dimensional structure of T4 thioredoxin is similar to that of *E. coli* thioredoxin (Söderberg et al., 1978), and a patch of negative charges has been observed in a corresponding position in the T4 molecule.[3]

(2) A region around Arg-73 in the 74–108 region is proposed to involve a second binding interaction. This is based on the low activity of thioredoxin-T′ (Slabý & Holmgren, 1979). Furthermore, a mutant thioredoxin (*E. coli* tsnC 7007), with the permissible Gly-92 exchanged to Asp-92,[4] has a dramatically changed reactivity with thioredoxin reductase.

(3) Lysine residues of the 1–37 sequence appear essential as seen from the chemical modification of thioredoxin-C-(1–37). Of special interest is Lys-36 which is a conserved residue[2] which may stabilize a thiolate ion base pair in thioredoxin-$(SH)_2$. In contrast, the folding of thioredoxin-C′ was possible from the lysine-modified thioredoxin-C-(1–37).

The model, in Figure 11, is a first attempt to describe the three-dimensional complementarity between thioredoxin and thioredoxin reductase. As can be seen, it leaves some areas of the thioredoxin surface free. In fact, when thioredoxin is bound to gene 5 as a subunit of phage T7 DNA polymerase (Mark & Richardson, 1976), it retains its activity with thioredoxin reductase.[4]

References

Ambler, R. P. (1965) *Biochem. J. 96*, 32P.
Anfinsen, C. B., & Scheraga, H. A. (1975) *Adv. Protein Chem. 29*, 205–300.
Berglund, O. (1969) *J. Biol. Chem. 244*, 6306–6308.
Davis, B. J. (1964) *Ann. N.Y. Acad. Sci. 121*, 404–427.
Dyckes, D. F., Creighton, T. E., & Sheppard, R. C. (1973) *Nature (London) 247*, 202–204.
Hall, D. E., Baldesten, A., Holmgren, A., & Reichard, P. (1971) *Eur. J. Biochem. 23*, 328–335.
Holmgren, A. (1968) *Eur. J. Biochem. 6*, 475–484.
Holmgren, A. (1972) *FEBS Lett. 24*, 351–354.
Holmgren, A. (1980) in *Dehydrogenases Requiring Nicotinamide Coenzymes* (Jeffrey, J., Ed.) Birkhäuser Verlag, Basel (in press).
Holmgren, A., & Reichard, P. (1967) *Eur. J. Biochem. 2*, 187–196.
Holmgren, A., & Sjöberg, B. M. (1972) *J. Biol. Chem. 247*, 4160–4164.
Holmgren, A., Söderberg, B. O., Eklund, H., & Brändén, C. I. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 2305–2309.
Laurent, T. C., & Killander, J. (1964) *J. Chromatogr. 14*, 317–330.
Light, A., Taniuchi, H., & Chen, R. F. (1974) *J. Biol. Chem. 249*, 2285–2293.
Mark, D., & Richardson, C. C. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 780–784.
Ornstein, L. (1964) *Ann. N.Y. Acad. Sci. 121*, 321–349.
Sjöberg, B.-M., & Holmgren, A. (1972) *J. Biol. Chem. 247*, 8063–8068.
Slabý, I., & Holmgren, A. (1975) *J. Biol. Chem. 250*, 1340–1347.
Slabý, I., & Holmgren, A. (1979) *Biochemistry* (preceding paper in this issue).
Söderberg, B.-O., Sjöberg, B.-M., Sonnerstam, U., & Brändén, C.-J. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 5827–5830.
Thelander, L. (1970) *J. Biol. Chem. 245*, 6026–6029.

[4] A. Holmgren, G.-B. Kallis, and B. Nordström, unpublished experiments.

Effect of an Induced Conformational Change on the Physical Properties of Two Chemotactic Receptor Molecules†

R. Suzanne Zukin,* P. R. Hartig, and D. E. Koshland, Jr.

ABSTRACT: The physical properties and conformational dynamics of the *Salmonella typhimurium* ribose and galactose receptors have been examined. Studies involving circular dichroism, fluorescence, absorption spectroscopy, and sedimentation analysis show that the two receptor proteins have different morphologies and exhibit diverse responses to sugar binding. The ribose receptor lacks both tryptophan and disulfide residues, and the galactose receptor lacks disulfides and has only a single tryptophan residue. By virtue of these fortuitous properties, the conformational changes induced in these proteins by sugar binding can be dissected by utilizing a variety of physical probes. A ligand-induced conformational change in the ribose receptor is shown by circular dichroism and fluorescence spectroscopy, which reveal spectral changes assignable to tyrosine, phenylalanine, and methionine residues. A conformational change in the galactose receptor has been demonstrated by fluorescence spectroscopy involving the distant reporter group method, which shows changes assignable to tryptophan and methionine sites and which is corroborated by sedimentation analysis. It is clear that there are extensive conformational changes in the two receptor proteins and that the different physical methods provide complementary information on the nature of these changes.

Physical probes have been widely used as tests of conformational change in protein molecules (Konev, 1967; Yu, 1977; James, 1978; Stryer, 1978). Upon ligand binding to a protein site, a signal associated with the probe may change in response either to direct interactions or to delocalized refolding of the macromolecule. Alternatively, physical properties of a reporter group may remain unchanged in reactions in which a ligand-induced conformational change does occur.

