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Involvement of the Histidine Protein (HPr) of the Phosphotransferase System in Chemotactic Signaling of *Escherichia coli* K-12

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It is known that in mutants of *Escherichia coli* lacking the histidine protein (HPr) of the carbohydrate: phosphotransferase system, all substrates of the system can be taken up in the presence of the fructose-regulated HPr-like protein FPr (gene *fruF*). Although this protein fully substituted for HPr in transport and phosphorylation, we found that it was not able to complement efficiently for HPr in mediating chemotaxis toward phosphotransferase system substrates. Furthermore, transport activity and chemotaxis could be genetically dissected by the exchange of single amino acids in HPr. The results suggest a specific role of HPr in chemotactic signaling. We propose a possible link of signal transduction pathways for phosphotransferase system- and methyl chemotaxis protein-dependent substrates via HPr.

In enterobacteria, the best-known chemoreceptors are the methyl chemotaxis proteins, which mediate the response to ions, amino acids, and several sugars such as maltose, galactose, and ribose (12, 18). Other carbohydrates, transported by the phosphoenolpyruvate-dependent phosphotransferase system (PTS), are sensed by a different type of chemoreceptors, the enzymes II (EIIs). These membrane-bound proteins catalyze the uptake and concomitant phosphorylation of their substrates. The EIIs differ in their substrate specificity but are similar in their general biochemical properties and molecular structure. Some of them function together with a closely associated enzyme III (EIII) (22, 27). The chemoreceptor activity and the transport-phosphorylation reaction seem to be obligatorily coupled and have thus far never been dissected by mutating the EIIs (13, 15, 17).

The EIIs are phosphorylated as the result of two subsequent phosphoryl transfer reactions catalyzed by the soluble protein kinases enzyme I (EI; encoded by *ptsI*) and HPr (histidine protein, encoded by *ptsH*) with phosphoenolpyruvate as the primary phosphoryl donor. These general PTS components are necessary for the uptake of all PTS carbohydrates, and mutation in either of the corresponding genes results in a pleiotropic transport-defective phenotype. The degradation of D-fructose, however, is not affected by *ptsH* mutations because of the fructose-regulated expression of an HPr-like protein (also called pseudo-HPr or FPr) (5, 8, 25, 26) with significant primary sequence similarity to HPr (7). If constitutively expressed, FPr can generally substitute for HPr (6, 8, 11). The soluble components of the PTS constitute an essential part of the signal transduction pathway (2, 13, 15). It has been suggested that an alteration in their phosphorylation level during the uptake of substrates triggers an intracellular chemotactical signal, perhaps by controlling the activity or the phosphorylation level of a hypothetical phosphoryl chemotaxis protein (14, 15). The proposed role of adenylate cyclase and cyclic GMP in the transfer of sensory information in PTS chemotaxis (3, 4) has not been confirmed in subsequent studies (31, 33).

In the present study we reinvestigated the involvement of the general PTS components in chemotactic signaling by mutating the gene *ptsH* encoding HPr. In null mutants, the activity of this protein was substituted for by a constitutively expressed FPr. These mutants, although normal for transport and phosphorylation, showed essentially no chemotaxis toward PTS substrates, indicating a chemotaxis function of HPr that can be fulfilled only partially by FPr. The analysis of *ptsH* mutants with single amino acid substitutions (kindly provided by R. Eisermann and W. Hengstenberg) corroborated the conclusion that HPr has an important function in signal transduction.

MATERIALS AND METHODS

Bacteria and plasmids. All mutants used in this study are derivatives of *Escherichia coli* JWL184-1 (F⁻ *thi-1 argG6 metB1 hisG1 lacY1 galT6 xyl-7 supE44 rpsL104 gutAp49 mtlAp49 gatR49*) (15). The *pts*⁺ and *ptsH* alleles were transferred by P1 transduction with the help of a *zfb-1*::Tn*10* insertion originally taken from strain JLV86 (linkage, about 20%) (32). Tetracycline-susceptible derivatives were selected as described by Maloy and Nunn (19). Plasmid pBCP37-9 encodes the gene *fruF* of *Salmonella typhimurium* (7). The construction of plasmids coding mutated HPr (provided by R. Eisermann and W. Hengstenberg) will be described elsewhere. The mutations resulted in the exchange of Pro-11 to Glu (HPr11PE), of Phe-48 to Met and Lys-49 to Gly (pHPr48/49), and of Glu-68 to Ala (pHPr68EA), or in the deletion of the C-terminal amino acid 85 (pHPrdel85). The vector for pHPr11PE, pHPr68EA, and pHPrdel85 is pT7-6 (30), and the vector for pHPr 48/49 and pHPr is pUC19. Strains and plasmids are listed in Table 1.

