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Signal Transduction in Chemotaxis Mediated by the Bacterial Phosphotransferase System

Friedrich Titgemeyer

Department of Biology, University of California at San Diego, La Jolla, California 92093-0116

Abstract Gram-negative bacteria are able to respond chemotactically to carbohydrates which are substrates of the bacterial phosphoenolpyruvate:sugar phosphotransferase system (PTS). The mechanism of signal transduction in PTS-mediated chemotaxis is different from the well-studied mechanism involving methyl-accepting chemotaxis proteins (MCPs). In PTS-mediated chemotaxis, carbohydrate transport is required, and phosphorylation seems to be involved in both excitation and adaptation. In this review the roles of the components of the PTS in chemotactic signal transduction are discussed. © 1993 Wiley-Liss, Inc.

Key words: enteric bacteria, phosphotransferase system, chemotaxis, signal transduction, protein phosphorylation

Motile bacterial cells are able to respond to environmental changes by moving towards attractants and away from repellents. This chemotactic behavior was first described by Pfeffer in 1885 [Pfeffer, 1881–1885] and is the focus of molecular biological research concerning sensory transduction in procaryotes. The bacterial system provides a model for sensory transduction in higher organisms [Koshland, 1980]. Procaryotic and eucaryotic signal transduction have several features in common. In most cases integral membrane proteins act as receptors for certain stimuli. The signals are then transmitted through the membrane to the cytoplasm where protein kinases may act as mediators that transduce the signals to the responding protein complexes. One of the chemotactic systems in bacteria is the phosphoenolpyruvate (PEP): sugar phosphotransferase system (PTS). Phosphotransferase (PT) chemotaxis has been detected in various Gram-negative [Adler and Epstein, 1974; Lengeler, 1975; Bassler et al., 1991] and Gram-positive [Thoelke et al., 1990] bacteria. In this communication the data available on the signal transduction pathway in PT chemotaxis in enteric bacteria will be summarized, and the possible roles of the different PTS components will be discussed. Since little is known about PT chemotaxis in other bacteria, and since the mechanism of chemotaxis in the Gram-positive organism *Bacillus subtilis* seems to be different from that in the enterics, chemotaxis in non-enterics will not be considered here.

COMPARISON BETWEEN METHYL-ACCEPTING CHEMOTAXIS PROTEIN (MCP)-MEDIATED CHEMOTAXIS AND PTS-MEDIATED CHEMOTAXIS

The molecular basis for signal transduction in MCP-mediated chemotaxis has been studied in detail [for a more detailed discussion of this topic the reader is referred to the symposium contribution of Lukat and Stock and to Borkovich and Simon, 1990; Bourret et al., 1991]. In *Escherichia coli* four different integral membrane chemosensor proteins (Tsr, Tar, Trg, and Tap) have been described. These proteins bind attractants (amino acids, sugars, and oligopeptides) and also sense repellents (pH, Co^{2+}, Ni^{2+}, and hydrophobic amino acids). In the absence of a stimulus each MCP forms a complex with two other chemotaxis proteins, CheW and CheA. The latter protein is an ATP-dependent autokinase which phosphorylates the CheY and CheB proteins. Phosphorylated CheY (CheY-P) interacts with the switch which controls the direction of flagellar rotation and alters the swimming behavior of the cell from smooth swimming (counterclockwise rotation) to tumbling (clockwise rotation). Dephosphorylation of CheY-P

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Address reprint requests to Dr. Friedrich Titgemeyer, Department of Biology 0116, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0116, E-mail: FRITZ@JEEVES.UCSD.EDU.

