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Evidence for a Conformational Change in the *Escherichia coli* Maltose Receptor by Excited-State Fluorescence Lifetime Data†

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ABSTRACT: The initial signaling event during maltose chemoreception in *Escherichia coli* is identified with a delocalized ligand-induced conformational change in the maltose binding protein. Substantiation for the conformational change involves a new application of the "distant reporter group technique" [Zukin, R. S., Hartig, P. R., & Koshland, D. E., Jr. (1977a) *Proc. Natl. Acad. Sci. U.S.A. 74*, 1932–1936] utilizing excited-state fluorescence lifetime measurements. Binding of maltose to its receptor results in changes in the microenvironment of the two tryptophan residues of the receptor protein and of an experimentally attached reporter group, 5-(iodoacetamido)fluorescein. The minimum distance between the two tryptophans from efficiency of fluorescence energy transfer theory is 17 Å; the minimum distance from the farther tryptophan to the fluorescein is 50 Å. Thus, the maltose receptor is shown to undergo molecular rearrangements at distant sites upon ligand binding. The general feature of conformational change as the initial signaling event during chemoreception in the enteric bacteria is discussed.

The initial event of stimulus transduction during sensory perception in many neural and microbial systems is that of receptor activation. The molecular mechanism of this activation has been generally assumed to be a ligand-induced conformational change. The evidence for conformational changes, however, has usually been circumstantial. It is thus of interest to develop new methods to obtain more direct evidence.

Bacterial chemotaxis, a complete behavioral system contained within a single cell, provides a sensory system that is particularly amenable to a detailed molecular study. This system has been shown to consist of discrete chemical receptors (Adler, 1969; Aksamit & Koshland, 1974; Zukin et al., 1977b), a transmission mechanism to relay information from the receptors to the flagella (Adler et al., 1973; Parkinson, 1974), and a motor response (Strange & Koshland, 1976; Macnab, 1978). Conformational changes in bacterial chemoreceptors have been postulated as an obligatory first step leading to interaction with the signal transmission apparatus (Strange & Koshland, 1976). One of the only purified receptor systems in which a conformational change has been definitely demonstrated is that of the galactose receptor (Zukin et al., 1977a). The distant reporter group method was used to detect delocalized protein perturbations by steady-state fluorescence spectroscopy. This type of application was limited, however, to proteins which have a single tryptophan residue, and thus a more general approach is needed if more complex proteins containing multiple tryptophan residues are to be studied.

The technique of excited-state fluorescence lifetime measurements provides tryptophan spectral data which can be resolved into as many as three components assignable to individual residues (Yashinsky, 1972; Grinvald & Steinberg, 1976). Lifetime measurements thus provide the potential of detecting changes in polarity or hydrophobicity in the microenvironments of specific tryptophans. Application of the distant reporter group method with fluorescent lifetime techniques can therefore be used to detect delocalized polypeptide refolding in a large number of protein systems.

The protein chosen for development of this approach was the maltose receptor of *Escherichia coli* involved in bacterial sensing and transport. The maltose binding protein was purified and shown to be the product of the *malE* gene involved in maltose transport by Kellerman & Szmelcman (1974). It was identified as the maltose chemoreceptor by Hazelbauer (1975), and its binding properties have been studied extensively (Kellerman & Szmelcman, 1974; Schwartz et al., 1976; Szmelcman et al., 1976). The maltose receptor is a monomer of approximately 37 000 molecular weight (Kellerman & Szmelcman, 1974) and binds a single maltose residue per molecule of protein with a K_D of 2×10^{-6} M. The present study describes fluorescence lifetime studies of the two tryptophan moieties of the maltose receptor and of the reporter group 5-(iodoacetamido)fluorescein (5-IAF)[1] (Zukin et al., 1977a; Hartig et al., 1977a,b) specifically attached at a single

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[1] Abbreviations used are: 5-IAF, 5-(iodoacetamide)fluorescein; AF, acetamidofluorescein; $NaDodSO_4$, sodium dodecyl sulfate.

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protein site. These data are used to elucidate conformational events at specific protein sites during receptor activation.

Materials and Methods

The maltose binding protein was isolated and purified from *E. coli* wild-type strain K12 by a modification of the method of Kellerman & Szmelcman (1974). One hundred liters of *E. coli* K12 were grown to stationary phase and harvested. Bacteria were subjected to osmotic shock by the method of Willis et al. (1974), and the shock fluid was fractionated by ion-exchange chromatography on a QAE-Sephadex column (2.5 × 50 cm) by using a stepwise gradient of 0–250 mM KCl in 10 mM Tris-HCl buffer containing 2 mM $MgCl_2$ and 0.02% sodium azide (buffer B) and then on a second identical column by using a linear gradient of 50–500 mM KCl. Elution from the second QAE-Sephadex column yielded 40 mg of the purified protein. The final protein preparation was determined to be homogeneous by sodium dodecyl sulfate ($NaDodSO_4$)–polyacrylamide gel electrophoresis. This technique gave a single sharp band with no trace of impurity. The molecular weight of the maltose receptor was estimated by $NaDodSO_4$–polyacrylamide gel electrophoresis to be 35 000, in corroboration of the previously reported value (Kellerman & Szmelcman, 1974). Protein concentration was determined by the method of Lowry et al. (1951), and binding activity was determined by equilibrium dialysis (Kellerman & Szmelcman, 1974); 1 mol of maltose was found to bind per mol of protein.