Media and growth conditions. Bacteria were grown in the minimal and complex media described previously (13) with the following additions (if required): amino acids, 20 mg liter^{-1}; thiamine, 5 mg liter^{-1}; carbohydrates, 0.2%; kanamycin, 25 mg liter^{-1}; ampicillin, 100 mg liter^{-1}; cyclic AMP (cAMP), 1 mM. Soft agar plates contained KH_2PO_4-K_2HPO_4 (10 mM), $(NH_4)_2SO_4$ (1 mM), $MgSO_4$ (1 mM), 0.25% Difco

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TABLE 1. *E. coli* K-12 strains and plasmids used in this study

Strain or plasmid	Genotype	Parent or construction	Reference or source
E. coli			
JWL184-1	*pts*$^+$		15
LBG1605	*ptsH5 trp ilv rpsL*		24
JLV92	*ptsH5 zfb*::Tn*10*	JWL184-1	This study
JLV92M	*ptsH5 zfb*::Tn*10 fruR*	JLV92 Mtl$^+$	This study
GGL101	*ptsH5 fruR*	JLV92M Tet^s	This study
GGL102	*pts*$^+$ *zfb*::Tn*10 fruR*	GGL101	This study
Plasmids			
pHPr11PE			R. Eisermann
pHPr48/49			R. Eisermann
pHPr68EA			R. Eisermann
pHPrdel85			R. Eisermann
pHPr			R. Eisermann
pBCP37-9	*fruF*$^+$		7

agar, and the carbohydrate to be tested at a final concentration of 0.3 mM. For growth rate determinations, cells were grown in a rotary shaker at 37°C, and the increase of the A_{420} was followed (1 A_{420} unit equals 5×10^9 bacteria per ml).

Chemotaxis assays. The capillary tube test of Adler (1) for chemotaxis was performed with slight modifications as described previously (15). For qualitative chemotaxis tests, about 5×10^8 bacteria in a volume of 5 μl were transferred on soft agar plates, incubated at 30°C for 16 to 24 h, and checked for the formation of chemotaxis rings. Optical assays were performed by observing the bacterial behavior under a microscope and recording on videotape as described before (33).

Transport assays. Standard uptake assays for D-[^{3}H]mannitol (5 μM), D-[^{3}H]glucitol (25 μM), and D-[^{14}C]fructose (17.5 μM) have been described in detail elsewhere (13).

RESULTS

Isolation and characterisation of *ptsH* pseudorevertants. The involvement of the general PTS proteins HPr and EI in signal transduction cannot be studied by simply mutating the corresponding genes *ptsH* and *ptsI*, because both activities are indispensable for the phosphorylation of the EIIs and their functioning as primary chemoreceptors. In *ptsH* null mutants, this problem can be avoided by expressing the HPr-like protein FPr. This protein (encoded by *fruF*) is under the control of the fructose repressor FruR, and mutations in the repressor gene result in constitutive expression of FPr and a pleiotropic PTS$^+$ phenotype in an HPr-negative background (6, 8, 11).

The *ptsH5* mutation of strain LBG1605, which has never been observed to revert and therefore is believed to be a deletion (24), was transferred into strain JWL184-1, a wild-type strain for chemotaxis expressing constitutively the *mtl* and *gut* operons, including the corresponding EIIs. The resulting strain, JLV92, showed the expected PTS$^-$ Fru$^+$ phenotype (Table 2). Phenotypic PTS$^+$ revertants could be obtained on MacConkey-mannitol plates as represented by JLV92M (Table 2) or less frequently on other carbon sources. [To compensate for the lowered levels of cAMP in *pts* mutants, these revertants were selected in the presence of 1 mM cAMP to avoid the accumulation of unrecognized *crp*(In) (cAMP-independent cAMP receptor protein) mutations that have been found to affect PTS chemotaxis (33).]