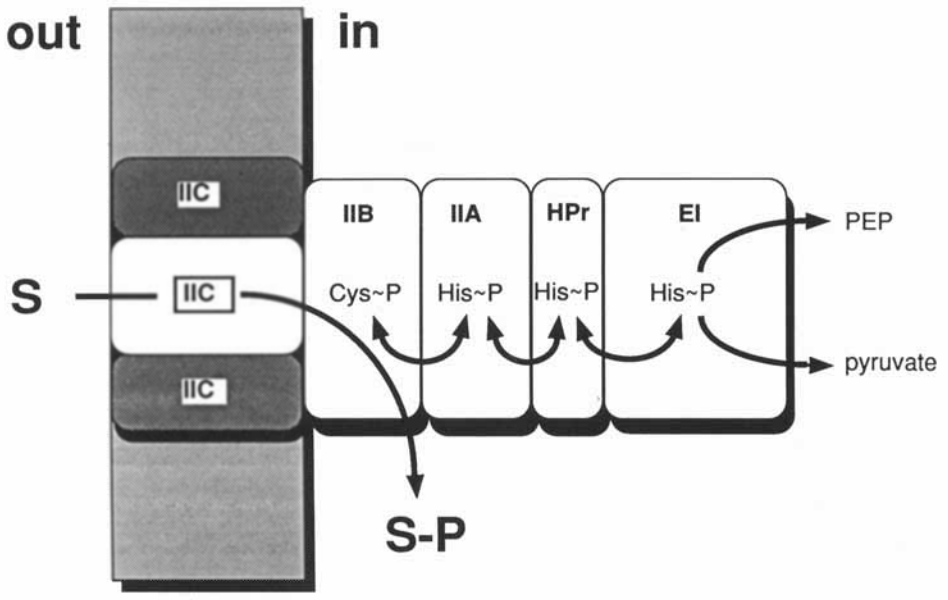

Fig. 1. General scheme for phosphoryl transfer via the protein constituents of the phosphotransferase system. The hydrophobic part of the IIC domain is crosshatched. In the hydrophilic part of the IIC domain the substrate binding site is indicated by a box. For abbreviations and further explanation see text (Modified from Lengeler et al., 1992 with permission).

and subsequent diffusion of CheY away from the switch is caused by the CheZ protein. CheZ and CheY together form the tumble regulator of the flagellar motor. The result of these protein interactions in the absense of a stimulus is the random movement of a bacterial cell. During excitation, an MCP binds the stimulating molecule and transmits this signal via a conformational change through the membrane to CheA and CheW, thereby reducing the phosphorylation activity of a sequestered CheA protein. Consequently, less CheY-P is formed, resulting in longer periods of smooth swimming. In the adaptation process two proteins, CheR and CheB, are involved. CheR alters the excitation activity of an MCP by methylating glutamyl residues in the cytoplasmic domain of the MCP. CheB, a methyl esterase which is activated by phosphorylation through CheA, reverses this process. The MCP's only function in sensing stimuli. Uptake and metabolism of an attractant are generally separate from the chemotactic response, and the former functions are encoded by genes distant on the chromosome from the chemotaxis genes.

In contrast to the mechanism described above, PTS-mediated chemotaxis correlates with transport. Two general proteins, Enzyme I (EI) and HPr, the carbohydrate-specific Enzyme IIABC complexes (IIABC), PEP, and a carbohydrate substrate are involved [Fig. 1; for a general review see Postma et al., 1985; Reizer et al., 1988]. A phosphoryl group is transferred from PEP to EI, and then to HPr. Phospho-HPr serves to energize many integral membrane IIABC enzyme complexes. The IIABC proteins catalyze both transport and chemoreception. Analysis of protein sequences derived from more than 20 different genes coding for IIABC proteins revealed that they may exist as a single polypeptide (i.e., $IIABC^{Mannitol}$). Alternatively, they may be split into two polypeptide chains (i.e., $IIA^{Glucose}$ and $IICB^{Glucose}$) or they may consist of three, distinct, functional proteins (IIA, IIB, and $IIC^{Cellobiose}$) [Lengeler et al., 1990; Saier and Reizer, 1992]. The IIA domain is phosphorylated by phospho-HPr on a histidyl residue, and this domain subsequently phosphorylates the IIB domain on a cysteyl or histidyl residue, depending on the protein. The only hydrophobic domain, IIC, is believed to function in substrate binding and transport. During transport, the substrate is phosphorylated, receiving a phosphoryl group from the IIB domain of the complex.