Physical probes of conformation change provide a dynamic picture of protein interactions not possible from X-ray crystallographic analysis. Such probes are of particular interest

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in the case of receptor proteins which may be present in exceedingly small quantities and therefore require sensitive methods of study. Thus, the calibration of a variety of physical probes for a particular receptor system may elucidate in a comprehensive way the molecular events associated with ligand–protein and protein–protein interactions.

The systems chosen here for such a calibration are the *Salmonella typhimurium* galactose and ribose receptors involved in bacterial sensing and transport. These chemosensory proteins are well suited for this study in several ways. First, the ligand-activated receptors have been shown to interact with a common macromolecule involved in sensory signaling. This signaling component has been tentatively identified as the *trg* gene product in both *Escherichia coli* (Ordal & Adler, 1974) and *Salmonella* (Strange & Koshland, 1976). Thus, the receptors must have in common some region of three-dimensional structure. Second, both receptors are water-soluble, periplasmic proteins of 30 000 daltons (Aksamit & Koshland, 1972; Anraku, 1968; Zukin et al., 1977b), yet preliminary studies indicate that there is little homology in their amino acid sequences (Hogg et al., 1977). Thus, it is likely that the ligand-activated receptors have regions of similar three-dimensional structure which enable interaction with the same signaling site. Third, evidence for a ligand-induced delocalized conformational change in the galactose receptor was presented by Boos and co-workers (Boos et al., 1972; McGowan et al., 1974) and was demonstrated by the distant reporter group technique (Zukin et al., 1977a). Spectroscopic evidence presented here indicates that the ribose receptor also undergoes molecular rearrangements when ligand binds. Finally, the ribose receptor lacks both tryptophan and disulfide residues and the galactose receptor lacks disulfides and contains only a single tryptophan residue (Zukin et al., 1977b). This situation greatly simplifies the analysis of the CD[1] spectra and the assignment of both fluorescence and CD spectral features to specific amino acid residues. We have therefore examined a number of physical properties in these two receptors and observed spectroscopic manifestations of similarities and differences in structure and conformational dynamics.

Methods

Ribose binding protein and galactose binding protein were purified from *S. typhimurium* strain ST1 as previously described (Aksamit & Koshland, 1972; Zukin et al., 1977b). Protein concentration was determined by the method of Lowry (Lowry et al., 1951); sugar binding activity was determined in each case by the nitrocellulose filter assay (Lever, 1972), using radiolabeled sugars. [^{14}C]Ribose (55 mCi/mmol) and [^{14}C]galactose (52 mCi/mmol) used in these assays were obtained from Amersham/Searle.

Labeling of the *Salmonella* galactose receptor has been described previously (Zukin et al., 1977a); the ribose receptor was labeled by the same method. The dye 5-iodoacetamidofluorescein was obtained from Dr. Richard Haugland (Hamline University, St. Paul, Mn). The labeling ratio was determined from the protein concentration and the absorbance of the attached dye at 490 nm. An extinction coefficient of 42 000 at 490 nm was used for the attached label (Zukin et al., 1977a), and a correction for light scattering was made. Methionine sulfone derivatives were prepared by using performic acid reagent according to the method of Hirs (1967). The amino acid compositions of the native and 5-AF-labeled proteins and their methionine sulfone derivatives were determined with a Beckman Model 120C amino acid analyzer.

Steady-state fluorescence measurements were performed on a Hitachi Perkin-Elmer MPF 2A fluorescence spectrophotometer at ambient room temperature.

Circular dichroism was measured at room temperature with a signal-averaging spectropolarimeter which has been previously described (Sutherland et al., 1974). Between 26 and 40 scans were averaged for each spectrum. In the case of near-UV CD spectra, measurements were made in small-volume fluorescence cuvettes of 1-cm path length and were corrected for the optical activity of the cuvette. A path length of 0.5 mm in strain-free quartz cuvettes was used for far-UV CD spectra. Sample absorbances were generally below 1.0 over the range of recording. A constant slit width of 0.8 mm for far-UV spectra and 0.4 mm for near-UV spectra with a scan speed of 0.5 mm/s was used to provide a system resolution of $\sim$1.5 nm in all spectra. Spectra are reported as the differential molar extinction coefficient for left minus right circularly polarized light per mole of protein. Absorption spectra were recorded on a Cary 118 spectrophotometer. Sedimentation coefficients were determined by sedimentation equilibrium and sedimentation velocity analysis at 20 °C with a Beckman Model E analytical ultracentrifuge. Samples of the protein were dialyzed extensively against physiological saline buffer, pH 6.0, at 4 °C for 72 h.

Results

Circular Dichroism Studies of the Salmonella Ribose and Galactose Receptors. The far-UV CD spectrum of the ribose binding protein from *S. typhimurium* is shown in Figure 1a. The spectrum has a negative maximum from 205 to 216 nm. The secondary structure of the purified ribose receptor was estimated by the method of Greenfield & Fasman (1969) to be 50% α helix, 30% β sheet, and 20% random coil. Addition of ribose to ribose binding protein at levels that saturate the binding did not result in any detectable change in the far-UV CD spectrum of this protein.

Figure 1b shows the far-UV CD spectrum of *Salmonella* galactose binding protein. One negative maximum of 216 nm and a shoulder at 205 nm were observed. The secondary structure of the purified galactose receptor was estimated to be 35% α helix, 25% β sheet, and 40% random coil. These values may be compared with those which we calculated by using this same method from the CD spectrum previously published for the *E. coli* galactose binding protein (Boos et al., 1972): 20% α helix, 50% β sheet, and 30% random coil.