The maltose chemoreceptor protein was modified by treatment with a 1000-fold excess of 5-(iodoacetamido)-fluorescein (a gift of Richard Haugland, Hamline University) in 10 mM potassium phosphate buffer, pH 6.8, for 24 h in the dark. The labeled protein was then purified by passage through a Sephadex G-25 column equilibrated with the same buffer and was subsequently dialyzed against buffer B for 5 days at 4 °C to remove carbohydrate contamination caused by passage through the Sephadex column.

The labeling ratio was determined from the protein concentration and the absorbance of the modified protein at 490 nm. A molar extinction coefficient of 42 000 for the attached fluorescein label at 490 nm was used (Zukin et al., 1977a). Steady-state fluorescence measurements were performed on a Perkin-Elmer Hitachi Model MPF-3 fluorescence spectrometer at 10 °C. Steady-state polarization measurements were made by using the standard polarization accessory for the spectrophotofluorimeter and were normalized for a constant grating transmission. Lifetime measurements were performed on an Ortec 9200 nanosecond fluorescence spectrometer at 10 °C. An Optitron Model NR-1 lamp operating under 200 psi of N_2 with a Bausch & Lomb monochromator at 296 nm was utilized as the excitation source with a combination of Corning 0-52 and 7-37 filters (which together select for the region from 340 to 360 nm) to isolate the emission. Data analysis was by the Method of Moments (Isenberg & Dyson, 1969). The extent of energy transfer was determined by measurement of the excited-state lifetime of the energy donor, tryptophan, in the presence and absence of the acceptor, 5-AF, and also independently from the excitation spectrum of fluorescein bound to the protein. Tryptophan quantum yields were calculated from the ratio of the observed fluorescence lifetimes to the value for the indole natural lifetime (15 ns; Longworth, 1971).

The amino acid compositions of the native and 5-AF-maltose receptors and their methionine sulfone derivatives were determined by Dr. H. Steinman by using a Beckman Model 120 C amino acid analyzer. The methionine sulfone derivatives were prepared by using performic acid reagent according to the method of Hirs (1967).

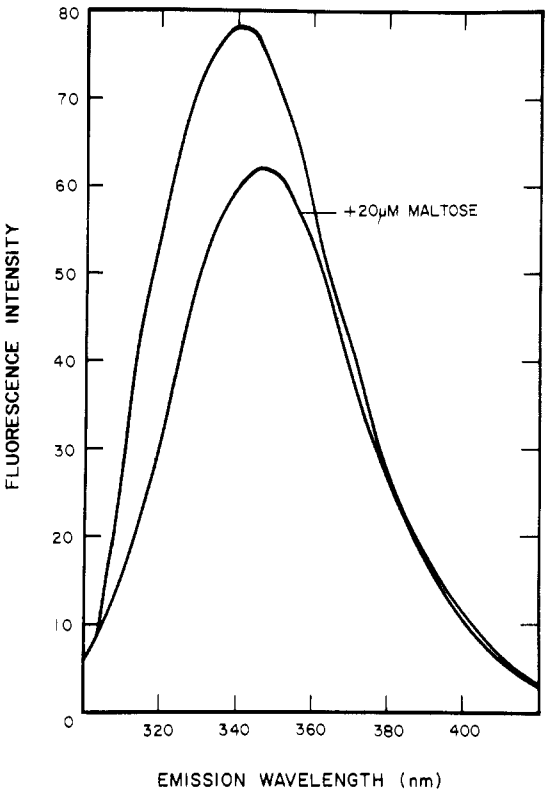

FIGURE 1: Fluorescence emission spectrum (uncorrected) of the *E. coli* maltose receptor in the presence and absence of 10^{-4} M maltose. Spectra were recorded on a Perkin-Elmer Hitachi Model MPF-2A fluorescence spectrometer at ambient temperature with an excitation wavelength of 288 nm. Maltose binding protein (60 μg/mL) was in buffer A (10 mM Tris, pH 7.3, 2 mM $MgCl_2$, and 0.02% NaN_3).

