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ORIGINAL PAPER

J. van der Vlag · P. W. Postma

Regulation of glycerol and maltose uptake by the IIA^{Glc}-like domain of II^{Nag} of the phosphotransferase system in *Salmonella typhimurium* LT2

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Abstract In *Enterobacteriaceae* the nonphosphorylated form of IIA^{Glc} of the phosphoenolpyruvate-dependent phosphotransferase system (PTS) can inhibit the uptake and subsequent metabolism of glycerol and maltose by binding to, and inhibiting, glycerol kinase and the MalK protein of the maltose transport system, respectively. In this report we show that the IIA^{Glc}-like domain of the membrane-bound II^{N-acetylglucosamine} (II^{Nag}) of the PTS can replace IIA^{Glc} in a *Salmonella typhimurium* *crr* mutant strain that lacks all soluble IIA^{Glc}. The inhibition was most severe in cells which were partially induced for the glycerol or maltose uptake systems. The *Streptococcus thermophilus* lactose transporter LacS, which also contains a IIA^{Glc}-like domain, could not replace IIA^{Glc}. Neither II^{Nag} nor LacS could replace IIA^{Glc} in activation of adenylate cyclase.

Key words Phosphotransferase system · Inducer exclusion · *Salmonella typhimurium* · Glucose PTS · Regulation of transport

Introduction

Phosphoenolpyruvate: carbohydrate phosphotransferase systems (PTS) are responsible for the uptake and concomitant phosphorylation of numerous PTS carbohydrates in *Enterobacteriaceae*. The transfer of the phospho group of phosphoenolpyruvate (PEP) to the PTS carbohydrates is catalyzed by the general cytoplasmic proteins enzyme I and HPr, and a number of carbohydrate-specific enzyme II complexes (EIIs). The enzyme II complex of the glucose PTS, which catalyzes the transport and phosphorylation of glucose and its

analogue methyl α -glucoside (α MG), consists of the cytoplasmic IIA^{Glc} and the membrane-bound IICB^{Glc} proteins.

The PTS plays also an important role in the regulation of the metabolism of several non-PTS carbon sources, e.g. glycerol, maltose, melibiose, lactose, and Krebs cycle intermediates. The central regulatory molecule, IIA^{Glc}, can, like the other PTS proteins exist in the phosphorylated or the nonphosphorylated form. Nonphosphorylated IIA^{Glc} binds to, and inhibits, the uptake systems for glycerol, maltose, melibiose and lactose, and, as a consequence, prevents the induction of various operons (PTS-mediated inducer exclusion). Phosphorylated IIA^{Glc} is required for the activation of adenylate cyclase. The resulting increase of the intracellular cAMP level promotes the transcription of numerous metabolic genes or operons (for a review, see Postma et al. 1993).

Although most *Escherichia coli* *crr* mutants, which lack IIA^{Glc}, are unable to transport α MG and are resistant to inducer exclusion, α MG uptake and PTS-mediated inducer exclusion could still be demonstrated in *Salmonella typhimurium* *crr* strains that completely lacked the soluble IIA^{Glc} (Nelson et al. 1982). A membrane-bound, IIA^{Glc}-like, activity was detected in these *crr* mutants, and this activity could be inhibited by anti-IIA^{Glc} antiserum (Scholte et al. 1982). Prompted by the fact that several EIIs from gram-negative and gram-positive organisms contain IIA^{Glc}-like domains (for a summary, see Postma et al. 1993, and legend to Fig. 1), it was shown in subsequent studies that the IIA^{Glc}-like domains in II^{Nag} (specific for N-acetylglucosamine) and II^{Bgl} (specific for β -glucosides) of enteric bacteria could restore the transport and phosphorylation of glucose and α MG in IIA^{Glc}-deficient *crr* strains of *E. coli* (Vogler et al. 1988; Schnetz et al. 1990). This result implies that these IIA^{Glc}-like domains can interact with, and transfer their phospho group to, IICB^{Glc}. The question remained whether the regulatory functions of IIA^{Glc}, i.e. inhibition of non-PTS uptake

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systems by IIA^{Glc} and activation of adenylate cyclase by phospho-IIA^{Glc}, could be restored in *crr* strains by these IIA^{Glc}-like domains present in several EIIs.