Secondary structures predicted here for the *Salmonella* galactose receptor are strikingly similar to that determined by crystallographic analysis for the *E. coli* arabinose receptor protein [35% α and 20% β (Quiocho et al., 1977)]. This finding supports the hypothesis that many of the periplasmic receptors share common features of gross morphology. Immunological cross-reactivity had previously been shown between the *E. coli* arabinose and galactose receptor (Parsons & Hogg, 1974) and also between the galactose receptors of *E. coli* and *Salmonella* (Zukin et al., 1977b). These immunological results suggest antigenic homology between the *E. coli* arabinose protein and the *Salmonella* galactose protein. Regions of similarity would therefore be expected in their secondary or tertiary structure, and this is what is found. The periplasmic binding proteins, which include the ribose and sulfate binding proteins, have all been shown to be elongated ellipsoids of axial ratios in the range 2:1–4:1 and to have molecular weights of $\sim$30 000 (Pardee, 1968; Aksamit & Koshland, 1972; Oxender, 1972; Phillips et al., 1976; Quiocho et al., 1977; Zukin et al., 1977b).

[1] Abbreviations used: CD, circular dichroism; 5-AF, 5-acetamidofluorescein.

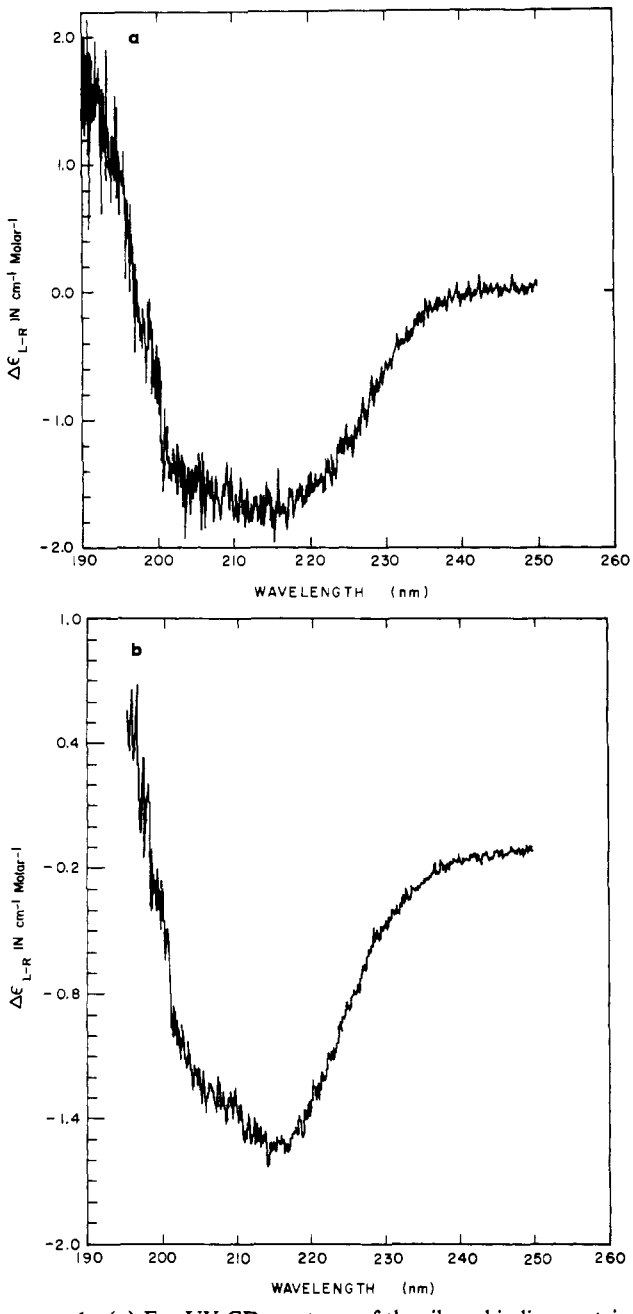

FIGURE 1: (a) Far-UV CD spectrum of the ribose binding protein. Protein concentration was 2 μM in 150 mM NaCl and 10 mM sodium phosphate, pH 6.0. (b) Far-UV CD spectrum of the galactose binding protein. Protein concentration was 3.3 μM in 150 mM NaCl and 10 mM sodium phosphate, pH 6.0.