Results

Effect of Maltose on Intrinsic Tryptophan Fluorescence of E. coli Maltose Receptor. The intrinsic steady-state fluorescence of the purified maltose binding protein was examined in the presence and absence of saturating maltose (Figure 1). Maltose causes a 6-nm red shift and a 21% quenching of the tryptophan fluorescence emission spectrum. In contrast, no such changes are observed in the control upon addition of glucose, a sugar that shows no affinity for the protein (Kellerman & Szmelcman, 1974). The tryptophan content of this protein was determined to be two residues per mol of protein (Table I). The receptor emission spectrum therefore represents the sum of the fluorescence of the two tryptophan moieties; changes in this emission spectrum reflect a superposition of the changes in the microenvironment of the two residues. On the basis of model system results, the red shift observed upon addition of ligand is consistent with a change for one or both of the tryptophans and a net shift to a more polar environment (Konev, 1967). A similar shift and quenching were also observed by Szmelcman et al. (1976) for the *E. coli* maltose receptor. No change in the receptor absorption spectrum was observed upon addition of saturating maltose.

In order to determine the effect on the individual tryptophan residues of maltose binding to the receptor, the protein excited-state fluorescence lifetimes were examined. In Figure 2 is shown the nanosecond emission kinetics of the intrinsic fluorescence of the maltose receptor in the presence and absence of substrate. The observed time course of the

Table I: Amino Acid Compositions of Some Chemosensory Receptors

amino acid	*S. typhimurium*, ribose receptor (mol/ 29000 g)[a]	*E. coli*, galactose receptor (mol/ 35000 g)[a]	*E. coli*, maltose receptor (mol/ 35000 g)[b]
Lys	26.0	27.3	24.3
His	3.0	3.0	2.6
Arg	5.8	5.7	5.0
Asp	35.6	43.4	33.5
Thr	12.2	12.2	14.6
Ser	8.0	11.0	10.5
Glu	26.1	25.2	29.1
Pro	8.2	8.0	15.0
Gly	23.7	19.4	24.2
Ala	36.7	37.7	31.3
Val	25.5	26.1	15.4
Met	4.9	5.2	3.3
Ile	12.0	13.2	14.5
Leu	24.0	21.2	20.8
Tyr	2.7	5.0	12.1
Phe	6.7	5.8	18.2
Cys	0	0	0
Trp	0	3.5	2.2[a]

[a] Zukin et al. (1977b). [b] Tryptophan was detected by the method of Barman & Koshland (1967) and also by excited-state fluorescence lifetime analysis.

Table II: Excited-State Fluorescence Lifetimes of *E. coli* Galactose Receptor and Its 5-AF-Labeled Analogue

	Trp fluorescence lifetime (ns)	
	T_1	T_2
E. coli maltose receptor	4.4	9.8
E. coli maltose receptor + 10^{-4} M maltose	3.5	12.1
5-AF-maltose receptor	2.9	9.7

fluorescence decay fits a curve calculated for two excited-state lifetimes of 4.4 and 9.8 ns (Table II) which have been assigned arbitrarily to the two tryptophans. Thus, the tryptophan molecules of the maltose receptor would appear to be in environments of different polarity and would be expected to have different emission spectra. In contrast, in the case of the very similar galactose receptor of *Salmonella typhimurium* (Zukin et al., 1977b), a protein which contains one tryptophan residue per molecule of protein, a good fit of the fluorescence decay to a single exponential was observed (R. S. Zukin, P. R. Hartig, and D. E. Koshland, Jr., unpublished experiments). The value for the indole natural fluorescence lifetime is 15 ns (Longworth, 1971). Both lifetimes of the maltose receptor tryptophans exhibit changes upon binding of maltose to receptor at saturating levels: the 9.8-ns component lengthens to 12.1 ns and the 4.4-ns lifetime is quenched to 3.6 ns; in addition, the early part of the trace is noticeably steeper in the presence of maltose, while the latter portion is less steep. These changes most likely indicate that one tryptophan is shifted to a more hydrophilic environment and the other is shifted to a more hydrophobic environment (Zukin et al., 1977a; Konev, 1967). In addition, the tryptophan lifetime changes would indicate a 10% increase in the quantum yield of the protein upon binding of maltose. This prediction is in contrast to the observed quenching of the protein fluorescence emission spectrum upon binding of sugar. Such an apparent difference may arise because the lifetime data measured under these conditions preferentially weigh the contribution of the tryptophan molecule associated with the longer wavelength

