891–1898.
Ha, J.-H., Spolar, R. S., & Record, M. T., Jr. (1989) *J. Mol. Biol. 209*, 801–816.
Jacob, F., & Monod, J. (1961) *J. Mol. Biol. 3*, 318–356.
Kunkel, T. A., Roberts, J. D., & Zakour, R. A. (1987) *Methods Enzymol. 154*, 367–382.
Lakowicz, J. R. (1983) *Principles of Fluorescence Spectroscopy*, Plenum Press, New York.
Matthews, K. S. (1987) in *DNA:Protein Interactions and Gene Regulation* (Thompson, E. B., & Papaconstantinou, J., Eds.) pp 13–19, University of Texas Press, Austin.
McKay, D. B., Pickover, C. A., & Steitz, T. A. (1982) *J. Mol. Biol. 156*, 175–183.
Müller-Hill, B. (1983) *Nature 302*, 163–164.
O'Gorman, R. B., Dunaway, M., & Matthews, K. S. (1980) *J. Biol. Chem. 255*, 10100–10106.
Pace, H. C., Lu, P., & Lewis, M. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 1870–1873.
Pilz, I., Goral, K., Kratky, O., Bray, R. P., Wade-Jardetzky, N. G., & Jardetzky, O. (1980) *Biochemistry 19*, 4087–4090.
Pilz, I., Schwarz, E., Wade-Jardetzky, N., Bray, R. P., & Jardetzky, O. (1982) *FEBS Lett. 144*, 247–249.
Quiocho, F. A. (1986) *Annu. Rev. Biochem. 55*, 287–315.
Riggs, A. D., Bourgeois, S., Newby, R. F., & Cohn, M. (1968) *J. Mol. Biol. 34*, 365–368.
Royer, C. A., Gardner, J. A., Beechem, J. M., Brochon, J.-C., & Matthews, K. S. (1990) *Biophys. J.* (in press).
Sams, C. F., Vyas, N. K., Quiocho, F. A., & Matthews, K. S. (1984) *Nature 310*, 429–430.
Sams, C. F., Hemelt, V. B., Pinkerton, F. D., Schroepfer, G. J., Jr., & Matthews, K. S. (1985) *J. Biol. Chem. 260*, 1185–1190.
Schmitz, A., Schmeissner, U., Miller, J. H., & Lu, P. (1976) *J. Biol. Chem. 251*, 3359–3366.
Schneider, J. M., Barrett, C. I., & York, S. S. (1984) *Biochemistry 23*, 2221–2226.
Sommer, H., Lu, P., & Miller, J. H. (1976) *J. Biol. Chem. 251*, 3774–3779.
Steitz, T. A., Richmond, T. J., Wise, D., & Engelman, D. (1974) *Proc. Natl. Acad. Sci. U.S.A 71*, 593–597.
Stryer, L., & Haugland, R. P. (1967) *Proc. Natl. Acad. Sci. U.S.A. 58*, 719–726.
Tu, S.-C., Wu, C.-W., & Hastings, J. W. (1978) *Biochemistry 17*, 987–993.
Whitson, P. A., & Matthews, K. S. (1986) *Biochemistry 25*, 3845–3852.