In this study we report that the IIA^{Glc}-like domain of II^{Nag}, but not a similar domain in the *Streptococcus thermophilus* lactose permease LacS (Poolman et al. 1989), can replace IIA^{Glc} in a *S. typhimurium crr* strain in inducer exclusion. These domains are not able to restore the activation of adenylate cyclase.

Materials and methods

Bacterial strains and plasmids

The following *S. typhimurium* strains were used: SB3507 (*trpB223*) and PP2005 (*crr-307::Tn10 trpB223*; Scholte et al. 1982). PP2178 (*crr-307::Tn10 nagE142 trpB223*), a derivative of PP2005, was selected by resistance to streptozotocin as described by Lengeler (1980). The following plasmids were used: pJFHE119 [ampicillin resistance (Ap^r); Fürste et al. 1986]; pAVL1 (Ap^r) containing the *nagE*⁺ gene of *Klebsiella pneumoniae* (Vogler and Lengeler 1988; Vogler et al. 1988); pEKS8 (Ap^r) containing the *S. thermophilus lacS*⁺ gene (Poolman et al. 1989). This last plasmid was kindly provided by B. Poolman (University of Groningen, The Netherlands). Plasmids were isolated and transformed as described by Sambrook et al. (1989).

Growth conditions

Cells were grown overnight in minimal salts medium A (Postma 1977), supplemented with 0.4% D,L-lactate, L-tryptophan (20 µg/ml), and (when required) ampicillin (50 µg/ml). Cells were harvested by centrifugation and diluted 5–10 times in fresh minimal salts medium A, containing tryptophan (20 µg/ml), ampicillin (50 µg/ml), and 0.5% glycerol or 0.2% maltose. After growth for the indicated periods on glycerol or maltose, glucose was added to a final concentration of 10 mM and cells were allowed to grow for an additional hour. Cells were harvested and washed twice with minimal salts medium A prior to uptake experiments. All cultures were incubated at 37°C.

Uptake experiments, preparation of cell extracts and *in vitro* phosphorylation experiments

Transport of labelled carbohydrates was performed at room temperature as described by Postma (1977). For the preparation of cell extracts, cells were ruptured by passage through an Aminco French pressure cell at 1100 kg/cm². High-speed supernatants and membrane fractions were prepared as described previously (Ruijter et al. 1991). II^{Nag} activity was determined, as described by Roseman et al. (1982), in the membrane fractions at 37°C, by measuring PEP-dependent phosphorylation of 0.5 mM N-acetyl-D-[1-¹⁴C]glucosamine with the high-speed supernatant from PP2178 (*crr-307::Tn10 nagE142*) as a source of the soluble PTS proteins enzyme I and HPr. Immunoblotting was performed as described (Towbin et al. 1979). The antiserum against LacS was a gift from B. Poolman, University of Groningen, The Netherlands.

Protein and dry weight determinations

Dry weights were determined as described previously (Herbert et al. 1971). Protein concentrations were determined on a Cobas BIO

autoanalyzer (Roche, Basel, Switzerland) by using the bicinchoninic acid assay (Smith et al. 1985), with bovine serum albumin (BSA) as a standard.

Chemicals

[U-¹⁴C]methyl α-glucoside (5.68 GBq/mmol), [U-¹⁴C]Maltose (20 GBq/mmol) and N-acetyl-D-[1-¹⁴C]glucosamine (2.11 GBq/mmol) were obtained from Amersham International, Amersham, U.K. and [U-¹⁴C]glycerol (5.7 GBq/mmol) was obtained from NEN Research products, Du Pont, Boston, Mass. Streptozotocin was obtained from Boehringer, Mannheim, Germany. PEP, 2-deoxyglucose, and bicinchoninic acid were obtained from Sigma Chemical Co., St. Louis, Mo.