TABLE 2. Growth and transport activities of HPr- and FPr-containing strains

Strain	Genotype	Growth[a]			Transport[b] (nmol min^{-1} mg of protein^{-1})		
		Mtl	Fru	Mal	Mtl (ui)	Fru (ui)	Fru (i)
JWL184-1	*pts*$^+$ *fruR*$^+$	+	+	+	12.8	2.5	40.0
JLV92	*ptsH fruR*$^+$	−	+	−	<0.1	3.5	39.0
JLV92M	*ptsH fruR*	+	+	(+)	11.6	54.0	34.0
GGL102	*pts*$^+$ *fruR*	+	+	+	13.2	36.0	27.0

[a] The growth was tested on MacConkey agar plates containing 1% (wt/vol) either D-mannitol (Mtl), D-glucitol or galactitol (data not shown), D-fructose (Fru), or maltose (Mal): +, colony coloration dark red or good growth; (+), pink colonies; −, white colonies or no growth.

[b] Transport activity for D-mannitol (5 μM) and D-fructose (17.5 μM): ui, uninduced, cells pregrown in glycerol; i, induced, cells pregrown in D-fructose.

As seen by test crosses into the wild type, JLV92M clearly retained the *ptsH5* allele of its parent. Transport measurements showed fully restored D-mannitol and constitutive D-fructose uptake (Table 2), indicating that the constitutively expressed fructose enzymes, including FPr, indeed compensate for the *ptsH* lesion. In this strain, FPr was not able to restore completely the growth on non-PTS carbohydrates such as maltose (see Discussion). When a *pts*$^+$ allele was reintroduced into JLV92M (to give strain GGL102; Table 2), the maltose-positive phenotype was used to distinguish the *pts*$^+$ transductants phenotypically.

Test of FPr function in chemotaxis. On hexitol soft agar plates, wild-type cells exhibit two sharp chemotaxis rings due to their response to mannitol (outer ring) and glucitol (inner ring). In contrast, a less clear ring formation was observed for the *ptsH* pseudorevertant JLV92M (Fig. 1A); cell growth (generation time on mannitol, 55 min versus 61 min for the wild type; Table 3), motility, and chemotaxis via methyl chemotaxis proteins (data not shown) were not affected. The addition of cAMP did not improve the chemotactic response. Surprisingly, the response toward fructose (the authentic phosphorylation pathway of the fructose system was completely present) was greatly reduced in the mutant (Fig. 1B). In capillary assays with mannitol and fructose the response of JLV92M essentially did not exceed the background level (Fig. 2). The chemotaxis defect to fructose was also observed in strain JLV92 (Fig. 1B and 2B) and thus is not due to the *fruR* mutation. Chemotaxis behavior was examined in the light microscope. The addition of either mannitol or fructose to a final concentration of 0.2 mM to wild-type cells resulted in immediate smooth swimming for more than 1 min. In strains JLV92 and JLV92M, however, the presence of these carbohydrates, even at higher concentrations, produced no significant response. When a *pts*$^+$ allele was introduced into the mutants, the chemotaxis was completely restored (e.g., in strain GGL102; Fig. 1). These results show that FPr, although able to compensate fully for HPr activity in transport, did not restore chemotaxis functions in the mutants. The failure of FPr to efficiently mediate PTS chemotaxis might be due either to the lack of a chemotaxis-specific activity in FPr or to the insufficient expression of FPr activity. To distinguish between both possibilities, plasmid pBCP37-9, a multicopy plasmid encoding the FPr from *S. typhimurium* (7), was introduced into JLV92 and JLV92M. The lack of recombination between the *fru* genes from *S. typhimurium* and the chromosomally encoded *fru* genes of its host together with the high copy number of pBCP37-9 causes overexpression of

