Sites of Covalent Modification in Trg, a Sensory Transducer of *Escherichia coli*

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The Trg protein mediates chemotactic response of *Escherichia coli* to the attractants ribose and galactose. Like other transducers, Trg is a transmembrane protein that undergoes post-translational covalent modification. The modifications are hydrolysis (deamidation) of certain glutamine side chains to create glutamate residues and methylation of specific glutamates to form carboxyl methyl esters. Analysis of radiolabeled, tryptic peptides by high performance liquid chromatography and gas-phase sequencing allowed direct identification of the modified residues of Trg. The protein has 5 methyl-accepting residues. Four, at positions 304, 310, 311, and 318, are contained in a 23-residue tryptic peptide ending in lysine. The fifth, at position 500, is within a 25-residue tryptic peptide ending in arginine. At two sites, 311 and 318, glutamates are deamidated to create methyl-accepting glutamates. There is not a required order of modification among the sites. However, there is a substantial preference for methylation on the arginine peptide and, among sites on the lysine peptide, for the middle pair. Comparison of sequences surrounding modified residues identified in this work for Trg and previously for Tsr and Tar suggests a consensus sequence for methyl-accepting sites of Ala/Ser-Xaa-Xaa-Glu-Glu*-Xaa-Ala/OH-Ala-OH/Ala, where OH signifies Ser or Thr and the asterisk marks the site of modification.

Chemotaxis is a behavioral response of an organism to changes in the chemical environment. The chemotactic behavior of the closely related bacteria *Escherichia coli* and *Salmonella typhimurium* has been investigated extensively (see articles in Eisenbach and Balaban, 1985). In the absence of a chemical gradient, these organisms move through their environment in a random walk, consisting of smooth swimming (corresponding to counter-clockwise rotation of flagella) punctuated by uncoordinated tumbles (corresponding to clockwise rotation) that result in random reorientation of the direction of movement. An increase in attractant concentration or decrease in repellent concentration suppresses tumbles, while either a decrease in attractant or an increase in repellent concentration enhances the tumble frequency. The result of this behavior is net progress of the bacterium up a concentration gradient of attractant or down a gradient of repellent.

There are four chemotactic transducers present in *Escherichia coli*, Tsr, Tar, Trg, and Tap (see Simon et al., 1985 for a recent review). These integral membrane proteins each bind specific ligands. Trg mediates tactic response to galactose and ribose by recognition of ligand-occupied galactose- and ribose-binding proteins, respectively. Changes in occupancy of ligand-binding sites initiate an excitatory signal, resulting in an alteration of the rotational bias of the flagellar motor. An adaptational process reestablishes rotational bias and thus the pattern of swimming to the pre-stimulus state. Adaptation to a positive stimulus is linked to methylation of specific glutaryl residues by a cytoplasmic methyltransferase which is encoded by the *cheR* gene. Adaptation to a negative stimulus is characterized by demethylation of these esters, as well as hydrolysis of specific glutaminyl residues (deamidation) and is mediated by a *cheB*-encoded methylase. Transducers contain multiple sites for methylation (Boyd and Simon, 1980; Chelsky and Dahlquist, 1980; DeFranco and Koshland, 1980; Engström and Hazelbauer, 1980) and deamidation (Rollins and Dahlquist, 1981; Sherris and Parkinson, 1983; Harayama et al., 1982). These sites have been characterized in detail for Tsr and Tar (Kehry and Dahlquist, 1982a, 1982b; Kehry et al., 1983a; Terwilliger and Koshland, 1984; Terwilliger et al., 1986a, 1986b). A preliminary study of covalent modification of Trg (Kehry et al., 1983b) indicated that the nature of the tryptic peptides containing sites of modification was similar to those derived from Tsr and Tar. However, the patterns of [3H]methyl-labeled tryptic peptides derived from Trg was considerably more complex than the corresponding peptide maps of Tsr or Tar. In this report, we present a detailed analysis and identification of the sites of covalent modification in the Trg protein.

EXPERIMENTAL PROCEDURES

Bacterial Strains—All strains used are derivatives of *E. coli* K12. CP362 (Park and Hazelbauer, 1966a) is a derivative of OW1 that contains Δtar-7286, Δ(tar-tap)5201, and Δtgr-100. The miniEII-producing strains TH1219 (tsr-49, Δ(tar-tap)5201), TH1326 (Δ(tap-cheB)2241), and TH1370 (Δ(cheA-cher)2231) have been described (Harayama et al., 1982). The trg-containing plasmid, pMC2, was constructed by introducing the 2.2-kilobase 

* HindII fragment containing *trg* into pUC13 that had been cleaved with BamHI and *Smal.