Results and discussion

Expression and activity of II^{Nag} and LacS protein in *S. typhimurium* strains

To investigate the possible contribution of the IIA^{Glc}-like domains, present in II^{Nag} and the LacS protein, to PTS-mediated regulation of glycerol and maltose metabolism, we first studied the expression and activity of these proteins in the *S. typhimurium crr* strain PP2178 (*crr-307::Tn10 nagE142*) containing plasmids pAVL1 (II^{Nag}) or pEKS8 (LacS). The functional expression of enzyme II^{Nag} in an *E. coli crr nagE*/pAVL1 strain was previously reported and it was shown with an anti-IIA^{Glc} antiserum that II^{Nag} was present only in the membrane fraction (Vogler et al. 1988). Similar results were obtained with the strains used in this study. II^{Nag} was detected immunologically in the membrane fractions of SB3507 (wild type) and PP2005 (*crr*) but not in the membrane fraction of PP2178 (*crr nagE*), unless this last strain contained plasmid pAVL1. The levels of II^{Nag} in PP2005, induced by growth on N-acetylglucosamine, and uninduced PP2178/pAVL1 were approximately similar (data not shown).

S. typhimurium strain PP2005, which lacks all soluble IIA^{Glc}, was still able to transport (and phosphorylate) αMG at a rate of 22% of that of the wild-type strain (Table 1). Growth in the presence of N-acetylglucosamine (Nag) increased this rate to about 60% of the wild-type level, while the rate decreased to less than 7% of the wild-type level when a *nagE* mutation was introduced in addition to the *crr* mutation (strain PP2178). Transformation of PP2178 (*crr nagE*) with plasmid pAVL1 restored the rate of αMG uptake to about 60% of that of the wild type (Table 1). The rate of Nag uptake and phosphorylation in the different strains showed a similar pattern (Table 1). Residual Nag uptake and phosphorylation in PP2178 was most probably due to II^{Man}, which is more or less constitutively expressed in *S. typhimurium* (Stock et al. 1982). The residual αMG uptake observed in PP2178 could not be due to II^{Man} under our experimental conditions because II^{Man} has a very low affinity (25 mM as

Table 1 Uptake of methyl α -glucoside and N-acetylglucosamine, and PEP-dependent phosphorylation of N-acetylglucosamine

Strain/plasmid (genotype)	Inducer	Uptake ^a		Phospho- rylation Nag
		α MG	Nag	
SB3507/pJFHE119 (<i>crr</i> ⁺ <i>nagE</i> ⁺)	-	29.2	3.0	58
PP2005/pJFHE119 (<i>crr</i> ::Tn10 <i>nagE</i> ⁺)	-	6.5	3.8	52
PP2005/pJFHE119 (<i>crr</i> ::Tn10 <i>nagE</i> ⁺)	Nag	17.2	7.2	116
PP2178/pJFHE119 (<i>crr</i> ::Tn10 <i>nagE</i>)	-	2.0	1.8	23
PP2178/pAVL1 (<i>crr</i> ::Tn10 <i>nagE/nagE</i> ⁺)	-	18.3	9.5	121
PP2178/pEKS8 (<i>crr</i> ::Tn10 <i>nagE/lacS</i> ⁺)	-	3.0	2.1	19

^a Cells were grown for 2 h on glycerol, where indicated, 10 mM Nag was added as an inducer. Uptake of 0.5 mM [U-¹⁴C] methyl- α -glucoside (α MG, specific activity 200 dpm/nmol) and 0.1 mM [1-¹⁴C]Nag (specific activity 800 dpm/nmol) was performed as described and uptake is expressed as nmol/min per mg dry weight. PEP-dependent phosphorylation of 0.5 mM [1-¹⁴C]Nag (specific activity 400 dpm/nmol) was determined in membrane fractions and is expressed as nmol/min per mg membrane protein

measured by PEP-dependent in vitro phosphorylation) for α MG (Stock et al. 1982). From the results presented in Table 1 we conclude that II^{Nag} contributed about 70% to the residual α MG uptake in *S. typhimurium crr* strains.

The rate of α MG uptake in strain PP2178 harbouring plasmid pEKS8 (*lacS*⁺) was not significantly different from the rate observed in strain PP2178/pJFHE119. As expected, the LacS protein did not stimulate the rate of Nag uptake and phosphorylation (Table 1). An anti-IIA^{Glc} antiserum did not react with membrane fractions of *E. coli* strains harbouring plasmid pEKS8 (B. Poolman personal communication). Expression of the LacS protein from plasmid pEKS8 was shown *via* immunoblotting using an anti-LacS antiserum. The expected 51 kDa band was observed in the membrane fraction of strain PP2178 containing plasmid pEKS8. The anti-LacS antiserum did not cross-react with purified IIA^{Glc} or II^{Nag}-containing membranes (data not shown).