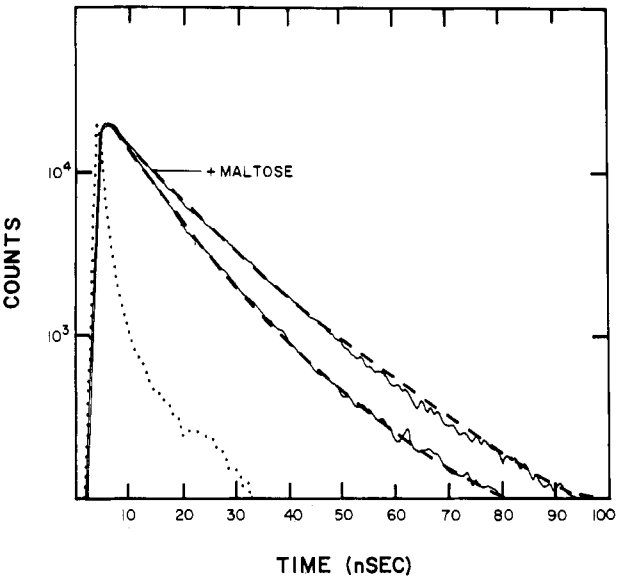

FIGURE 2: Nanosecond emission kinetics of tryptophan in *E. coli* maltose receptor in the presence and absence of 10^{-4} M maltose. In the absence of added maltose, the observed fluorescence kinetics (—) fit a curve calculated for two excited-state lifetimes of 4.43 and 9.84 ns (---); in the presence of saturating maltose, the data are resolved into lifetime components of 3.56 and 12.09 ns: (···) light pulse.

Table III: Amino Acid Analyses of the Maltose Receptor of *E. coli* and Its 5-AF-Labeled Analogue

amino acid	maltose binding protein	5-AF-maltose binding protein
lysine	24.3	23.0
histidine	2.6	2.8
aspartate	33.5	33.3
cysteine	0	0
methionine	4.7	3.8
(carboxymethyl)lysine	0	0
(carboxymethyl)histidine	0	0
methionine sulfone[a]	4.7	3.9
cysteic acid[a]	0.02	0.02

[a] Value reported is for the performate-treated protein.

of emission and, as Figure 1 indicates, the quenching is dominant on the short wavelength side (<340 nm) of the emission spectrum.

Effect of Maltose on Fluorescein Fluorescence of the Dye-Modified Maltose Receptor. In order to differentiate between a mechanism in which the sugar maltose interacts directly with the two tryptophans when it binds with no conformational change and a mechanism that does involve a ligand-induced conformational change, the maltose receptor was modified with an additional chromophore. The purified maltose receptor was reacted with 5-IAF to produce a covalently modified protein (5-AF-maltose binding protein), as shown by comigration of fluorescence at 520 nm with a single protein band during $NaDodSO_4$–polyacrylamide gel electrophoresis. Modification proceeded to a fixed value; at saturation, measurement of the absorbance at 490 nm of the protein in 8 M urea showed one fluorescent group per molecule of protein, as calculated from the molar extinction coefficient of the free dye in urea. Amino acid analyses of the protein before and after modification established that a single methionine residue, and no other residue, was modified by this reagent (Table III). Modification of one methionine was shown both by comparison of the methionine contents of maltose binding protein and 5-AF-maltose binding protein and

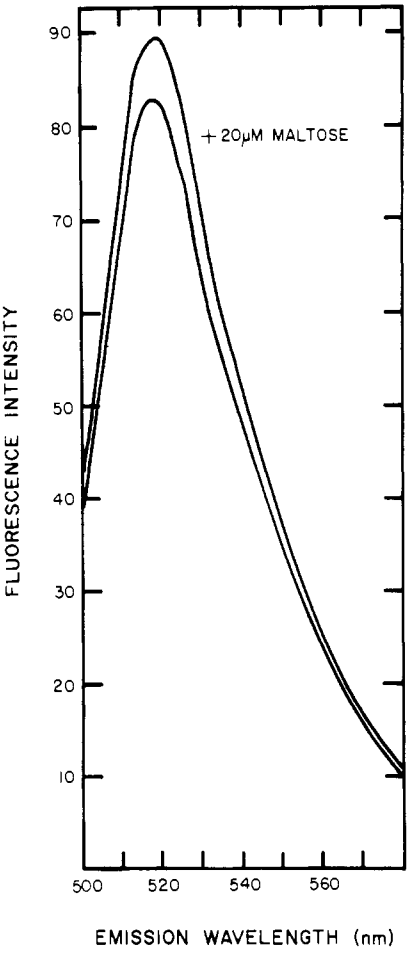

FIGURE 3: Fluorescence emission spectra (uncorrected) of the 5-AF-modified maltose receptor from *E. coli* in the presence and absence of 10^{-4} M maltose. Spectra were recorded at ambient temperature with an excitation wavelength of 460 nm. The 5-AF-maltose protein (10 μg/mL) was in buffer A.

more rigorously by comparison of the methionine sulfone content of the performate-treated proteins. The recoveries of lysine, histidine, and all other amino acids were found to be quantitative in agreement with the absence of peaks in the positions of elution volumes identified with ε-*N*-(carboxymethyl)lysine or 1-(or 3)-(carboxymethyl)histidine (Gundlach et al., 1959). The dye-modified maltose receptor exhibited full sugar-binding activity.