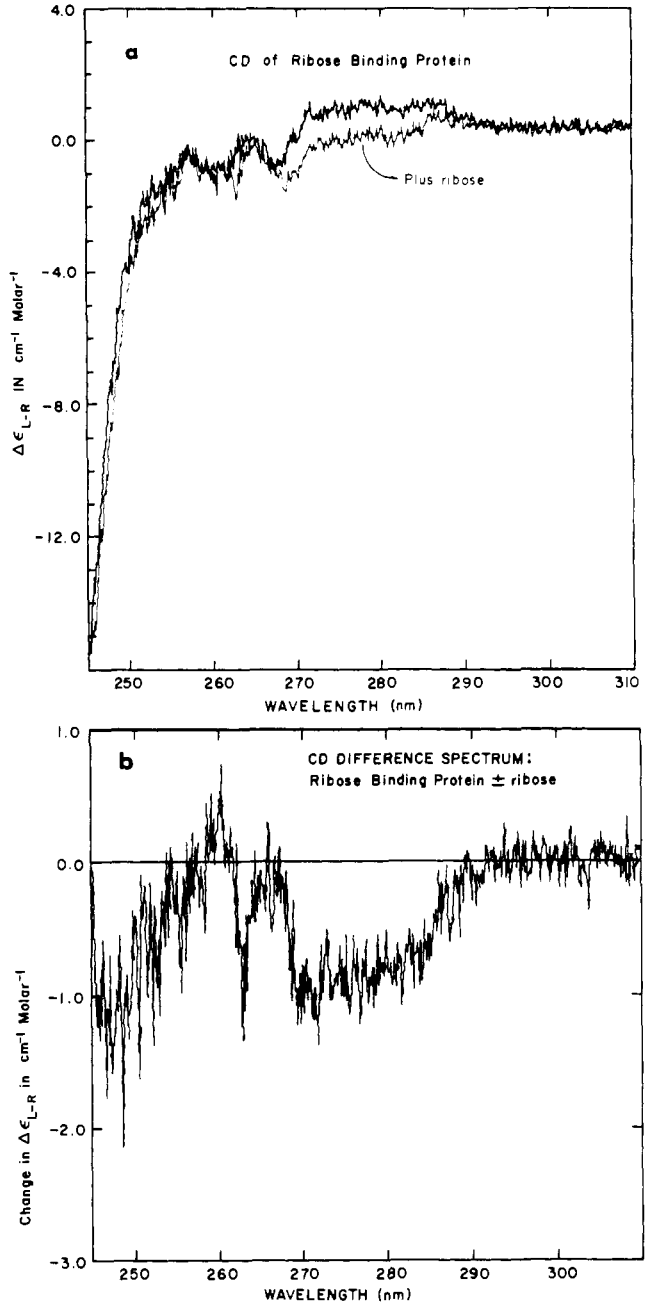

FIGURE 2: (a) Near-UV CD spectrum of the ribose binding protein in the presence and absence of 0.5 mM ribose. Protein concentration was 20 μM in 150 mM NaCl and 10 mM sodium phosphate, pH 6.0. (b) Near-UV CD difference spectrum for the addition of 0.5 mM ribose to the ribose binding protein. The spectrum for ribose binding protein without ribose in Figure 3 was substracted from the spectrum with added ribose.

The near-UV CD spectrum of *Salmonella* ribose binding protein (Figure 2a) exhibits negative maxima at 261 and 268 nm. The spectrum between 270 and 290 nm can be assigned to the three tyrosyl residues of this protein because neither tryptophan nor cysteine is present in *Salmonella* ribose binding protein (Aksamit & Koshland, 1972). The region between 257 and 268 nm exhibits sharp fine structure which is uniquely characteristic of phenylalanine (Strickland et al., 1970). Sharp phenylalanine transitions near 262 and 268 nm have been frequently observed in proteins (Strickland, 1974) and apparently represent vibronic components of a mixed π–π^* transition of the aromatic chromophore (Goodman et al., 1968). In the ribose receptor, these two phenylalanine vibronic components appear as weak bands of the same sign (negative) as expected for a single vibronic progression (Strickland et al., 1970).

The difference spectrum obtained by subtracting the CD spectrum of the ribose receptor in the presence of 5×10^{-4} M ligand from that of the protein alone (Figure 2b) shows a broad negative peak from 270 to 290 nm and a sharp negative peak at 262 nm. A similar sharp 268-nm phenylalanine peak decrease may be present but unresolved at the edge of the broad tyrosine change. These results demonstrate a change in the microenvironment of one or more of the three tyrosines and also one or more of the seven phenylalanine residues upon ribose binding. Addition of the nonbinding sugar sucrose at a 10-fold higher concentration resulted in no detectable spectral changes.

A detailed interpretation of the CD spectral change of phenylalanine and tyrosine residues in the ribose receptor is

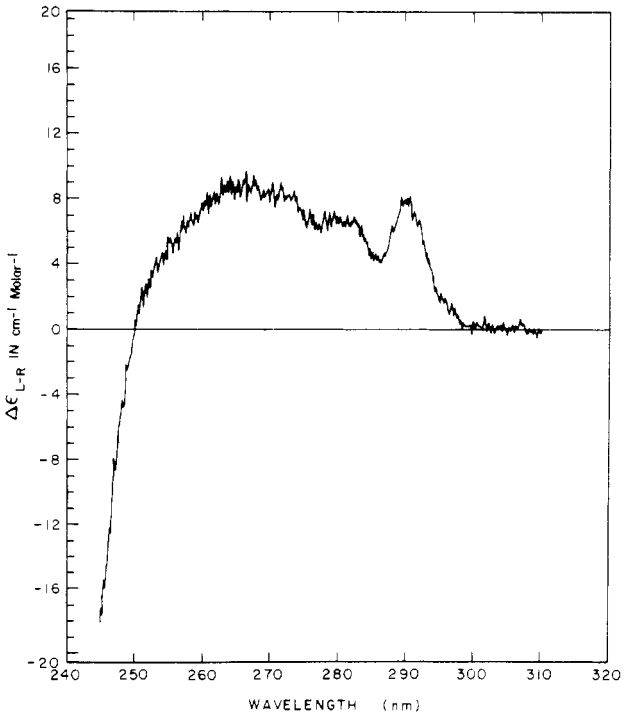

FIGURE 3: Near-UV CD spectrum of the galactose binding protein. Protein concentration was 10 μM in 150 mM NaCl and 10 mM sodium phosphate, pH 6.0.

difficult. Since CD spectra of amino acid residues in proteins are largely attributable to local environmental asymmetries (Adler et al., 1973), it might be expected that a CD decrease arises from a decreased local asymmetry. Alternatively, in analogy to peroxidase C, the ribose receptor may undergo changes upon binding of ribose which alter the distribution of conformations in the vicinity of the phenylalanine residues as well as change the asymmetry. Moreover, the phenylalanine CD band at 262 nm is sensitive to solvent interaction and is slightly more prominent in nonpolar environments (Simmons et al., 1969). Thus, the increase in the phenylalanine CD on ribose binding may arise in part from an increased hydrophobicity in its environment.