In Table I the most striking features of the two chemotactic systems are summarized and compared. In both pathways, excitation is triggered by phosphorylation. However, adaptation through methylation, as found in MCP-medi-

TABLE I. Comparison of MCP-mediated and PTS-mediated Chemotaxis

	MCP	PTS
Excitation	P	P
Adaptation	M	P
Attractants	+	+
Repellents	+	−
Transport	−	+
Signal	+++	+

P, phosphorylation; M, methylation; (+), chemotactic response; (−) no chemotactic response. For further explanation see text.

ated chemotaxis, has never been observed in PTS-dependent chemotaxis. In agreement with this fact, mutants lacking the CheR and CheB proteins as well as mutants lacking all MCPs exhibit normal PT chemotaxis [Niwano and Taylor, 1982]. Further, the PTS is unable to respond to repellents. When chemotactic responses of the two systems to their substrates are compared, those involving the MCPs are at least five times stronger than those involving the PTS [Vogler and Lengeler, 1987].

WHAT ARE THE ROLES OF THE PTS COMPONENTS IN PT CHEMOTAXIS?

Each component of the PTS has been studied with regard to its function in chemotaxis [for a more detailed discussion of this topic the reader is referred to Lengeler and Vogler, 1989; Taylor and Lengeler, 1990]. The IIABC proteins are the receptors for PTS stimuli. It has been reported that the affinities of one Enzyme II complex for its substrates are almost identical for functional transport and chemotaxis. Mutant Enzyme II proteins with higher K_m values for their substrate are reduced in both transport and chemotaxis, while in null mutants lacking a particular Enzyme II, both functions are abolished in a genetic background where no other Enzyme II for the substrate is present.

The requirement of phosphorylation for chemotaxis was investigated in mutants lacking the *ptsI* gene coding for EI but expressing different Enzymes II constitutively. These mutants are able to bind and sometimes transport the substrate in its unphosphorylated form, but they are unable to phosphorylate or respond to it chemotactically. These results suggest that chemoreception and phosphorylative transport are positively correlated.

If IIABC proteins interact with other chemotactic components to process the signal for chemotaxis, it should be possible to isolate IIABC mutants which are defective in transport function but not in chemotaxis and vice versa. In spite of this prediction extensive mutagenesis experiments conducted in several laboratories have not been successful in selecting such mutants. This fact has led to the postulate that transport and chemotactic functions are inextricably linked.

Other results exclude the possibility that the product of the transport reaction, the carbohydrate-phosphate, is the molecule mediating the chemotactic signal. Mutants constitutively expressing the uptake system for carbohydrate-phosphates do not trigger a chemotactic response when a carbohydrate-phosphate is added to the cells [Pecher et al., 1983]. Further, degradation of the sugar-phosphate is not required for chemotaxis. This was proven by studying the effects on chemotaxis of PTS-specific sugar analogs (i.e., 2-deoxyglucose and methyl-α-glucoside), which cannot be metabolized. These analogs were transported and caused a clear chemotactic response [Lengeler et al., 1981].

When the effects of the general energy coupling proteins, EI and HPr, were studied genetically, it was found that mutations which abolish the function of these proteins abolish chemotaxis and transport for most or all substrates of the PTS [Lengeler et al., 1981]. In a recent publication, Grübl et al. [1990] reported results which appeared to achieve the functional dissection of transport from chemotaxis. In mutants lacking HPr but constitutively expressing the *fruF* gene, encoding a fructose-specific HPr-like protein (FPr), normal transport, and growth, but no chemotaxis, were observed with various substrates of the PTS. FPr was not able to restore chemotaxis unless it was overexpressed. An HPr mutant which had a substitution in the prolyl residue at position 11 (P11E) showed the same phenotype when a plasmid containing this *ptsH* mutation was transformed into an *E. coli ptsH* negative strain. The authors concluded that prolyl residue 11 in HPr is not essential for transport but is critical for transmitting the chemotaxis signal.