The fluorescence emission spectrum of the reporter group on the protein showed a 3-nm shift and an 8% enhancement upon binding of maltose at saturating levels (Figure 3). Changes of this type are consistent with shifting of the fluorescein to a more hydrophobic environment (Hartig et al., 1977a; Hartig & Sauer, 1979).

Distance between the Reporter Groups. In order to prove the occurrence of a delocalized conformational change, distances between each of the tryptophan residues and the fluorescein dye in the 5-AF-maltose receptor were calculated by using the theory of Förster (1966). The Förster equation (eq 1) relates the distance, R, between two fluorophores which

$$\frac{9(\ln 10)Q_D K^2}{128\tau^5 N n^4 \tau_D R^6} J\bar{\nu} = E \qquad (1)$$

undergo excitation transfer to the transfer efficiency E; J is the spectral overlap integral between the donor emission and the acceptor absorption, Q_D is the quantum yield of the donor in the absence of the acceptor, n is the index of refraction of the intervening medium, and K^2 is the orientation factor of the donor and acceptor transition moments (Förster, 1966). This theory has been tested in well-defined model systems (Latt et al., 1965; Stryer & Haughland, 1967) and has been used to deduce distances in various biological systems.

The molecular orientation factor K^2 was calculated from steady-state polarization by using the Weber-modified Perrin equation (Weber, 1952) as previously described (Zukin et al., 1977a) and the modeling approach of Dale & Eisinger (1974, 1976). The model chosen for the 5-AF-labeled maltose binding protein assumes that each tryptophan energy donor executes motion over an area approximated by the surface area of a cone. This assumption of restricted motion for the maltose receptor tryptophans is based on findings in other protein systems (Munro et al., 1979; Weber, 1952) that the indole moiety of tryptophan in a protein is not completely rigid. The fluorescein (5-AF) residue, attached to the polypeptide backbone by a methionine "arm", is assumed to execute rapid motion throughout the volume of a cone. The half-angle describing these cones is calculated from the fluorescence polarization of the respective chromophores.

The measured polarization for tryptophan in the modified receptor is 0.10; P_0 for immobilized tryptophan is 0.3 (Weber, 1966). Thus, the tryptophans of the maltose receptor exhibit some freedom of motion. The measured polarization for 5-AF bound to the maltose receptor is 0.03. This value is very close to the polarization observed for the free dye in water, 0.017 (Hartig et al., 1977a). The theoretical value of fluorescence polarization for this dye randomly immobilized on a protein of 35 000 molecular weight with a 2:1 axial ratio for a prolate ellipsoid [in analogy to other periplasmic binding proteins characterized thus far (Pardee, 1966; Quiocho et al., 1977; Hartig et al., 1977b)] is 0.34 (Zukin et al., 1977a); thus, the reporter group has considerable independent freedom of motion. These polarization values were then used to calculate $P_{0(m)}$, the polarization of the fluorophore bound to the macromolecule when the macromolecule is immobilized, but the fluorophore is free to move within the restricted environment of its binding site. The polarization values together with the assumed values for the receptor shape were used as previously described (Zukin et al., 1977a) in the Weber equation (1952) that relates $P_{0(m)}$ to P for a dye attached with random orientation to a prolate ellipsoid. $P_{0(m)}$ values of 0.14 and 0.18 were determined for the short-lived and long-lived tryptophans, respectively; $P_{0(m)}$ for 5-AF bound to the maltose receptor was calculated to be 0.04. These values were applied according to the Dale and Eisinger theory (1974, 1976) to give cone half-angles of 30° and 25° for the short-lived and long-lived tryptophans, respectively, and a cone half-angle of 68° for 5-AF. Using these cone half-angle values, we calculated the limits of K^2 to be 0.35–1.4 for energy transfer between tryptophan (I) and the attached fluorescein residue, and the K^2 range is 0.35–1.5 for the tryptophan (II)–5-AF donor–acceptor pair.