The near-UV CD spectrum of the *Salmonella* galactose binding protein is shown in Figure 3. This spectrum is radically different from that of the ribose receptor and exhibits a broad positive peak from 250 to 300 nm, a shoulder at 280 nm, and a sharp peak at 290 nm. The peak at 290 nm is readily assignable to the single tryptophan of this protein and is due to the $0 - 0\ ^1L_b$ band of tryptophan (Strickland et al., 1969; Strickland, 1974). On the basis of this prominent transition at 290 nm and a $0 + 850$ cm^{-1} ^1L_b transition at 283 nm, we can assign the CD of the single tryptophan in the galactose receptor as a type 1 spectrum, in the nomenclature of Strickland et al. (1969). The broad positive peak centered at 268 nm most likely contains contributions from both tyrosine and tryptophan residues in this protein. The galactose receptor contains no cysteine or cystine residues, so no disulfide spectral contributions are present. Addition of saturating galactose results in no detectable CD spectral changes.

Fluorescence Studies of the Receptor Proteins. For investigation of the dynamics of the binding interaction between the sugar receptors and their respective chemoeffectors, the purified proteins were labeled with the dye 5-iodoacetamidofluorescein. This dye is a new sulfhydryl reagent with a number of special properties which make it useful as a biological probe. Covalent modification of the galactose receptor with the fluorophore has been described (Zukin et al., 1977a). Reaction of the protein with a 1000-fold excess of the dye for 24 h results in the labeling of a single methionine and of no other residues of the galactose protein. Reaction of the ribose receptor with dye under the same conditions also results in covalent binding of acetamidofluorescein to this protein, as shown by the comigration of fluorescence at 520 nm with the single protein band during sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The labeling reaction proceeded to a fixed value of 1.1 mol of dye per mol of protein, as determined from the measured absorbance at 490 nm of the protein in 8 M urea using the molar extinction coefficient of the free dye in urea. This result strongly suggests labeling of the ribose receptor at a single site, although peptide mapping experiments would more definitively resolve this issue. Amino acid analyses of the protein before and after modification established that a single methionine residue and no other residues were modified by this reagent (Table I). Modification of one methionine was shown both by comparison of the methionine content of the ribose receptor and 5-AF receptor and more rigorously by comparison of the methionine sulfone content of the performate-treated proteins using the method of Hirs (1967). 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 at elution volumes identified with ϵ-(carboxymethyl)lysine or 1- or 3-(carboxymethyl)histidine (Hirs, 1967). The ribose binding protein from *Salmonella* has no sulfhydryl residue (Aksamit & Koshland, 1972). Both the galactose and ribose receptors retained full sugar binding activity when modified with acetamidofluorescein. Since the fluorescein residue is a large bulky molecule, this result provides presumptive evidence that the site of modification is distant from the sugar binding site in the case of both proteins.

Table I: Amino Acid Analyses of the *S. typhimurium* Ribose Receptor and Its 5-Acetamidofluorescein-Labeled Analogue

amino acid	ribose binding protein	5-AF-ribose binding protein
lysine	26.0	26.0
histidine	3.0	3.0
aspartate	35.6	35.0
cysteine	0	0
methionine	3.9	3.0
(carboxymethyl)lysine	0	0
(carboxymethyl)histidine	0	0
methionine sulfone[a]	4.1	2.9

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

Binding of galactose to the 5-AF-labeled galactose receptor results in a 10% quenching of the fluorescence intensity and a 4-nm blue shift in the fluorescence emission spectrum (Figure 4a). By comparison with model studies (Konev, 1967), this result indicates movement of the fluorescein to a more hydrophilic environment upon ligand binding. There are no tryptophan residues in the ribose receptor protein of *S. typhimurium*. Attachment of the reporter group 5-AF has permitted examination of localized refolding of the polypeptide chain in this ligand–receptor complex. Binding of saturating ribose to the 5-acetamidofluorescein-modified ribose receptor results in a 14% increase in fluorescence intensity and a 4-nm blue shift in the emission spectrum of the dye (Figure 4b). Studies with 5-IAF in solution (Hartig et al., 1977) suggest that this increase in fluorescence intensity is indicative of transfer of the fluorescein to a more hydrophobic environment when ribose binds to the ribose receptor.

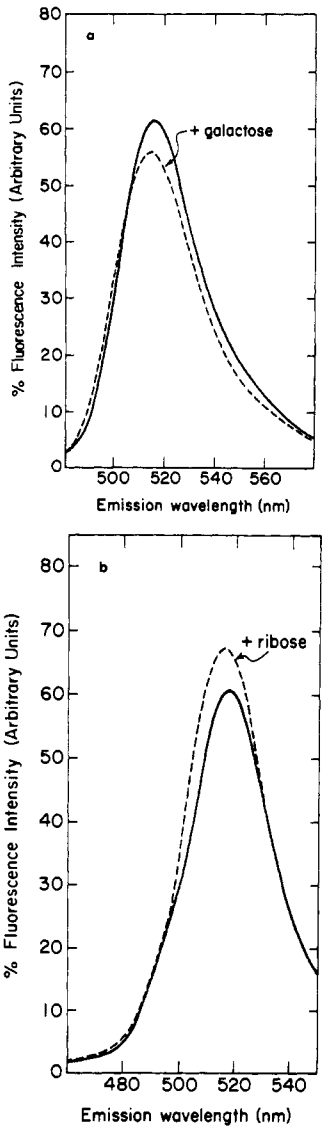

FIGURE 4: Fluorescence emission spectra (uncorrected) of (a) the 5-AF-modified galactose receptor from *Salmonella* in the presence and absence of 10^{-4} M galactose and (b) the 5-AF-modified ribose receptor in the presence and absence of 10^{-4} M ribose. Spectra were recorded at ambient temperature with an excitation wavelength of 460 nm. Receptor proteins (10 μg/mL) were in 10 mM sodium phosphate buffer (pH 7.0) and 150 mM NaCl.