Chemicals—L-[methyl-3H]Methionine (12 and 80 Ci/mmol), [3S]methionine (1000 Ci/mmol), and L-[3H]arginine (15 Ci/mmol) were purchased from Du Pont-New England Nuclear. Ethanol present in the L-[methyl-3H]methionine solutions was removed under vacuum before use in labeling experiments. Iodomethane, phosphoric acid,
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and acetonitrile (HPLC grade) were obtained from Baker Chemical Co. β-D-Allose, diphenylcarbamyl chloride-treated trypsin, and N-ethylmorpholine were from Sigma. All other chemicals were reagent grade.

Preparation of Radiolabeled Trg—Labeling in vivo using L-[methyl-3H]methionine was as described (Engström and Hazeltaiuer, 1980). Cells were grown in the presence of 0.4% ribose to insure full induction of ribose-binding protein and the labeling procedure involved stimulation with 10 mM β-D-allose to induce the adaptational increase in methylation of Trg. Preparation of labeled protein for analysis of tryptic peptides by HPLC was done by adding 0.5–1 mCi of 12 Ci/mmol of L-[methyl-3H]methionine to 1.25 × 10^6 cells in 5 ml to yield a final methionine concentration of 8.5–17 μM. For labeled material with high specific activity, methionine at 26 Ci/mmol was added to yield a final concentration of 4 μM. Minicells were prepared and radiolabeled as described (Kehry et al., 1983b). Preparation of Trg protein containing a specific radiolabeled amino acid involved adding 40 μCi of [3H]arginine or 50 μCi of [35S]methionine to 50 μl of minicells, harboring a trg-containing plasmid, at an optical density equivalent to 2 × 10^6 cells/ml (95 μg of protein in 50 μl). SDS-polyacrylamide gel electrophoresis was performed in conditions optimized for resolution of the electrophoretic forms of Trg (Kehry et al., 1983b). The HPLC buffer (4.4 ml lane, approximately 40 μg of whole cell protein (1.25 × 10^6 cells) or 95 μg of minicell protein was applied. Usually gels were poured with one, very wide sample lane. Following electrophoresis, gels were stained for 10 min (0.04% Coomassie Brilliant Blue, 25% isopropl alcohol, 10% acetic acid), destained for 15 min (10% acetic acid), and, for ‘H-labeled material, treated with Amplify (Amersham Corp.), then dried at 60 °C. The positions of minicells, harboring a trg-containing plasmid, at an optical density of decreasing the probability of methyl ester hydrolysis during sequencing. However, later sequence analysis under standard conditions had improved initial yield and coupling efficiency.

RESULTS

Modifications of Trg—The pattern of covalent modifications of the Trg protein was studied by analysis of radiolabeled, tryptic peptides using HPLC. Peptides that include sites of modification on Trg are eluted from a reversed-phase column much later in a gradient of acetonitrile than most tryptic peptides of the protein and thus are well resolved in the tryptic map generated by HPLC (Kehry et al., 1983b). In the analyses reported here, the shape of the eluting gradient of acetonitrile was designed to compress the early region of the map where many peptides appear and to expand the later region in which peptides carrying modification sites are found (see “Experimental Procedures”). Thus fraction numbers and spacings between peptides do not correspond to those in the previous study (Kehry et al., 1983b). Sufficient Trg protein for analysis was obtained by using cells and minicells harboring multicopy plasmids carrying the trg gene. Methyl-accepting sites were examined using Trg protein that had acquired [3H]methyl groups in intact, viable cells. Analyses of deamidation and of effects of amino acid substitutions on properties of protein or peptides were performed using Trg protein that had incorporated radiolabeled amino acids during synthesis in intact minicells. Radiochemically pure protein was obtained by excising appropriate regions of SDS-polyacrylamide gels.

Modifications of Trg catalyzed by the CheR and CheB enzymes are reflected in alterations in the mobility of the transducer protein in SDS-polyacrylamide gel electrophoresis (Harayama et al., 1982; Kehry et al., 1983b). Trg synthesized in strains lacking both modification enzymes appears on gels as a single band with an apparent molecular weight of approximately 60,000. An active CheB enzyme results in three slower migrating forms of Trg that have undergone one or two deamidations. Active CheR and CheB enzymes combine to generate seven [3H]methyl-labeled, electrophoretic forms of Trg, the four described above and three forms that migrate more rapidly than unmodified Trg (Fig. 1). The forms are numbered in order of increasing mobility, with band 4 at the position of unmodified polypeptide; band 7 is often hardly detectable.