The transfer E was determined from measurements of the quantum yield (Q) and the excited-state lifetime (τ) of the energy donor (tryptophan) in the presence and absence of the 5-acetimidofluorescein energy acceptor (Wu & Stryer, 1972) (i.e., in the fluorescent-labeled (f) and unmodified or native (n) protein, respectively)

$$E = 1 - (Q_f/Q_n) \qquad (2)$$

$$E = 1 - (\tau_f/\tau_n) \qquad (3)$$

The excited-state lifetime of one tryptophan (I) is 4.4 ns in the native protein and 2.9 ns in the modified protein (Table

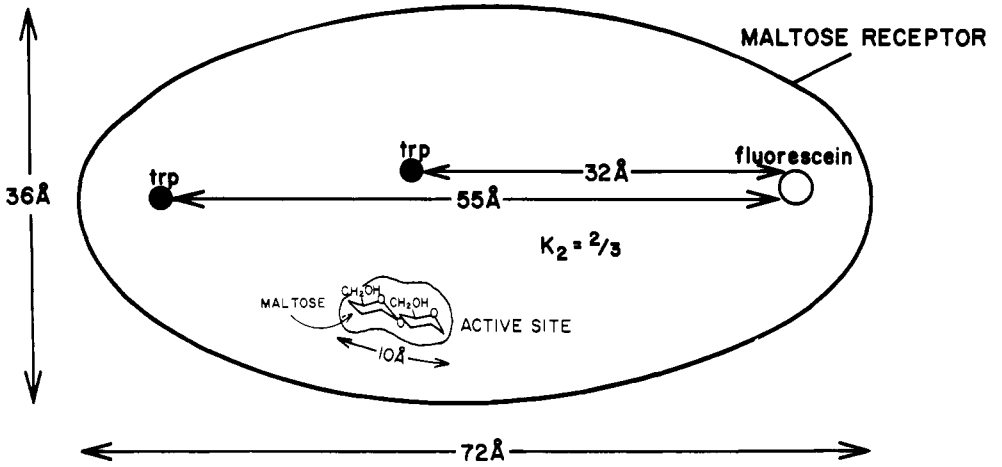

FIGURE 4: Schematic representation of the *E. coli* maltose receptor showing the dimensions of the protein, based on an axial ratio of 2:1 and the distances from the two tryptophans to the 5-AF labeling site as calculated from fluorescence energy transfer measurements. The model includes the single maltose binding site near the center of the long axis in analogy to the very similar *E. coli* arabinose receptor (Quiocho et al., 1977).

II). The lifetime of the second tryptophan (II) decreased from 9.8 ns in the unlabeled protein to 9.7 ns in the 5-AF-galactose receptor; this change is within the experimental error of the measurement. From eq 2 and 3, the transfer efficiencies from the tryptophans I and II to 5-AF are calculated to be 35 and $\leq$3%, respectively. Equations 2 and 3 assume that the local conformations of the energy donors are not altered by modification with the fluorescent dye, 5-AF (Lakowicz & Weber, 1973). That the local conformation of tryptophan is unaltered is shown by the similar spectral shapes (same λ_{max}) for Trp fluorescence in the native and dye-modified proteins. In addition, the average energy transfer efficiency from the two tryptophans to the acceptor, 5-AF, was determined to be 35% from the excitation spectrum of 5-AF bound to the protein. Addition of saturating maltose to the dye-modified receptor resulted in an energy transfer efficiency of 45%.

By use of the Förster eq 1 and by assumption of the accepted value of 1.4 for n (Pardee, 1966), R_0 (the tryptophan–fluorescein distance which corresponds to 50% fluorescence energy transfer and a K^2 value of $^2/_3$) is 28.9 Å for tryptophan I. R_0 for tryptophan II is 30.9 Å. In the case of tryptophan I a fluorescence transfer efficiency of 35% and a K^2 value of $^2/_3$ give a distance of 32 Å. The limits of K^2 calculated from the polarization give a range of 29–33 Å for the distance of this tryptophan of 5-AF. If the special case in which the tryptophan and fluorescein transition moments are parallel is eliminated, this distance range reduces to 29–30 Å. Tryptophan II is 55 Å (by assuming $K^2 = {}^2/_3$) from the fluorescein dye or 50 Å away, taking into account the limits of K^2.

Discussion

We have resolved two fluorescence excited-state lifetimes for the two tryptophan residues of the *E. coli* maltose receptor and determined them to be 4.4 and 9.8 ns. By fluorescence energy transfer measurements we have calculated that the tryptophan associated with the shorter lifetime is 31 ± 2 Å from the single acetamidofluorescein moiety of the 5-AF-modified maltose receptor, and the longer lived tryptophan (9.8-ns lifetime) is $\geq$50 Å from the attached dye. Thus, the minimum distance between the two tryptophan residues is 17 Å. Since (a) the maltose molecule is only 10 Å in length, (b) only one molecule of ligand binds per molecule of receptor, and (c) its binding perturbs the microenvironment of both tryptophans and the fluorescein chromophore, it is clear that the changes introduced at a minimum of two of the three sites are the result of an indirect effect. Hence, there must be a ligand-induced conformational change that is propagated through the receptor to distant sites which are a minimum of 50Å (from the farther tryptophan to 5-AF) apart.