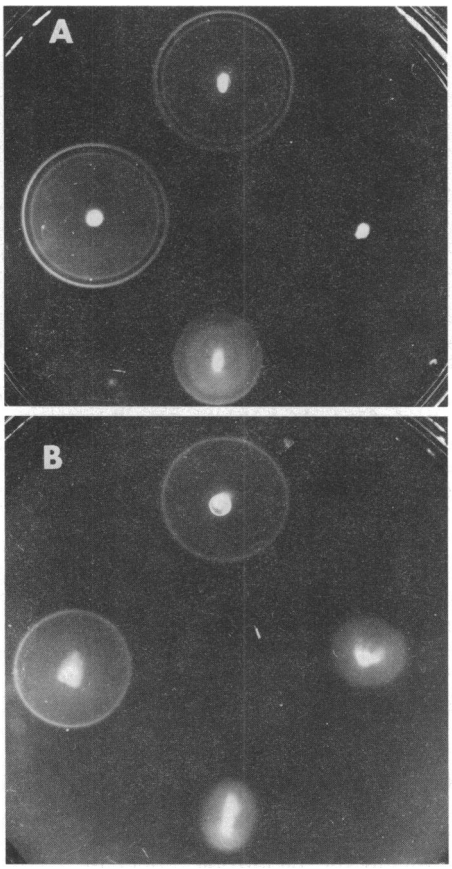

FIG. 1. Swarm plate tests of *ptsH* mutants. About 5×10^8 bacteria were transferred to a swarm plate containing (A) D-mannitol and D-glucitol (300 μM each) or (B) D-fructose (300 μM) and incubated at 30°C for 16 h. Strains (clockwise from the top): JWL184-1 (wild type), JLV92 (*ptsH*), JLV92M (*ptsH fruR*), and GGL102 (*ptsH*$^+$ *fruR*).

FPr. The plasmid restored full growth on all PTS carbohydrates and in contrast to the *fruR* mutation, even growth on non-PTS carbohydrates such as maltose together with normal transport activities. Subsequent chemotaxis assays revealed a positive response toward hexitols and fructose in strain JLV92M(pBCP37-9) (data not shown). These observations imply that FPr has a high phosphorylation activity for the different EIIs as tested by growth and transport tests but a low activity in chemotaxis signal transduction. This conclusion is corroborated by the observation that the isogenic *fruR*$^+$ strain JLV92(pBCP37-9) did not show a chemotactic response. It is only in a *fruR* mutant background that pBCP37-9 overexpresses FPr at the maximal level, as has been shown by Geerse et al. (7). Thus, the low level activity of FPr in chemotactic signaling seems to require its maximal expression to compensate for the chemotaxis defect caused by the loss of HPr. The observations imply furthermore that neither EI nor the various EIIs are direct intermediates in signal transduction. Their turnover during transport mediated by FPr must be at a normal level (even in *fruR* mutants lacking pBCP37-9) as judged from the high growth, transport, and phosphorylation activities observed (Table 2), and yet no chemotaxis could be seen.

Effects of *ptsH* point mutations on transport and chemotaxis. The fact that FPr could complement the transport-phosphorylation activity of HPr but not its chemotaxis function (except under conditions of severe FPr overexpression) suggests that the two functions of HPr are not necessarily coupled and might be separated by mutating the HPr gene. Existing point mutations causing single or double amino acid substitutions in HPr were introduced into strain JLV92 (*ptsH*) on multicopy plasmids and tested for chemotaxis. The wild-type plasmid and two of the mutant plasmids (pHPr68EA and pHPrdel85) fully restored PTS chemotaxis in this background, whereas two other plasmids (pHPr48/49 and pHPr11PE) did not (Fig. 3). Both plasmids, but especially pHPr48/49, also conferred reduced transport activity and slower growth (Table 3). As a consequence of inefficient glucitol uptake, only one chemotaxis ring (reflecting the response toward mannitol) was observed on hexitol swarm plates. If this plasmid was introduced into strain JLV92M (unfortunately resulting in a highly unstable transformant), growth on hexitols was essentially reconstituted by the phosphorylation activity of FPr, and under these conditions chemotaxis activity like that of the wild type was regained (Table 3). Thus the mutation in pHPr48/49 caused primarily a defect in the transport-phosphorylation function of HPr. The chemotaxis function instead was unaffected and still able to complement the otherwise chemotaxis-negative strain JLV92M. The characteristics of the HPr mutation encoded by pHPr11PE were different: growth rate and transport in JLV92(pHPr11PE) were reduced to 30 to 50%, whereas virtually no chemotaxis toward hexitol was observed (Fig. 3, Table 3). The severe effect on chemotaxis was unexpected because the diminished transport activity indicated the presence of EII activity, which should allow at least some chemotaxis. The chemotaxis defect could not be relieved by reconstituting the full growth and transport activity via FPr in strain JLV92M (Table 3). We therefore concluded that the HPr11PE mutation affected the transport-phosphorylation function of HPr only to a minor extent but greatly affected the chemotaxis function of this protein.