Regulation of glycerol and maltose uptake by II^{Nag} and the LacS protein

The degree of inhibition of glycerol and maltose uptake by the glucose analogue 2-deoxyglucose (2DGlc) was determined in the *crr* mutant PP2005 and the *crr nagE* double mutant PP2178, as well as in PP2178 transformed with pAVL1 (*nagE*⁺) or pEKS8 (*lacS*⁺).

The rate of glycerol uptake in strain PP2005, grown for 2 h on glycerol, was inhibited by 11% by 2DGlc and

the degree of inhibition increased to 34% when cells were grown for a shorter period on glycerol (Table 2). The corresponding values for PP2178, 6% and 13%, were significantly lower. When PP2178 was transformed with plasmid pAVL1, the inhibition of glycerol uptake by 2DGlc increased from 15% (cells grown for 2 h on glycerol) to 52% (cells grown for 30 min on glycerol). Inhibition of maltose uptake by 2DGlc in PP2178 also increased from 10% (with control plasmid pJFHE119) to 46% (with pAVL1). From the results presented in Table 2 we conclude that II^{Nag} is the major contributing factor to the inhibition of glycerol and maltose uptake by PTS carbohydrates (PTS-mediated inducer exclusion) observed in *S. typhimurium crr* strains that lack all soluble IIA^{Glc}.

In strain PP2178/pEKS8 (*crr nagE/lacS*⁺) the inhibition of glycerol and maltose uptake by 2DGlc was low and similar to the inhibition observed in the control strain PP2178/pJFHE119 (Table 2).

To investigate a possible role of the IIA^{Glc}-like domains present in II^{Nag} and the LacS protein in the stimulation of adenylate cyclase, the strains listed in Table 2 were grown on minimal medium A plates containing succinate or citrate as sole carbon source. *Crr*⁻ mutants are unable to grow on these carbon sources due to the low intracellular cAMP levels. Except for the wild type, no growth was observed, unless 5 mM cyclic AMP was included in the medium. From this result we conclude that the IIA^{Glc}-like domains of II^{Nag} and the LacS protein were not able to stimulate adenylate cyclase sufficiently, if at all, to restore growth of *crr* mutants on citrate and succinate.

Conclusions

The interaction between soluble IIA^{Glc} and several non-PTS enzymes, such as the lactose and melibiose carrier, glycerol kinase and the MalK component of the maltose transport system, which results in inactive complexes, has been well documented (for a review, see Postma et al. 1993). Inducer exclusion in *crr* strains of *S. typhimurium* lacking soluble IIA^{Glc}, has been reported previously (Nelson et al. 1982) but it was not known which enzyme was responsible for this phenomenon. Here we report that the IIA^{Glc}-like carboxy-terminal domain of the membrane-bound II^{Nag} is able to replace IIA^{Glc} in *crr* mutants and to mediate the inhibition of glycerol and maltose uptake by the PTS carbohydrate 2-deoxyglucose (inducer exclusion).

II^{Nag}-mediated inhibition of glycerol and maltose uptake was most clearly observed in *S. typhimurium crr* mutants which were partially induced for glycerol kinase or the MalK protein (Table 2). It has been shown previously that the extent of inducer exclusion is determined both by the level (and phosphorylation state) of IIA^{Glc} and the level of its target proteins (for a review, see Postma et al. 1993). Thus, the degree of

Table 2 Inhibition of glycerol and maltose uptake in *crr* mutants

Strain/plasmid	Inhibition of glycerol uptake (%) ^a			Inhibition of maltose uptake (%) ^a
	30'	60'	120'	30'
SB3507/pJFHE119 (<i>crr</i> ⁺ <i>nagE</i> ⁺)	100	100	100	100
PP2005/pJFHE119 (<i>crr</i> ::Tn10 <i>nagE</i> ⁺)	34	18	11	36
PP2178/pJFHE119 (<i>crr</i> ::Tn10 <i>nagE</i>)	13	9	6	10
PP2178/pAVL1 (<i>crr</i> ::Tn10 <i>nagE</i> / <i>nagE</i> ⁺)	52	23	15	46
PP2178/pEKS8 (<i>crr</i> ::Tn10 <i>nagE</i> / <i>lacS</i> ⁺)	15	11	7	12