Absorption Spectroscopy Studies. Information concerning conformers of the ribose and galactose receptors of *Salmonella* was also sought by absorption spectrometry and by sedimentation analysis. Addition of saturating ribose to the ribose receptor does not result in any detectable change in the absorption spectrum of this protein, a result which suggests that the sugar does not bind directly to either phenylalanyl or tyrosyl residues. In addition, binding of saturating galactose to its receptor results in no detectable difference in the protein absorption spectrum. The absence of any change in the tryptophan absorption in the case of the galactose receptor may be significant in light of other reports on the corresponding *E. coli* receptor in which the interaction of a sugar or other carbohydrate with a tryptophan residue at the receptor site was shown to result in specific changes in the tryptophan absorption spectrum (McGowan et al., 1974). It is possible that the relationship of the tryptophan residue to the sugar binding site is significantly different in these two bacteria.

Sedimentation Analyses. Sedimentation velocity analyses of the *Salmonella* galactose and ribose receptors performed in an analytical ultracentrifuge afforded values of $s_{20,w}$ = 3.1 and 2.7 S, respectively. Visualization of a single component for each receptor protein provided further evidence that the protein samples were homogeneous ones. Addition of saturating galactose (10^{-4} M) to the galactose protein resulted in no detectable change in the $s_{20,w}$ value; addition of 10^{-4} M ribose to the ribose receptor resulted in a 4.5% decrease of $s_{20,w}$ to 2.6 S. No such change was observed upon addition of nonbinding sugar at 10^{-4} M to the ribose receptor. Errors of SEM = ±0.5% in these values have been calculated. The change in the case of the ribose protein is small but may indicate that this receptor undergoes a ligand-induced conformational change to a slightly more compact or spherical conformation. Boos et al. (1972) reported that 10^{-4} M galactose or glucose produces an increase in the electrophoretic mobility of the galactose binding protein during polyacrylamide gel electrophoresis. This finding is consistent with a refolding of this receptor also to a more spherical conformation in the presence of binding sugars.

Table II: Effect of Chemoeffector on Structure Indicators Intrinsic to or Experimentally Attached to Receptor Molecules

property measd	changes in properties on binding of chemoeffector: ribose receptor	galactose receptor
far-UV CD	none	none
near-UV CD	decrease	none
absorption spectra	none	none
intrinsic Trp fluorescence	*a*	increase
added fluorescent reporter group on Met	increase	decrease
fluorescence quenching by KI of Trp of reporter group	*a* *b*	increase
fluorescence lifetimes of Trp of reporter group	*a* decrease	decrease decrease
sedimentation coeff	decrease	none

[a] Molecule contains no tryptophan residues. [b] Not measured.

Frictional coefficients can be determined for the two proteins from these values and the molecular weights, determined independently by sodium dodecyl sulfate gel electrophoresis and corroborated by sedimentation equilibrium analyses. A frictional coefficient ratio (f/f_0) of 1.1 corresponding to the case of no hydration sphere was calculated for the galactose receptor; this value is consistent with an axial ratio of 2–4:1, assuming a prolate ellipsoid shape in analogy to other known periplasmic binding proteins such as the *E. coli* sulfate and arabinose binding proteins (Pardee, 1968; Phillips et al., 1976). An axial ratio of 4:1 was reported for the sulfate binding protein (Pardee, 1968) and 2:1 for the arabinose binding protein (Phillips et al., 1976). In the case of the *Salmonella* ribose receptor, our data show a frictional coefficient ratio of 1.2 or an axial ratio of 2–4:1.

Discussion

The mechanism of ligand activation of the *Salmonella* ribose and galactose receptors in sensory signaling has been examined by various biophysical approaches. Both intrinsic and experimentally attached reporter groups have been used. A comparison of these findings is summarized in Table II. In the case of the ribose receptor, chemoeffector binding produces distinctive changes in the near-UV CD spectrum assignable to both phenylalanyl and tyrosyl residues. Spectral changes are also observed in the fluorescent dye, 5-iodoacetamidofluorescein, attached to a single methionine residue. It is highly unlikely that these various residues are all concentrated at the single substrate binding site (Aksamit & Koshland, 1972).