TABLE 3. Chemotaxis, transport, and growth rate of HPr mutants

Strain	Chemotaxis[a]		Transport[b]		Generation time[c] (min)	
	Mtl	Gut	Mtl	Gut	Mtl	Gut
JWL184-1	+	+	12.5	12.7	61	60
JLV92	−[d]	−[d]	<0.1	<0.1	−[d]	−[d]
JLV92M	−	−	11.8	11.0	55	70
JLV92(pHPr11PE)	−	−	6.8	4.0	72	96
JLV92(pHPr48/49)	(+)	−	6.8	1.3	132	>200
JLV92(pHPr)[e]	+	+	16.9	12.7	60	57
JLV92M(pHPr11PE)	−	−	ND[f]	ND[f]	72	90
JLV92M(pHPr48/49)	+	+	ND[f]	ND[f]	73	129

[a] Qualitative chemotaxis assays, +, positive reaction; −, no reaction; (+), ring formation less clear, relatively short reaction in optical assays. Mtl, D-Mannitol; Gut, D-glucitol.

[b] Transport of D-mannitol (5 μM) and D-glucitol (25 μM) after pregrowth in glycerol.

[c] Doubling time in minimal medium containing 0.2% D-mannitol or D-glucitol. Plasmid-containing strains were grown in the presence of ampicillin (100 mg liter^{-1}).

[d] −, No growth after 12 h.

[e] Identical results were obtained with pHPr68EA and pHPrdel85.

[f] ND, Not determined. No reproducible results could be obtained due to the instability of this plasmid in the *fruR* background.

DISCUSSION

The general PTS components are essential for chemotactic signaling (2, 15). Although their precise role in the signal

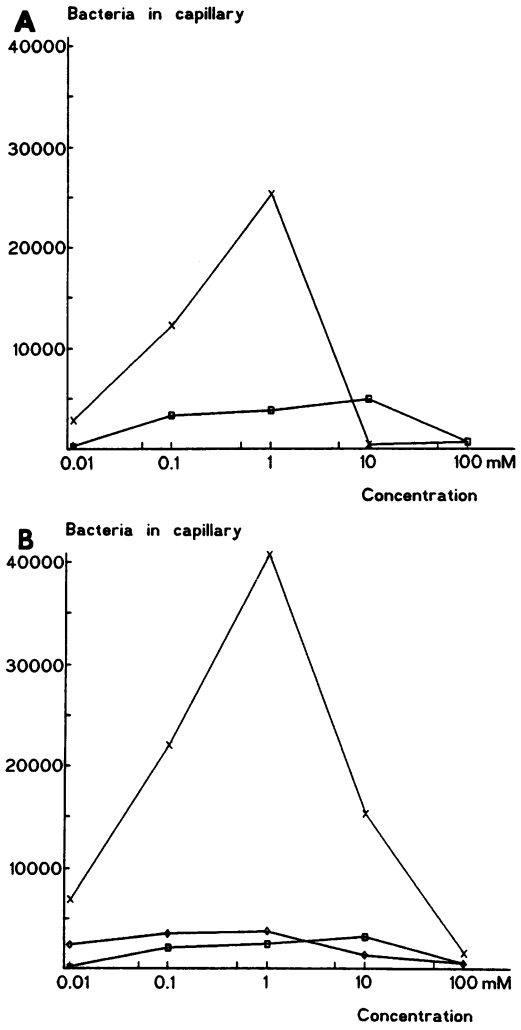

FIG. 2. Chemotaxis toward PTS carbohydrates in capillary tube tests by the following strains: ×, JWL184-1 (wild type); ◇, JLV92 (*ptsH*); □, JLV92M (*ptsH fruR*). (A) D-Mannitol chemotaxis. (B) D-fructose chemotaxis. Corrected numbers of bacteria per capillary tube are given relative to the number in a 1 mM L-aspartate tube, where the level of accumulation were between 430,000 and 700,000 cells.

transduction pathway is not understood, they are believed to integrate stimuli emanating from different EIIs and to generate the signal as a result of alterations in their level of phosphorylation, possibly involving a hypothetical phosphoryl chemotaxis protein (14, 15, 21). In the present study, *ptsH* mutations of *E. coli* were used to show that HPr has a function in chemotaxis that is different from its activity in phosphoryl transfer to the EIIs. This finding was supported by two lines of evidence; (i) the HPr-like protein FPr restored all transport activities in *ptsH* null mutants to full levels but poorly restored chemotaxis toward PTS substrates, and (ii) *ptsH* point mutations were found that specifically inactivated either the transport or the chemotaxis activities of HPr.