Identity of the K1 Peptide—A methionine- and lysine-containing tryptic peptide, termed K1, that contains two sites of modification was added to analysis of peptides from the four electrophoretic forms of Trg observed in cheR− cheB" strains (Kehry et al., 1983b).

\footnotetext[1]{The abbreviations used are: HPLC, high performance liquid chromatography; SDS, sodium dodecyl sulfate.}

FIG. 1. Multiple electrophoretic forms of the Trg protein. The figure is a fluorograph of an SDS-polyacrylamide gel, optimized for resolution of Trg, containing a sample of [3H]methyl-labeled protein from CP362, which has deletions in the chromosomal copies of ter, tar, tap, and trg, but harbors the multicopy plasmid, pMG2, that contains the wild type trg gene. Only one of the segment, including the Trg bands is shown. The bands are numbered in order of increasing electrophoretic mobility.
Fig. 2. Maps of tryptic peptides from [3H]methyl-labeled Trg protein synthesized from wild type and mutant genes. Trg was labeled with [3H]methyl during synthesis in minicells derived from a cheR cheB strain and harboring pMG2 or its derivatives carrying a mutated trg. Protein bands excised from SDS-polyacrylamide gels were digested with trypsin and the peptides separated by HPLC. The bottom trace is the complete pattern of [3S]methionine-labeled peptides derived from wild type protein. The peak corresponding to unmodified K1 peptide is labeled (K0) and arrows mark the positions of once-deamidated (K1) and twice-deamidated (K2) forms of the K1 peptide. The two insets show the regions containing a difference from the map of wild type protein in the maps of mutant proteins having alanine in place of Gln311 (middle). Shifted peaks corresponding to unmodified K1 peptide from the mutant proteins are labeled K0 and the position of the corresponding wild type peptide is indicated by an arrow.

Fig. 2, the elution positions in the modified gradients used in these studies are indicated for the unmodified (K10), once-deamidated (K11) and twice-deamidated (K12) forms of the K1 peptide. The identity of the K1 peptide was established by analyses of proteins containing specific amino acid substitutions that had been generated by oligonucleotide-directed mutagenesis of trg. Substitutions at residues 311 or 318 shifted the position of the unmodified K1 peptide in the chromatographic pattern (Fig. 2), indicating that the K1 peptide must include those residues and thus, from the deduced amino acid sequence of Trg (Bollinger et al., 1984), must be the 23-residue tryptic peptide from Thr312 through Lys331 (see below). This peptide corresponds to homologous sequences in Tar and Tar that include sites of deamidation and methylation (Kehry et al., 1983a; Terwilliger and Koshland, 1984).

[3H]Methyl-labeled Tryptic Peptides—As reported previously (Kehry et al., 1983b), analysis by HPLC reveals many peaks of radioactivity after trypsin digestion of [3H]methyl-labeled Trg. Fig. 3 provides a representative sample of many analyses. Nine different peaks were identified and were labeled T1 to T9 in order of elution. Only two peaks of radioactivity, T4 and T7, appeared in chromatographic pattern (Fig. 2), indicating that the K1 peptide is labeled KO and the position of the corresponding wild type peptide is indicated by an arrow.

The peaks do not necessarily correspond to the peaks labeled T0 to T9 in the preliminary study (Kehry et al., 1983b).

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Fig. 4. Effects of base-catalyzed demethylation on \(^{3}H\) methyl-labeled peptides. Peptides in specific radioactive peaks from tryptic maps like those shown in Fig. 3 were treated with base at elevated temperature, resulting in hydrolysis of a proportion of methyl esters on the peptides. The treated samples were analyzed in the same chromatographic conditions used to generate the original peptide maps. The arrow in each panel marks the position of the particular peak of radioactivity before treatment with base. The numbers identify peaks corresponding to those found in tryptic maps like those shown in Fig. 3. Aging of columns results in slight shifts in the elution position of the entire set of peaks and thus numbered peaks vary slightly in fraction number. Analysis of peaks T7 (A), T6 (B), and T8 (C) are shown.