In Figure 4 is shown a schematic representation of the *E. coli* maltose receptor showing the distance from the two tryptophan residues to the 5-AF labeling site as calculated from fluorescence energy transfer measurements. The model shows the three fluorophores aligned such that the tryptophans are at a minimum distance from one another. The model also includes the dimensions of the protein (long axis, 72 Å; short axis, 36 Å), based on an axial ratio of 2:1 by analogy to the very similar *E. coli* arabinose receptor (Munro et al., 1979) and *Salmonella* galactose receptor (Zukin et al., 1977a; Hartig et al., 1977b). The single maltose binding site is centrally located in analogy to the arabinose receptor (Quiocho et al., 1977). The arrangement of the two tryptophans and the 5-AF moiety illustrated here is only one of the many possible arrangements consistent with the interatomic distances calculated for this triplet. The greatest possible distance between the two tryptophans is the long axis of the prolate receptor molecule or 72 Å.

The distant reporter group method for estimating molecular distances utilizing either steady-state fluorescence or excited-state fluorescence lifetimes requires placing of a chromophore at a position too distant to allow direct interactions with the ligand. Any combination of intrinsic or experimentally attached fluorophores can serve equally well. The approach requires only that a minimum distance be established between at least one pair of dyes and is therefore less demanding than mapping positions on protein surfaces by fluorescence energy transfer measurements. If that minimum is far greater than the size of the ligand (in this case, 17, 29, or 50 Å vs. 10 Å), the chance for an erroneous conclusion is very remote.

The above derivation makes a number of assumptions, many of which are based on experimental facts and have been summarized previously (Zukin et al., 1977a). It has been assumed that tryptophan executes motion throughout the surface area of a cone; this assumption is based on tryptophan data from other systems (Lakowicz & Weber, 1973) and on its measured polarization in this case. It has been assumed that 5-AF executes rapid motion throughout the volume of a cone within its binding site on the macromolecule. The fluorescein is attached to the peptide backbone through a series

of carbon–carbon single bonds of the acetamido function and the methionine side chain which provide flexibility for rapid motion. The observed polarization for the bound fluorescein is 0.034, a value which is much closer to $P_0 = 0.017$ for the free dye than to $P_0 = 0.35$ calculated for 5-AF immobilized on a prolate ellipsoid of a 2:1 axial ratio and 35000 molecular weight (Zukin et al., 1977a). Thus, 5-AF exhibits considerable independent motion in its binding site.

The motion of 5-AF in its binding site need not be isotropic for the modeling to be valid, as errors introduced by deviations from isotropic motion tend to cancel (Dale & Eisinger, 1974). Moreover, the calculated donor–acceptor distance range is not expected to be sensitive to the assumed receptor shape (Zukin et al., 1977a). Finally, the parameters used to calculate R_0 enter into the distance calculation only by their sixth root, so that uncertainties in these have little effect on the distance calculation. In particular, there is little uncertainty in the donor quantum yield or the spectral overlap integral (Wu & Stryer, 1972), as these are based on direct measurements. The only exception is the value of K^2 which has been thoroughly treated above. In conclusion, the various potential errors in estimation of the interdye distances will not reduce the minimum values by more than 10%.

Several findings independently corroborate the occurrence of a ligand-induced conformational change in the modified maltose receptor. First, addition of maltose to the receptor protein results in a 30% increase in the observed energy transfer efficiency from tryptophan to 5-AF. Second, labeling of a methionine with the large, bulky fluorescein molecule results in no detectable loss of binding activity. If sugar binding involved direct participation of this amino acid, a substantial loss of activity would be expected upon its chemical modification. Third, the distance between the fluorophores is consistent with the dimensions of the protein. A prolate ellipsoidal protein of molecular weight 35000 and an axial ratio 2:1 has dimensions 36 × 36 × 72 Å. Dimensions of 33 × 33 × 70 Å have been calculated for the very similar *Salmonella* galactose receptor (Zukin et al., 1977a; Aksamit & Koshland, 1974), and dimensions of 30 × 38 × 68 Å are observed for its antigenically related *E. coli* arabinose receptor (Quiocho et al., 1977). Finally, binding of sugar to the *E. coli* maltose receptor does not produce an alteration in the protein absorption spectrum. Such alterations have been reported for sugar binding to tryptophan in other systems (McGowan et al., 1974; Hyashi et al., 1963).

The *E. coli* maltose receptor has been shown to undergo a ligand-induced conformational change by fluorescent lifetime and energy transfer measurements and by a variety of independent data. The lifetime method has utilized the intrinsic protein fluorescence resolved into its two independent components and the fluorescence of an experimentally attached reporter group to reveal molecular rearrangements of distant protein sites. This multiple reporter group method extends the applicability of the previously described distant reporter group method to proteins with more than one tryptophan residue. The only requirement is that the individual lifetime components be resolved; computer programs have now been described that resolve protein fluorescence into as many as three tryptophan components.