In enterobacteria only the fructose system has its own HPr-like protein, FPr (5, 8, 22, 26). This protein was as effective as HPr in mediating PTS-dependent transport processes in vivo (6, 8, 11; this study). In vitro, however, the

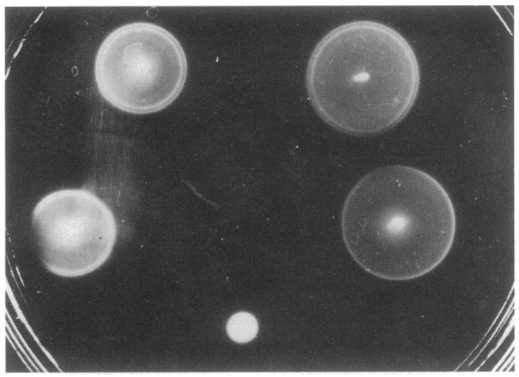

FIG. 3. Swarm plate test of strains expressing mutated HPr. In the background of JLV92 (*ptsH5*) the plasmid-encoded mutated HPr were tested on a swarm plate containing D-mannitol and D-glucitol (300 μM each) and ampicillin (100 mg liter^{-1}). Plasmids (clockwise from the top right): pHPr, pHPr48/49, pHPr11PE, pHPr68EA, and pHPrdel85.

substitution of HPr in carbohydrate phosphorylation needed significantly higher amounts of FPr; in particular, the FPr-dependent phosphorylation via EII^{Glc}-EIII^{Glc} was very poor (29). In *ptsH fruR* strains like JLV92M, the low phosphorylation of EIII^{Glc}, which in its unphosphorylated form inhibits the uptake of several non-PTS carbohydrates such as maltose (22), is probably the reason for the reduced growth rate on these carbon sources. This effect can be overcome by the introduction of plasmid pBCP37-9, indicating higher FPr activities (and thus elevated EIII^{Glc} phosphorylation) resulting from plasmid-encoded overproduction (Table 2).

In addition to the pleiotropic effect on PTS functions, mutations in *fruR* cause the inability to grow on carbon sources like acetate, pyruvate, and several Krebs cycle intermediates and inactivate the enzymes phosphoenolpyruvate carboxylase and phosphoenolpyruvate synthase (6, 8). This might affect the intracellular phosphoenolpyruvate concentration and the phosphorylation level of the PTS and finally result in an altered chemotaxis behavior. This possibility, however, is unlikely because the reintroduction of a *pts*$^+$ allele into JLV92M restores chemotaxis (Table 2), whereas the other *fruR* effects remain independent of the genetic background (6, 7).

In *E. coli* the DNA sequence of the *fruF* gene is not known. In the closely related species *S. typhimurium* the *fruF* and *ptsH* genes reveal a high degree of similarity of the FPr and HPr proteins (35% identical amino acids for the homologous domains), but a major difference is that *fruF* is fused to the gene encoding EIII^{Fru} (formerly *fruB*) (7). The failure of FPr in mediating efficient chemotaxis could therefore be due either to structural differences in the FPr part or to a sterical effect caused by the large EIII domain. In the latter case, free FPr that might be present in low amounts as a cleavage product (29) would be active in chemotaxis. Overproduction might provide sufficient free FPr and explain why strain JLV92M(pBCP37-9) but not its isogenic *fruR*$^+$ derivative regains chemotaxis. Although the actual FPr levels in these cells are not known, it may be that only the combined effects of plasmid-encoded overexpression and chromosomal repressor mutation, shown to give the highest expression levels (7), result in the formation of sufficient FPr for chemotaxis. It should be noted that the plasmid encodes FPr from *S. typhimurium*. In this species, *ptsH fruR* strains show a low chemotactic response toward PTS carbohydrates (B. Taylor, personal communication). It

is possible that the FPrs from both species are slightly different and that the residual chemotaxis activity, which is even observed in strain JLV92M (Fig. 1), is more pronounced when FPr from *S. typhimurium* mediates the response.