Table I

| Peptide | New radiolabeled species formed | Deduced number of methyl esters |
|---------|---------------------------------|--------------------------------|
| T1      | None                            | 1                              |
| T2      | None                            | 1                              |
| T3      | None                            | 1                              |
| T4      | None                            | 1                              |
| T5      | T3                              | 2                              |
| T6      | T4                              | 2                              |
| T7      | None                            | 1                              |
| T8      | T3 and T5                       | 3                              |
| T9      | T4 and T6                       | 3                              |

Fig. 5. Effects of treatment with iodomethane on \(^{3}H\) methyl-labeled peptides. Peptides in specific radioactive peaks from tryptic maps like those shown in Fig. 3 were treated with iodomethane, resulting in formation of cationic sulfonium ions on methionines that might be present. Treated samples were analyzed in the same chromatographic conditions used to generate the original peptide maps. The arrow in each panel marks the position of the particular peak of radioactivity before treatment with iodomethane.

In the published determination of the sequence for trg (Bollinger et al., 1984), three nucleotides in the region of position 1185 were not detected. The correct sequence, determined by high resolution analysis of a region of compression in the sequencing gels and by analysis with BsaHI1 has the codon GCG after the codon CGA at position 395. This results in the insertion of alanine in the deduced amino acid sequence of Trg at position 396, and a shift by one number in each of the subsequent residues. Thus Ghu\(^{35S}\) was numbered 509 in Bollinger et al. (1984).
Dahlquist, 1982a; Terwilliger and Koshland, 1984) and thus will be termed the R1 peptide of Trg.

In the preliminary description of modifications of Trg (Kehry et al., 1983b), differences between tryptic maps of [3H]arginine-labeled Trg from band 1 and band 4 suggested the possibility of a CheB-mediated modification on one or more arginine-containing peptides of Trg. This possibility has not been supported by subsequent investigations. Extensive characterization of patterns of tryptic peptides from Trg synthesized in the presence or absence of active CheB has provided no evidence for CheB-mediated modification of R1 or of any other arginine-containing peptide eluting in the vicinity of the K1 and R1 peptides.

Analysis of Peptides by Gas-Phase Sequencing—Selected [3H]methyl-labeled peptides were analyzed using a gas phase sequenator to determine which positions, within the peptide sequence, contained radiolabeled methyl esters. Peptides were purified by HPLC using the same conditions as for analytical experiments. Pooled material from several preparations was rechromatographed using a different solvent system to insure a pure, homogenous preparation of a particular peptide form and to place the peptide in a more volatile solvent. Material released at each cycle of the sequencing procedure was analyzed for radioactivity, not for the identity of the released amino acid. The monomethylated form of the R1 peptide, present in the T7 peak was analyzed by this procedure. The two insets show the regions containing a difference from the map of wild type protein in the maps of mutant proteins having Glu310 replaced by aspartate (top) or glutamine (middle). For the insets, an arrow marks the position of the wild type, unmodified peptide, R0.

FIG. 6. Maps of tryptic peptides from [3H]arginine-labeled Trg protein synthesized from wild type and mutant genes. Trg was labeled with [3H]arginine and analyzed as described in the legend for Fig. 2. The bottom trace is the complete pattern of [3H]arginine-labeled peptides derived from wild type protein. The two insets show the regions containing a difference from the map of wild type protein in the maps of mutant proteins having Glu310 replaced by aspartate (top) or glutamine (middle). For the insets, an arrow marks the position of the wild type, unmodified peptide, R0.

The T4 peak consists of a monomethylated (Table I) methionine-containing (Fig. 5) peptide with elution characteristics consistent with identity as a monomethylated, twice-deamidated K1 peptide. Studies of amino acid substitutions within the K1 sequence provide direct evidence that the T4 peak contains forms of the K1 peptide.8 Analysis of [3H]methyl-labeled T4 material with the sequenator revealed four methyl-accepting sites, corresponding to Glu304, Glu310, Glu311, and Glu318 (Fig. 7B). This pattern implies that two sites are generated by deamidation of glutamines to produce methyl-accepting glutamates. The peptides found in the chromatographic peak T4 are all monomethylated as determined by demethylation studies (Table I). Thus the observation of four different methylated sites among the population of molecules indicates that any of the four sites can be the sole methylated site on the K1 peptide and also that the chromatographic system does not resolve monomethylated peptides modified at different sites along the sequence. The predominance of radiolabel at positions 310 and 311 implies that, for the cells used, the steady state levels of methylation are not equal for the four sites. There is a strong preference, in these in vivo conditions, for a single methylation on K1 to be at the central pair of sites.