^a Cells were grown on glycerol or maltose for the indicated periods. Uptake of 0.5 mM [¹⁴C]glycerol and 0.5 mM [¹⁴C] maltose (specific activities 200 dpm/nmol) was determined in the absence and presence of 10 mM 2-deoxyglucose (2DGlc), as described. The rates of glycerol uptake without addition were 8.9, 18.3 and 25.7 nmol/min per mg (dry weight), as determined in the wild-type strain (SB3507/pJFHE119) grown on glycerol for 30', 60', and 120' respectively. The rate of maltose uptake was 8.8 nmol/min per mg (dry weight), as determined in the wild-type strain. The percentage inhibition of glycerol and maltose uptake was calculated by comparing the rates of uptake in the absence and presence of 2DGlc.

inhibition of glycerol or maltose uptake by PTS carbohydrates is higher in cells which are induced for a shorter period of time on glycerol (or maltose), i.e. have less of the non-PTS uptake systems.

Can other enteric EIIs replace IIA^{Glc}? Although α MG uptake and inducer exclusion in a *S. typhimurium crr* mutant stem to a large extent from II^{Nag}, the residual activity seen in a *crr nagE* double mutant may be due to the (obviously low) expression of other EIIs containing membrane-bound, IIA^{Glc}-like domains. Although (the normally cryptic) II^{Bgl} has been shown to be able to replace IIA^{Glc} in α MG transport (Vogler et al. 1988), it is an unlikely candidate since the *bglF* gene, encoding II^{Bgl}, is either cryptic or absent in *E. coli* K-12 and *S. typhimurium* LT2. We have reported previously that a mutation could be isolated in a *S. typhimurium crr nagE* mutant that completely abolished the residual α MG uptake and phosphorylation (Postma et al. 1989). The mutation mapped at a locus which in *E. coli*, contains the *cel* operon for cellobiose degradation (Kricker and Hall 1984). However, *E. coli* II^{Cel} does not contain a IIA^{Glc}-like domain (Parker and Hall 1990).

The LacS protein, another protein which contains a IIA^{Glc}-like domain, could not stimulate the uptake of α MG or mediate inhibition of glycerol or maltose uptake by 2DGlc in a *crr nagE* mutant. The observation that LacS cannot be phosphorylated by *E. coli* P-HPr *in vitro* (Poolman et al. 1990) may account for the inability to stimulate α MG transport. However, the nonphosphorylated LacS protein is also unable to inhibit the various non-PTS uptake systems. Interest-

ingly, the IIA^{Glc}-like domain of *Bacillus subtilis* II^{Glc}, which can be phosphorylated both by *E. coli* and *B. subtilis* P-HPr, has been shown to mediate both inducer exclusion and activation of adenylate cyclase (Reizer et al. 1992). It should be stressed, however, that in those experiments the carboxy-terminal domain of II^{Glc} was expressed as a separate, soluble protein, while normally it is fused to the IICB domain.

Why can II^{Nag}, but not LacS, replace IIA^{Glc}? Hurley et al. (1993) reported the crystal structure of glycerol kinase complexed with IIA^{Glc} and identified the residues of IIA^{Glc} that interact with glycerol kinase. Fig. 1 shows an alignment of *S. typhimurium* IIA^{Glc} and a number of IIA^{Glc}-like domains. Some of the residues of IIA^{Glc} that interact with glycerol kinase (indicated with # in Fig. 1), are conserved in all EII domains but others may differ. It cannot be excluded, however, that one or more of these non-conserved residues, present in IIA^{Glc}, are essential for interaction (e.g. Lys-44, which is also present in *K. pneumoniae* II^{Nag} and *B. subtilis* II^{Glc}).