Further support for the finding that transport and chemotaxis functions of HPr can be separated came from the analysis of HPr mutants in which both functions could be inactivated independently. The Pro-Glu amino acid exchange at position 11 caused a complete loss of chemotaxis but only partly affected transport. Thus the mutated HPr and the wild-type FPr cause a very similar phenotype. Interestingly, a Glu residue is also found at the corresponding position in FPr. The exchange of amino acids 48 and 49 of HPr caused the opposite mutant phenotype, primarily affecting transport but not chemotaxis. Glucitol transport was especially impaired in the mutant, which may reflect a rather low binding affinity of HPr to EII^{Gut}-$EIII^{Gut}$ as compared with that of EII^{Mtl}. These differences in HPr affinity probably also exist in the wild-type situation and might contribute to diauxic growth on mannitol-glucitol (16). Chemotaxis activity with the mutated HPr48/49 could be seen but only if transport-phosphorylation was complemented by FPr (in strain JLV92M), supporting the well-established observation that phosphorylation is indispensable for PTS chemotaxis (15). The fact that the introduction of pHPr48/49 into strain JLV92M restored chemotaxis not only to hexitols (Table 3) but also to fructose (data not shown) indicates again that FPr has no pronounced chemotaxis function and that stimuli triggered by the fructose system must also be mediated through HPr. HPr, in contrast, has a specific chemotaxis function effective in signaling of HPr- and FPr-dependent phosphorylation processes. Since the mutated HPrs are encoded by multicopy plasmids, it will be interesting to see how far the observed chemotaxis defects are depending on stoichiometric effects.

Recently it has been established by in vitro studies that the general chemotaxis proteins CheA, CheY, and CheZ (mutations in the corresponding genes cause a generally chemotaxis negative phenotype and thus mediate the chemotaxis response to any stimulus, whether derived from methyl chemotaxis proteins or from the PTS) cycle between phosphorylated and unphosphorylated forms. Signal transduction thus may involve the flow of phosphoryl groups through a cascade of phosphorylated chemotaxis proteins (10, 20, 28). This process has a remarkable resemblance to the flow of phosphoryl groups through the cascade of PTS proteins, and it can be expected that both pathways are intimately coupled during the processing of PTS signals. Any positive stimulus such as the addition of PTS substrates results in a decreased tumble probability. Since tumbling is triggered by the binding of the phosphorylated form of CheY to switch proteins (10, 23, 28, 34), PTS stimuli should lead to a net dephosphorylation of CheY. It is tempting to speculate that the dephosphorylation of the PTS during the uptake of carbohydrates causes a dephosphorylation of CheY through direct phosphoryl reactions between PTS and Che proteins. One possible target site for the interaction of the PTS is the protein CheA, which, like the PTS proteins, is phosphorylated at a His residue (9). An alternative possibility is that the PTS dephosphorylates CheY directly, thus acting in a manner similar to that of CheZ during the response to positive stimuli (14, 17). A series of experiments with intra- and intergenic hybrids between different EIIs as well as EIIs from various organisms as efficient chemoreceptors in *E. coli* chemotaxis toward PTS carbohydrates seems to rule out these molecules as the site of direct interaction of the PTS with the chemotaxis machinery (17, 32). This leaves HPr, which is constitutively expressed in the enteric bacteria, as the most likely candidate for this interaction, because EI, the other possible candidate, has normal activity and phosphoryl turnover during FPr-mediated transport-phosphorylation without triggering an efficient chemotactic response. A plausible explanation of all data presented is that the level of phosphorylation required for efficient transport is lower than the activity required for chemotaxis. Phospho-HPr reaches both levels in wild-type strains and even in many leaky mutants, whereas FPr reaches the chemotaxis level only under conditions of high overproduction. It remains to be shown whether HPr alleles that uncouple transport and chemotaxis activities of this protein affect two separate biochemical steps or whether uncoupling is apparent because their lowered activity is still sufficient for transport and EII phosphorylation but no longer for chemotactic coupling.

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