FIG. 7. Release of radioactivity from [3H]methyl-labeled tryptic peptides in cycles of amino acid sequencing reactions. Tryptic, [3H]methyl-labeled peptides were purified by HPLC as described under "Experimental Procedures" and submitted to analysis with a gas-phase polypeptide sequenator. The quantity of radioactivity released (minus background and corrected for an estimated coupling efficiency of 92%) at each cycle of cleavage is plotted versus the residue position, labeled with the deduced amino acid sequence of the appropriate peptide (see text). Residue numbers corresponding to the entire Trg sequence are indicated. Results are shown for monomethylated, R1 peptide in peak T7 (A), monomethylated, twice-deamidated, K1 peptides in peak T4 (B), dimethylated, once-deamidated K1 peptides in peak T5 (C), and monomethylated, not-deamidated K1 peptide in peak T1 (D).
The T5 peak consists of dimethylated peptide (Table I) with elution characteristics consistent with identity as once-deamidated, dimethylated K1 peptide. Analysis with the sequenator revealed that twice-methylated K1 peptide contained methyl esters at the same sites utilized in the array of monomethylated species (Fig. 7C). It appears that the first deamidation can occur at either site and that most once-deamidated, dimethylated K1 peptides are methylated at position 310.

Sufficient radiolabeled material for sequencing analysis of the T1 peak was obtained by use of a mutant protein, Trg-21, that is essentially not deamidated, but is methylated. The protein differs from the wild-type species by a single amino acid residue that is substantially distant from both the K1 and R1 regions of the protein sequence (Park and Hazelbauer, 1986b). Tryptic digestion of [³H]methyl-labeled mutant protein yielded essentially only two radiolabeled, chromatographic species, T7, the monomethylated form of R1, and T1, apparently the monomethylated, not deamidated form of K1. Analysis with the gas phase sequenator revealed that only a single site, Glu³⁰⁴, is methylated in the peptides in the T1 peak (Fig. 7D).

### DISCUSSION

**Sites of Modification**—The data presented here identify five methyl-accepting sites in the Trg transducer protein (Fig. 8). Four sites, residues 304, 310, 311, and 318, are in the central region of the protein, included in the K1 tryptic peptide. The fifth site is near the carboxyl-terminal at amino acid 500, within the R1 tryptic peptide. The sites will be referred to as 1–5 in order of residue number. Sites 3 and 4 correspond to glutamines that must be hydrolyzed to create glutamates before functioning as methyl-accepting residues. Deamidation at these two sites corresponds precisely to the number of CheB-dependent modifications previously determined for the K1 peptide (Kehry et al., 1983b). As shown in Fig. 8, the pattern of covalent modifications that occur on Trg is quite similar to the patterns documented for Tsr (Kehry and Dahlquist, 1982a, 1982b; Kehry et al., 1983a) and Tar (Terwilliger and Koshland, 1984; Terwilliger et al., 1986a, 1986b).

The identity of the amino acids in positions near a methyl-accepting residue is substantially conserved among the 13 identified sites (five in Trg and four each in Tsr and Tar). Alanine, serine, and threonine are the only amino acids found at position 4 on the amino side of the modified residue and at positions 2, 3, and 4 on the carboxyl side. The conserved positions can be expressed as a consensus sequence of Ala/Ser-Xaa-Xaa-Glu/Glu*-Xaa-Ala/Oh-Ala/Oh/Ala, where the asterisk marks the site of modification and the symbol "OH" signifies serine or threonine. If the sequence were contained in an α-helix, then the conserved residues would form a pocket surrounding the methyl-accepting site. The only deviations from the consensus sequence are site 1 in Tar, in which the conserved alanine at carboxyl position 3 is replaced by a serine, and site 4 in Tar and site 2 in Trg, in which the conserved glutamate is replaced by a glutamine and an isoleucine, respectively. The deviating sites in Tar exhibit substantially reduced rates of methylation and demethylation as well as reduced levels of steady state methylation (Terwilliger et al., 1986a). In contrast, there is substantial methylation of the nonconforming site in Trg (Fig. 7). There must also be a methylation site on Tsr that deviates from the consensus sequence. Kehry and Dahlquist (1982a) observed a tetramethylated form of the K1 peptide of Tar. Thus there is an unidentified, methyl-accepting site on the peptide, in addition to the three shown in Fig. 8. None of the three available glutamates is preceded by another glutamate, thus whatever the position of the fourth methyl-accepting site, it will lack the Glu of the consensus sequence. It seems reasonable that, in analogy with Trg, the fourth site on Tsr is the glutamate corresponding to Trg residue 310.