The purified HPrP11E protein was studied with respect to phosphoryl group-accepting activity using purified EI as the donor. Compared to the wild type protein, this mutant protein showed reduced activity (about 50%) [Eiser-

mann, 1989]. This fact indicates that the P11E mutation alters the phosphorylation rate of the PTS protein phosphoryl transfer chain. The defect in chemotaxis, which could also be due to the depressed activity of EI on HPrP11E, is consistent with the notion that either Enzyme I or HPr (or both) plays a direct role in chemotaxis.

Another approach was to study PTS components from nonmotile bacteria. Genes encoding different Enzyme II proteins from *Klebsiella pneumoniae* were transferred to *E. coli*. All of the encoded proteins served as chemosensors [Sprenger and Lengeler, 1984; Sprenger, 1985]. When sequence data became available the corresponding proteins from *K. pneumoniae* proved to share a high degree of sequence similarity (more than 90% identity) with the *E. coli* or *Salmonella typhimurium* proteins [Lengeler et al., 1992; Vogler and Lengeler, 1991]. No region could be identified suggestive of a binding site for a chemotaxis-specific protein. These results indicate that the IIABC complex is only indirectly involved in the signal transduction pathway.

The *ptsH* gene from *K. pneumoniae* has been cloned, sequenced, and studied in PTS-substrate dependent growth, transport, and chemotaxis [Titgemeyer et al., 1990]. The protein could fully replace *E. coli* HPr for both transport and chemotaxis activities. On the sequence level, only one conservative amino acid substitution (I63L) was detected, and the prolyl residue at position 11 was not altered. The EI protein was similarly studied and was found capable of replacing the *E. coli* protein in chemotaxis [Sprenger, 1985]. These results show that all PTS components from a non chemotactic organism can function normally in chemotaxis in the proper genetic background.

WHAT ADDITIONAL GENE FUNCTIONS ARE REQUIRED FOR PT CHEMOTAXIS?

This question was answered by investigating a mutant lacking all proteins of the MCP-pathway. This "gutted" mutant (lacking all MCPs, CheA, CheW, CheB, CheR, and CheZ) exhibited chemotaxis when CheA, CheW, and CheY functions were restored [Taylor et al., 1988; Conley et al., 1989]. In addition, the presence of CheZ seems to be important, because mutations in the *cheZ* gene showed a permanent tumbling phenotype. It is unknown whether these proteins are directly involved in receiving the signal from the PTS or indirectly interfere with their responses to the flagellar switch. These possibilities would be difficult to distinguish because mutations in either *cheA* or *cheY* generate the smooth swimming signal. However, it has been speculated that the PTS proteins, EI and HPr, might alter the phosphorylation state of either CheA or CheY directly [Taylor and Lengeler, 1990; Grübl et al., 1990]. This suggestion is consistent with the fact that the PTS proteins and CheA are phosphorylated on histidyl residues, favoring a connection via CheA rather than via CheY, which is phosphorylated on a aspartyl residue.

During excitation by a PTS substrate, HPr and EI molecules are primarily in their unphosphorylated states. They might therefore be able to accept phosphoryl groups from either CheA or CheY, resulting in smooth swimming and consequent positive chemotaxis. In experiments using purified proteins and radiolabeled ATP in vitro, this hypothesis has been tested. Several experiments using all possible combination of CheW, CheA, CheY, and CheZ, as well as EI and HPr in the presence and absence of MCP-containing membranes were performed in an attempt to detect ATP-dependent phosphorylation of HPr and/or EI, but no labeled PTS-protein could be detected (K.A. Borkovich and F. Titgemeyer, unpublished results). It is therefore possible that at least one other factor is required to complete the PT chemotactic signal transduction chain.

A MISSING LINK?

The postulated "missing link" protein was termed the "phosphoryl-chemotaxis-protein" ("PCP") by Lengeler [Lengeler et al., 1981; Lengeler, 1982]. He proposed signal transduction in PT chemotaxis from the PTS proteins via "PCP" to the tumble generator by direct or indirect phosphorylation/dephosphorylation of CheY. As shown in the model in Figure 2, all signals from the different EII chemoreceptors will be integrated by the general proteins of the PTS, EI and HPr. The signal which controls the tumble generator proteins, CheY and CheZ, is then transmitted from one of the two PTS proteins via "PCP" to one of the chemotactic proteins, most likely CheA or CheY.