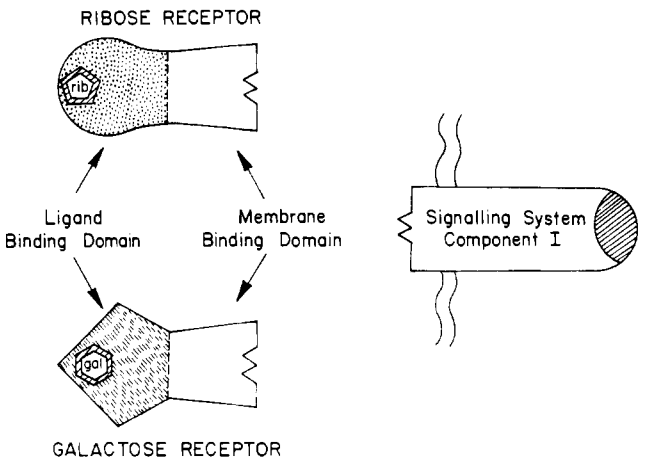

FIGURE 5: Hypothetical model for the structures of the ribose and galactose binding proteins. Both receptors contain a region of similar structure which contains the combining sites for a membrane-bound signaling component. A sugar binding region containing the attractant binding site is specific in structure for each receptor.

Therefore, a delocalized conformational change occurs in the ribose receptor. In the case of the galactose receptor, the distant reporter group technique (Zukin et al., 1977a) revealed changes upon sugar binding in the fluorescence of a single tryptophan residue and of a distant fluorescein dye also attached through a methionine moiety. This method demonstrated a ligand-induced conformational change that is propagated a minimum of 30 Å through the receptor molecule.

Several pieces of data independently corroborate the finding of an extensive conformational change in both of these receptors. First, modification of a methionyl residue with a large bulky fluorescein results in no detectable loss of biological activity in either receptor system. If sugar binding involved direct interaction with this amino acid, a substantial loss of activity would probably be associated with the chemical modification. Second, in the case of the ribose receptor, the small decrease observed in the sedimentation coefficient upon binding of ribose is suggestive of a shape change in this protein. Third, in the case of the galactose receptor, various other approaches including fluorescence quenching in the case of the *Salmonella* protein (Zukin et al., 1977a) and absorption spectroscopy and gel electrophoresis in the case of the *E. coli* protein (Boos et al., 1972) provided further evidence for ligand-induced molecular rearrangements.

The finding that ligand binding to the *Salmonella* receptors does not produce changes detectable by far-UV CD or absorption spectroscopy, or (in the case of the galactose protein) sedimentation analysis, can be explained in several ways. First, far-UV CD and sedimentation analysis generally detect gross morphological changes rather than more localized perturbations in the microenvironment of specific residues. Second, absorption spectrometry is generally less sensitive to changes in polarity in the environment of the probe than is fluorescence. Third, ligand-induced protein refolding may in many cases result in the exchange of like groups.

The calibration of various physical probes in the case of the *Salmonella* galactose and ribose receptors has provided a detailed molecular picture of a biological process. Because these are water-soluble, periplasmic proteins, the in vitro finding provides a good indication of receptor mechanism in vivo. Thus, the initial signaling event during galactose or ribose chemoreception has been identified with a delocalized conformational change. The initial event of stimulus transduction during sensory perception in many other microbial or neural systems is that of receptor activation. The evidence that this activation involves protein refolding, however, has usually been circumstantial.

Bacterial chemotaxis, a complete behavioral system contained within a single cell, provides a sensory system that is particularly amenable to a detailed molecular study. In this system sensory information is transmitted from the receptors to a central processing unit through focused pathways. Focusing provides (1) economy of processing components and (2) extensive feedback regulation. One mechanism of receptor focusing is a competition between ligand-activated receptor proteins for a common site (Strange & Koshland, 1976). Likely candidates for this site include the signaling component proteins which have been identified in the case of several classes of chemoreceptors (Springer et al., 1977; Silverman & Simon, 1977; Kondoh et al., 1979). Activated bacterial receptors may bind either to the signaling proteins directly or to other protein components which then associate with the signaling apparatus. A variety of biochemical events follow, including methylation (Kort et al., 1975) and demethylation (Stock & Koshland, 1978) of the signaling proteins.

A schematic representation of the *Salmonella* ribose and galactose receptors is shown in Figure 5. This hypothetical model shows the elongated shape of the receptors as demonstrated by ultracentrifugation analyses. It also shows the division of the protein into two domains as shown for the *E. coli* arabinose receptor (Quiocho et al., 1977). One of these domains could function as the ligand binding site and the other as a combining site for the *trg* signaling component. Clearly, the fractional α and β structures predicted by the CD data are consistent with common regions in the two receptors which could serve as the membrane binding site.

The receptor competition hypothesis of sensory signaling predicted that portions of the receptor molecules adopt similar three-dimensional structure upon ligand binding (Strange & Koshland, 1976). In this study, a variety of physical probes have been used to elucidate the molecular details of this ligand activation reaction which serves as the first step in chemosensory signaling.

References

Adler, A. J., Greenfield, N. J., & Fasman, G. D. (1973) *Methods Enzymol. 27*, 675–735.

Aksamit, R., & Koshland, D. E., Jr. (1972) *Biochem. Biophys. Res. Commun. 48*, 1348–1353.

Anraku, Y. (1968) *J. Biol. Chem. 243*, 3123–3127.

Boos, W., Gordon, A. S., Hall, R. E., & Price, H. D. (1972) *J. Biol. Chem. 247*, 917–924.

Goodman, M., Davis, G. W., & Benedetti, E. (1968) *Acc. Chem. Res. 1*, 275–281.

Greenfield, N. J., & Fasman, G. D. (1969) *Biochemistry 8*, 4108–4116.

Hartig, P. R., Bertrand, N. J., & Sauer, K. (1977) *Biochemistry 16*, 4275.

Hirs, C. H. W. (1967) *Methods Enzymol. 11*, 59–62.