LacS is distinguished from all other EII domains by substitutions in two of these (strongly) conserved positions—residues Leu-533 (Phe-71 in IIA^{Glc}) and Gly-556 (Asp-94 in IIA^{Glc}). Possibly, the Leu and/or Gly residue prevent binding of LacS to glycerol kinase and other target proteins. A more important clue may come from *E. coli* and *S. typhimurium crr* mutations that abolish inducer exclusion. These mutations are localized either in completely conserved positions (G47S, G47C, H75Q, and A76T; Presper et al. 1989; Zeng et al. 1992; Postma et al. 1994) or in residues that are not conserved at all (S78F and E97 K; see Zeng et al. 1989; Postma et al. 1994). Replacement of Glu-97 by a positively charged lysine residue in IIA^{Glc} abolished inducer exclusion but not α MG phosphorylation and stimulation of adenylate cyclase (Postma et al. 1994). Figure 1 shows that LacS and some other EII domains contain a lysine residue at the equivalent position of Glu-97, which may explain the failure of LacS to replace IIA^{Glc} in PTS-mediated inducer exclusion. If this explanation is correct, one can predict that EII^{Bgl} should also be deficient in PTS-mediated inducer exclusion.

Both II^{Nag} and LacS are unable to restore growth of *crr* mutants on succinate and citrate (i.e. to stimulate adenylate cyclase). In the case of LacS this is most likely to be due to the inability of *S. typhimurium* P-HPr to phosphorylate the LacS protein. II^{Nag} can be phosphorylated, however. In contrast, the IIA^{Glc}-like domain of the *B. subtilis* II^{Glc}, when expressed as a separate domain, is able to restore glucose-mediated repression of β -galactosidase in an *E. coli crr nagE* mutant (Reizer et al. 1992). The different capacities of the IIA^{Glc}-like domain of II^{Nag} and the *B. subtilis* enzyme IIA^{Glc} domain may be explained in several ways. Possibly an essential residue for interaction with, and activation of, adenylate cyclase is lacking in II^{Nag} but present in *B. subtilis* IIA^{Glc}. Alternatively, the