In the absence of deamidation, only one of the four methyl-accepting sites on the K1 peptide is active (Fig. 7D). The same observation has been made for Tsr (Kehry and Dahlquist, 1982b) and Tar (Terwilliger and Koshland, 1984), but explanations of the phenomenon were complicated by indications that only a single deamidation occurs on the K1 peptide of those two transducers (Kehry and Dahlquist, 1983b; Terwilliger and Koshland, 1984) and by incorrect identifications of methyl-accepting site 1 on Tar (Terwilliger et al., 1986a). For Trg, it is clear that, in the absence of deamidation, the single, active methyl-accepting residue in K1 is Glu³⁰⁴ and that two sites, Glu³¹¹ and Glu³¹⁸, are inactive because neither is deamidated. Among the inactive sites, only Glu³¹⁰ would be capable of forming a methyl ester, yet is not methylated. It seems reasonable that lack of an adjacent glutamate at position 311 inactivates the methyl-accepting capacity of Glu³¹⁰, perhaps by disrupting productive interaction with the methyltransferase.

**Preference among Sites**—There is not a strict order in which the four sites on the K1 peptide are methylated and demethylated since among monomethylated peptides, any of the four sites can carry the single methyl group (Fig. 7B). However, in the stimulated and adapted cells used in these studies, there is a substantial preference for the central pair, sites 2 and 3. A similar preference is observed for Tar; in stimulated and adapted cells, the glutamate corresponding to site 3 of Trg exhibits relative rates of methylation and demethylation that maintain the highest level of modification among the four
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sites (Terwilliger, et al., 1986a). Preference for site 2 of Trg would not be predicted from comparison with the consensus sequence, since the conserved glutamate at the adjacent amino position is replaced by an isoleucine. Apparently, activity of a methyl-accepting site is very dependent on the presence of a Glu-Glu pair (see previous paragraph) but the second Glu can follow as well as precede the modified residue.

Peak T7, containing monomethylated R1 peptide, occurred in the tryptic peptide maps of all electrophoretic forms of Trg and generally contained more radioactivity than any other peak (Fig. 3). This suggests that GluSW is a preferred site of methylation, to an extent that exceeds any of the sites on the K1 peptide. A similar preference for methylation on the R1 peptide was observed for Tsr (Kehry and Dahlquist, 1982a), but not for Tar (Terwilliger et al., 1986a), where a glutamine in place of the conserved, adjacent glutamate may drastically lower the efficiency of methylation at the residue corresponding to GluSW.

Complexity of Tryptic Maps of [3H]Methyl-labeled Trg—The maps of [3H]methyl-labeled tryptic peptides derived from Trg contain more peptide maps than the maps of Tsr or Tar (Kehry and Dahlquist, 1982a, 1982b). The analyses reported here revealed that the additional peaks occurred because methylated forms of the K1 peptide lacking one or both deamidations appeared as distinct species. The distinction may in part reflect the different origins of the transducer proteins analyzed. The Trg protein was from cells containing multiple, plasmid-carried copies of the transducer gene and single chromosomal copies of the genes for the modification enzymes, while Tar and Tsr were obtained from cells with single chromosomal copies of the genes for the particular transducer and for the enzymes. A relatively high ratio of transducer molecules to CheB deamidase could result in a substantial population of incompletely deamidated Trg proteins. The altered stoichiometry is likely to account for the presence of undeaminated, monomethylated K1 peptide (peak T1) in maps of some electrophoretic forms of Trg (Fig. 3). The corresponding form of K1 from Tsr or Tar was observed only in protein synthesized in cells lacking CheB activity (Kehry and Dahlquist, 1982b). An additional factor in the difference between the peptide maps of Trg and the other transducers may be the lack of resolution of once-deamidated and twice-deamidated forms of K1 peptides from Tsr and Tar. This possibility is consistent with resolution by HPLC of only a single deamidated form of K1 peptide from Tsr or Tar (Kehry and Dahlquist, 1982a, 1982b; Terwilliger and Koshland, 1984) even though the number of methyl groups found on maximally methylated forms of K1 implies that K1 peptide from both transducers can be deamidated twice (Kehry et al., 1983b; Terwilliger et al., 1986a). Differential resolution by HPLC of deamidated forms of Trg but not of Tsr or Tar could be related to the difference in placement of one of the two deamidated glutamines in Trg (Fig. 8). In any case, definition of the specific sites of covalent modification on the Trg transducer provides a basis for structural investigations of the functional significance of multiple covalent modifications of sensory proteins.

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