Hogg, R. W., Isihara, H., Hermondson, M. A., Koshland, D. E., Jr., Jacobs, J. W., & Bradshaw, R. A. (1977) *FEBS Lett. 80*, 377–379.

James, T. L. (1978) *Nuclear Magnetic Resonance in Biochemistry: Principles and Applications*, Academic Press, New York.

Kondoh, H., Ball, C. B., & Adler, J. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 260–264.

Konev, S. (1967) *Fluorescence and Phosphorescence of Proteins and Nucleic Acids*, Plenum Press, New York.

Kort, E. N., Goy, M. F., Larsen, S. H., & Adler, J. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 3939–3943.

Lever, J. E. (1972) *Anal. Biochem. 50*, 73–83.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.
McGowan, E. B., Silhavy, T. J., & Boos, W. (1974) *Biochemistry 13*, 993–999.
Ordal, G. W., & Adler, J. (1974) *J. Bacteriol. 117*, 517–526.
Oxender, D. L. (1972) *Annu. Rev. Biochem. 41*, 777–814.
Pardee, A. B. (1968) *Science 162*, 632–637.
Parsons, R. G., & Hogg, R. W. (1974) *J. Biol. Chem. 249*, 3608–3614.
Phillips, G. N., Jr., Mahajon, V. K., Siu, A. K. Q., & Quiocho, F. A. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 2186–2190.
Quiocho, F. A., Gilliland, G. L., & Phillips, G. N., Jr. (1977) *J. Biol. Chem. 252*, 5142–5149.
Silverman, M., & Simon, M. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 3317–3321.
Simmons, N. S., Barel, A. O., & Glazer, A. N. (1969) *Biopolymers 7*, 275–279.
Springer, M. S., Goy, M. F., & Adler, J. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 3312–3316.
Stock, J. B., & Koshland, D. E., Jr. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 3659–3663.
Strange, P. G., & Koshland, D. E., Jr. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 762–766.
Strickland, E. H. (1974) *CRC Crit. Rev. Biochem. 2*, 113–175.
Strickland, E. H., Horwitz, J., & Billups, C. (1969) *Biochemistry 8*, 3205–3213.
Strickland, E. H., Kay, E., & Shannon, L. M. (1970) *J. Biol. Chem. 245*, 1233–1238.
Stryer, L. (1978) *Annu. Rev. Biochem. 47*, 819–846.
Sutherland, J. C., Vickery, L. E., & Klein, M. P. (1974) *Rev. Sci. Instrum. 45*, 1089–1094.
Yu, N. T. (1977) *CRC Crit. Rev. Biochem. 4*, 229–280.
Zukin, R. S., Hartig, P. R., & Koshland, D. E., Jr. (1977a) *Proc. Natl. Acad. Sci. U.S.A. 74*, 1932–1963.
Zukin, R. S., Strange, P. G., Heavey, L. R., & Koshland, D. E., Jr. (1977b) *Biochemistry 16*, 381–386.

Structure of *Escherichia coli* Membranes. Phospholipid Conformation in Model Membranes and Cells As Studied by Deuterium Magnetic Resonance†

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ABSTRACT: The dynamic conformation of phospholipid molecules in membranes of *Escherichia coli* has been investigated by means of deuterium magnetic resonance. *E. coli* strains which were deficient in the synthesis of cardiolipin were grown in the presence of selectively deuterated elaidic acid, oleic acid, and palmitic acid. A total of 50–85% of the natural fatty acids were replaced by the deuterium-labeled analogues, and well-resolved deuterium magnetic resonance spectra of intact *E. coli* cells could be obtained in less than 1-h measuring time. The spectra reveal a striking similarity between the phospholipid conformation in a biological membrane and that of phospholipid model membranes. If the deuterium label is attached at the C-2 segment of the fatty acyl chains or at the cis double bond, the deuterium magnetic resonance spectra are rather unique and can be considered as spectral fingerprints of the phospholipid conformation in the fluid membrane. Almost identical fingerprints are observed for native *E. coli* membranes, for liposomes formed from extracted *E. coli* lipids, and for synthetic phospholipids. The phospholipid conformation in the fluid membrane as derived from these spectral patterns is closely related to the structural model suggested for phospholipids in the gel phase and in the crystalline state. The orientational fluctuations of the fatty acyl chain segments in the membrane are quantitatively described by the deuterium order parameters. A detailed order profile has been established for *E. coli* membranes by incorporating palmitic acid and oleic acid selectively deuterated at altogether 10 different carbon atoms. The shape of the curve drawn through deuterium order parameters of intact *E. coli* cells closely resembles that of synthetic 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine. It can be concluded that the order profiles characteristic of saturated and cis unsaturated fatty acyl chains are qualitatively not altered by the presence of membrane proteins. Due to instrumental limitations, lipids in the gel state or lipids tightly bound to membranous proteins cannot be resolved in the present experiments.

Studies in the past 10 years have lead to the consensus view that a fluid bilayer of phospholipids forms the basic two-dimensional matrix of most biological membranes into which and around which various proteins are situated. A more detailed molecular description of phospholipid arrangement in biological membranes requires knowledge about the conformation of both the polar and apolar parts of phospholipids in the bulk lipid phase and at the sites of interaction of lipids with intrinsic and peripheral proteins. The purpose of this study is to characterize the hydrocarbon chain conformation of phospholipids in a biological membrane and to establish similarities and differences between the native membrane and protein-free phospholipid bilayers. As a representative membrane system we have chosen *Escherichia coli* in which the hydrocarbon chain composition of the phospholipids can be manipulated by addition of fatty acids to the growth medium

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