		#####	#
STYIIAGLC	22	IVAPLSGEIVNIEDVDPVVF AEKIV GDGIAIKPTGNKMVAPVDGTIGKIF	71
KPNIINAG	503	LVSPITGDDVVALEQVPDEAF ASKAV GDGIAVKPTDNIIVVAPAAAGTVVKIF	552
ECOIIBGL	479	ICSPMTGEIVPLIHVADTT FASGL LKGKIAILPSVGEVRSVPAGRIASLF	528
ECHIIARB	485	LFSPPLAGEVLLLEQVADR TFASG VMGKGIARPTQGRLYAPVDGTVASLF	534
BSUIIGLC	552	FVSPITGEIHPITDVPDQ VFSGK MMGDGFAILPSEGI VVSPVRGKILNVF	601
SCAIIGLC	531	VYAPIKGEVVDISEVPD KVFSEK MMGDGIAIKPETGEVVAPFDGVVVMVF	580
CGLIIMAN	534	IVSPLEGKAIPLSEVPD PIFAAG KLPGIAIQPTGNTVVAPADATVILVQ	583
BLAIIGLC	526	ITSPLEGHAVPLSEVPD PIFAAG KLPGIAIEPTGNTVVAPADATVILVQ	575
SMUIISCR	517	LAAPLAGEAVELTSVND PVFS SEAMGKGIAPSGNTVYAPVDGTVQIAF	566
SSOIISCR	486	IASPLDGEAVELSKVND PVFS SEAMGKGIAPSGNTVYSPVNGTVQIAF	535
PPEIISCR	494	ISAPVTGASESLKQVND QVFS AEIMGKGAIVPSSDQVVAPADGVITVTY	543
PPERAFP	491	LATPIAGQVIPLAQVND PTFAAG TLDGFAIKPSDGRILAPFDATVRQVF	540
STHLACS	484	LVTPPTGYLVLDLSSVNDE HFASG SMGKFAIKPTDGAVFAPISGTIRQIL	533
		* * * * *	
		# # # # #	
STYIIAGLC	72	ETNHAFSIESDSGIEL FVHFG IDTVELKGEFGFKRIAEQGVRVKGDPVIE	121
KPNIINAG	553	NTNHAFCLETNNGAEIV VHMG IDTVALEGKGFKRLVEEGTDVKAGEPIE	602
ECOIIBGL	529	ATLHAIGIESDDGVEIL IHVGI DTVCLDGKFFSAHVNVGDKVNTGDRLLS	578
ECHIIARB	535	KTHHAIGLASRGAEVL IHVGI DTVRLDGRYFTPHVRVGDVVRQGDLLLE	584
BSUIIGLC	602	PTKHAIGLQSDGGREIL IHFGI DTVSLKGEFGFTSFVSEGDVREPQKLE	651
SCAIIGLC	581	PTKHAIGLESKDGI ELLHFG LETVKLEGGFDILVKENDNIVLGQPLMK	630
CGLIIMAN	584	KSGHAVALRLDSGVEIL VHVG LDTVQLGEGFTVHVERRQVQKAGDPLIT	633
BLAIIGLC	576	KSGHAVALRLDSGVEIL IHIG LDTVQLGEGFKVHVERKQVQKAGDPLIT	625
SMUIISCR	567	DTGHAYGIKSDNGAEIL IHIG DTVSMNGTGFDQKVAANQTVKVGDLVLT	616
SSOIISCR	536	ETGHAYGLKSDNGAEVL IHVGI DTVSMNGTGFDQKVAANQTVKVGDLVLT	585
PPEIISCR	544	DSHHAYGIKTTAGAEIL IHLG LDTVNLNGEHFTTNVQKGDVTHQGDLLGT	593
PPERAFP	541	TTRHAYGLVGDNGIVLL IHIG LGTVKLRGTGFISYVEEQGHVQGGDELLE	590
STHLACS	534	PTRHAYGIESDGVIVL IHVGI GTVKLNGEGFISYVEEQGDRVEVGQKLE	583
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Fig. 1. Alignment of *Salmonella typhimurium* IIA^{Glc} (residues 22–121) with the corresponding homologous regions of the IIA^{Glc}-like domains of several EIIs and the LacS protein. Residues of IIA^{Glc} that interact with glycerol kinase, as determined by Hurley et al. (1993), are indicated by #. Conserved residues are in **bold**. In addition, residues 47 and 76 are shown in *bold face*; they are altered by *crr* mutations that abolish inducer exclusion (Zeng et al. 1992; Postma et al. 1994). Residues conserved in all domains are indicated asterisks. STYIIAGLC, *S. typhimurium* IIA^{Glc} (PIR, A03405); KPNIINAG, *K. pneumoniae* enzyme II^{Nag} (PIR, S18607); ECOIIBGL, *E. coli* enzyme II^{Bgl} (SwissProt, P08722); ECHIIARB, *Erwinia chrysanthemi* II^{Arb} (SwissProt, P26207); BSUIIGLC, *B. subtilis* enzyme II^{Glc} (SwissProt, P20166); SCAIIGLC, *Staphylococcus carnosus* enzyme II^{Glc} (GenPept X80415); CGLIIMAN, *Clostridium glutamicum* II^{Man} (GenPept, L18874); BLAIIGLC, *Brevibacterium lactofermentum* II^{Glc} (GenPept, L18875); SMUIISCR, *Streptococcus mutans* II^{Scr} (SwissProt, P12655); SSOIISCR, *Streptococcus sobrinus* II^{Scr} (GenBank, L06791); PPEIISCR, *Pediococcus pentosaceus* II^{Scr} (GenPept, L32093); PPERAFP, *P. pentosaceus* RafP protein (GenPept, L32093); STHLACS, *S. thermophilus* LacS protein (SwissProt, P22733). *E. coli* IIA^{Glc} and II^{Nag} and *Lactobacillus delbrueckii* LacY are not shown but are very similar to *S. typhimurium* IIA^{Glc}, *K. pneumoniae* II^{Nag} and LacS from *S. thermophilus*, respectively. Three recent depositions (U15110, Z34526, and U15147) have not been included.

IIA^{Glc}-like domain in the membrane-bound II^{Nag} may have no access to adenylate cyclase, because, in contrast to the soluble *B. subtilis* IIA^{Glc} domain, its movement may be restricted by the nature of its covalent linkage to the IICB^{Nag} domain; mobility of the link is determined largely by the length and the flexibility of the linker sequence between the IIA^{Nag} and IICB^{Nag} domains (Vogler and Lengeler 1991; Schunk et al. 1992).

In summary, our results demonstrate that a membrane-bound EII, containing a IIA^{Glc}-like domain, can, like the soluble IIA^{Glc}, interact with both soluble proteins like glycerol kinase, and membrane-bound complexes like the maltose transport system.

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