The finding of a ligand-induced conformational change in the *E. coli* maltose receptor is of particular interest because maltose and aspartate chemoreception share a common signaling protein, the *tar* gene product (Springer et al., 1977; Silverman & Simon, 1977). By analogy to the *trg* signaling system for ribose and galactose chemotaxis, the conformational change in the maltose receptor could provide a combining site for the *tar* product. Ligand activation of the receptor would then serve as an obligatory first step in chemoreception which triggers the signaling apparatus. Because the maltose receptor is a water-soluble, periplasmic protein, these in vitro studies provide a good indication of its actual mechanism in vivo. Clearly, isolation and purification of the *tar* signaling component protein(s) and, ultimately, reconstitution of the entire chemoreception apparatus can further elucidate the molecular details of the early events in chemoreception. Finally, it is of interest that conformational changes have now been demonstrated in two distinct bacterial receptor systems as the analogies between these systems provide evidence that ligand-induced conformational changes are a common mechanism for receptor activation during chemotaxis.

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Variations in the Sialic Acid Compositions in Glycoproteins of Mouse Ascites Tumor Cell Surfaces†

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ABSTRACT: Sialic acid removable by *Vibrio cholerae* neuraminidase from four TA3 mammary carcinoma ascites sublines of the strain A mouse and six TA3-Ha/A.CA hybrid cell lines, in ascites form, resulting from fusion of TA3-Ha cells and normal fibroblasts of the A.CA mouse and adapted for growth in the strain A mouse [Klein, G., et al. (1972) *J. Exp. Med. 135*, 839], consisted of mixtures of *N*-acetylneuraminic acid and *N*-glycolylneuraminic acid (NeuNGl). Total cell-surface sialic acid ($\mu g/10^9$ cells) and proportion of NeuNGl (%) averaged, respectively, as follows: TA3-St, 270 μg, 20%; TA3-Ha, 620 μg, 7%; TA3-MM/1, 850 μg, 12%; TA3-MM/2, 1200 μg, 12%; TA3-Ha/A.CA/3B, 700 μg, 13%; TA3-Ha/A.CA/4, 870 μg, 19%; TA3-Ha/A.CA/6, 1180 μg, 17%; TA3-Ha/A.CA/7, 910 μg, 38%; TA3-Ha/A.CA/10, 470 μg, 17%; and TA3-Ha/A.CA/11, 850 μg, 6%. Fractions of effluents obtained by gel filtration chromatography of glycopeptides cleaved from viable cells by proteolysis were analyzed for carbohydrate composition and proportion of NeuNGl. Glycopeptide fractions from the same cells possessed markedly different proportions of NeuNGl. The proportion of NeuNGl increased consistently with the percent of mannose in the carbohydrate moiety and decreased with the percent of *N*-acetylgalactosamine and with the apparent molecular weights (by gel filtration). It was concluded that, in the TA3 tumor system, the proportion of NeuNGl in the sialic acid of cell surface glycoproteins of an ascites cell line correlates directly with the proportion of *N*-glycosyl-linked carbohydrate chains in the glycoproteins.

Many diverse functions have been attributed to sialic acid at the mammalian cell surface. These include the demonstration that masking of penultimate galactose residues in cell-surface glycoproteins may prolong the existence of erythrocytes (Aminoff et al., 1977), platelets (Greenburg et al., 1977), or lymphocytes (Woodruff & Gesner, 1969) in the circulation. Sialic acid may serve as a receptor site for myxo- and paramyxoviruses (Scheid & Choppin, 1974), and it may act as an essential component of both the M and N blood group specific determinants at the surface of erythrocytes (Lisowska & Duk, 1976). It has been shown to bind calcium ions at the cell surface by virtue of its three-carbon hydroxylated segment (Jaques et al., 1977); and it has been suggested that the property of aggregation in mammalian cells is modified by the presence of its negatively charged carboxylic acid group (Deman & Bruyneel, 1975).

Sialic acid does not appear to play a general or universal role in malignancy. Indeed, the concentration of sialic acid may vary widely from one tumor cell type to another, and in the case of certain cells grown in vitro its concentration may be greater in normal than in transformed cells (Jeanloz & Codington, 1976). Yet, the reduced allotransplantability of many tumor cell types at low inocula (10^3 to 10^4 cells per mouse) after sialic acid removal by neuraminidase is well documented (Jeanloz & Codington, 1976; Rios & Simmons, 1973; Bekesi et al., 1976). This effect appears to be primarily due to the exposure of new immunogenic galactose residues, which may also serve as receptors for cytotoxic γM antibodies. However, the removal of sialic acid residues from TA3-Ha mammary carcinoma cell surfaces was not found to exert any detectable effect upon transplantability in foreign mouse strains at inocula of 10^5 to 10^6 cells per mouse (Sanford et al., 1973).

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