Identification of a Possible Nucleotide Binding Site in CheW, a Protein Required for Sensory Transduction in Bacterial Chemotaxis*

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CheW is an essential component of the system which mediates chemotaxis in Salmonella typhimurium and Escherichia coli. Here we report the nucleotide sequence of the cheW gene as well as the purification and characterization of the CheW protein. The DNA sequence predicts a protein of 18,000 molecular weight. The pure protein exhibits an apparent molecular weight of 18,000 during sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Molecular sieve chromatography under nondenaturing conditions indicates a molecular weight of approximately 35,000, however. This result suggests that CheW is a homodimer. The predicted amino acid sequence between Thr-128 and Asp-160 fits a consensus exhibited by many proteins which bind purine nucleotides.

During bacterial chemotaxis, stimulatory ligands bind to receptor proteins at the cell periphery (for reviews see refs. 1-4). Over 10 different receptors have been identified in Escherichia coli and Salmonella typhimurium, each binding a distinct set of attractant and repellent stimuli. Information from this network of detectors is received by a processing system within the cytosol which acts, in turn, to modulate flagellar function and effect appropriate motor responses to changing extracellular conditions. Genetic studies have shown that the products of three genes, cheA, cheY, and cheW, are required to transduce information from the receptors into a signal which controls the flagellar motor. The signal transduction process also appears to require ATP, GTP, or a related purine nucleotide, since cells depleted of nucleoside triphosphates (5, 6) or starved for purines (7) behave the same as mutant strains defective in cheA, cheY, or cheW.

In this communication we present the nucleotide sequence of the S. typhimurium cheW gene and the purification and preliminary characterization of the CheW protein. Analysis of the sequence indicates that CheW probably binds a purine nucleotide and suggests that CheW may be the locus for the nucleotide requirement in chemotaxis.

EXPERIMENTAL PROCEDURES

Subclones of the S. typhimurium-pUC12 hybrid plasmid, pME1,† were used to sequence the cheW gene and to overproduce the CheW protein. This plasmid (Fig. 1) contains an 8.4-kilobase genomic S. typhimurium PstI fragment which extends from cheA to flaM. For sequencing, M13 subclones of pME1 were constructed by inserting a 3.1-kilobase PstI-SmaI fragment modified with BamHI linkers into the BamHI site of the polylinker region of M13mp10. Processive deletions through the inserts were generated using exonuclease III as described elsewhere.† The complete nucleotide sequence of cheW was determined on both strands by the dideoxynucleotide method (8), using [α-32P]dTTP, DNA polymerase I Klenow fragment and the deleted phage plus strands as templates.

The S. typhimurium CheW protein was purified from an E. coli F′* strain, HB101 (9), containing a CheW expression vector, pME105. The latter was constructed by inserting a 0.95-kilobase DraI-SalI fragment of pME1 into the SmaI and SalI sites in the pUC12 polylinker region. The signal transduction process also appears to require ATP, GTP, or a related purine nucleotide, since cells depleted of nucleoside triphosphates (5, 6) or starved for purines (7) behave the same as mutant strains defective in cheA, cheY, or cheW.

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† The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) J02656.

† A. Stock, R. Riggelman, and J. Stock, manuscript in preparation.
RESULTS

The nucleotide sequence of the S. typhimurium cheW gene and flanking regions, together with the deduced amino acid sequence of the CheW protein, is shown in Fig. 2. The predicted product is highly homologous to the corresponding protein in E. coli (12) with only 11 conservative changes among 167 residues. Five of the 11 differences occur between Val-73 and Glu-81, while the remainder are relatively scattered. A Gly-X-Gly-X-X-Gly sequence characteristic of nucleotide binding proteins (13, 14) is located between residues 133 and 138. In addition, the sequences flanking this glycine-rich region (Thr-128 to Asp-160) fit the consensus described by Wierenga et al. (14) as predictive of the $\beta$-a motif, which is characteristic of these proteins (Fig. 3). The only significant deviation occurs in the length of the predicted loop connecting the $\alpha$ helix to the second $\beta$-strand. In known structures this closely is less than 1 in 106 (14).

DISCUSSION

Over the past few years there has been a rapid accumulation of information relating protein sequences to characteristic features of tertiary structure. Perhaps the best example of this is the $\beta$-a motif associated with nucleotide binding sites of Walker type (15). The only deviation (*) from the sequence fingerprint occurs at Glu-129.

![Fig. 4. Sodium dodecyl sulfate-15% polyacrylamide gel electrophoresis of fractions from the purification of CheW.](image-url)
domains of proteins which interact with purine nucleotides such as NAD, FAD, ATP, and GTP (13, 14, 16). It is possible to identify this structure within a sequence and thereby discern a probable site of nucleotide interaction. Proteins of this type have frequently been associated with receptor-mediated regulation in vertebrate tissues. Examples include the tyrosine kinase domains of the insulin, epidermal growth factor, and platelet-derived growth factor receptors (17), as well as their product (22, 23). The nucleotide sequence encoding 215 residues at the C terminus of the E. coli CheA protein has also been reported (12), and we have sequenced a region of the Salmonella cheA gene which encodes 608 residues at the C terminus of the protein (unpublished results). None of these sequences exhibit a nucleotide binding fingerprint. The molecular weight of CheA appears to be approximately 76,000 (24). Therefore, there are approximately 80 codons at the extreme N terminus whose sequence remains to be determined. Three additional proteins are involved in chemotaxis, the identity of the nucleotide and the chemistry of its interactions can only be determined by studying the CheW protein. To address these questions we have purified CheW and have begun to investigate its properties.

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