When the role of adenylate cyclase in PT chemotaxis was investigated, a mutation was found which appeared to cause a transport-chemotaxis uncoupled phenotype. The muta-

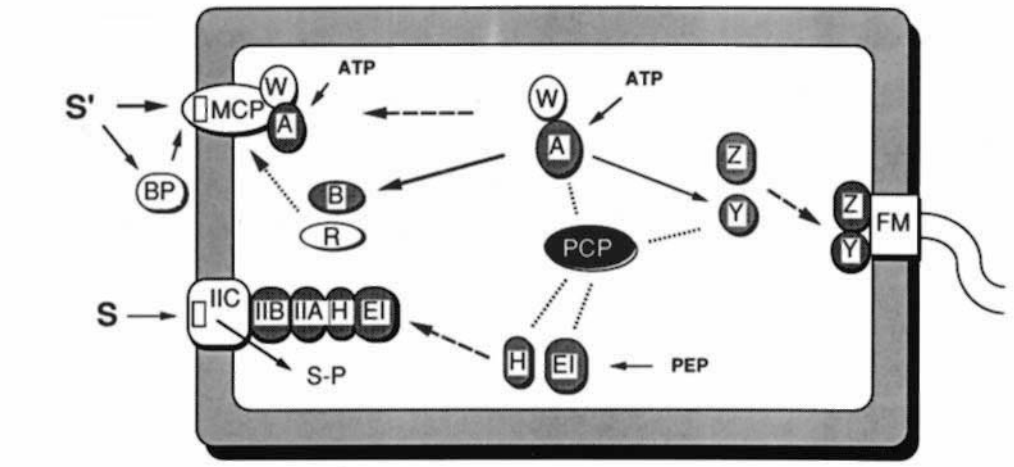

Fig. 2. Model for signal transduction in PTS-mediated and MCP-mediated chemotaxis. Proteins involved in phosphorylation and dephosphorylation are crosshatched. Dashed arrows indicate protein diffusion. Solid arrows show the flow of phosphoryl groups. Dotted arrows indicate protein-protein interactions involved in methylation and demethylation. Other dotted lines indicate the possible flow of phosphoryl groups which have not been experimentally verified. S', attractant or repellent binding to an MCP or indirectly to an MCP via a solute binding protein, BP; S, PTS-substrate; S-P, carbohydrate phosphate; A, CheA; W, CheW; B, CheB; R, CheR; Y, CheY; Z, CheZ; FM, flagellar motor; H, HPr. For additional abbreviations and further explanation see text.

tion was mapped near or within the *crp* gene locus [Vogler and Lengeler, 1987]. Since *crp* encodes the cAMP-catabolite activator protein, CAP, which acts as a positive regulator for gene expression, the authors considered that this specific *crp* allele might be unable to promote expression of a gene required for PT chemotactic signal transduction.

In a recent publication another hint as to the nature of the "missing link" has been provided [Lukat et al., 1992]. It was found that CheB and CheY can be directly phosphorylated by small phosphoryl donors. Moreover, it was demonstrated that CheY is phosphorylated by acetylphosphate. These results support the observation of Wolfe et al. [1988] that an intermediate in acetate metabolism might play a role in triggering a chemotactic signal. Since acetylphosphate is a substrate for acetate kinase, a protein which can act as a phosphoryl donor for EI in vitro [Fox et al., 1986], there might be a chemotactic connection between the PTS and CheY through acetate kinase and acetylphosphate or another small phosphoryl donor.

These possibilities can be tested by employing available biochemical and genetic methods in order to clarify the pathway of signal transduction in PT chemotaxis. Whatever the exact mechanism is, it is clear that in enteric bacteria a balanced network of proteins exist in which phosphorylated and nonphosphorylated forms are intricately balanced to guarantee the proper state of phosphorylation for PTS-mediated chemotaxis.

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