Antonie van Leeuwenhoek **78:** 253–261, 2000.

Glucose kinase of *Streptomyces coelicolor* A3(2): large-scale purification and biochemical analysis

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Key words: carbon catabolite repression, glucose kinase, *Streptomyces*

Abstract

Glucose kinase of *Streptomyces coelicolor* A3(2) is essential for glucose utilisation and is required for carbon catabolite repression (CCR) exerted through glucose and other carbon sources. The protein belongs to the ROK-family, which comprises bacterial sugar kinases and regulators. To better understand glucose kinase function, we have monitored the cellular activity and demonstrated that the choice of carbon sources did not significantly change the synthesis and activity of the enzyme. The DNA sequence of the *Streptomyces lividans* glucose kinase gene *glkA* was determined. The predicted gene product of 317 amino acids was found to be identical to *S. coelicolor* glucose kinase, suggesting a similar role for this protein in both organisms. A procedure was developed to produce pure histidine-tagged glucose kinase with a yield of approximately 10 mg/l culture. The protein was stable for several weeks and was used to raise polyclonal antibodies. Purified glucose kinase was used to explore protein-protein interaction by surface plasmon resonance. The experiments revealed the existence of a binding activity present in *S. coelicolor* cell extracts. This indicated that glucose kinase may interact with (an)other factor(s), most likely of protein nature. A possible cross-talk with proteins of the phosphotransferase system, which are involved in carbon catabolite repression in other bacteria, was investigated.

Abbreviations: CCR – carbon catabolite repression; PEP – phosphoenolpyruvate; PTS – phosphoenolpyruvate-dependent sugar:phosphotransferase system

Introduction

Streptomycetes are high-GC Gram-positive soil bacteria with a complex mycelial life cycle (Chater & Hopwood, 1983; Hopwood et al. 1995; Chater 1998). When nutrients become limited they are able to undergo several morphological stages, thereby producing secondary metabolites including antibiotics, immunosuppressants, and insecticidals (Chater 1993; Bibb 1996). Because of their economic and medical relevance, research has focussed mainly on secondary metabolism resulting in the identification of numerous antibiotic biosynthesis pathways (Baltz 1998). Although the production of secondary metabolites is linked to the availability of nutrients, relatively little is known about how streptomycetes sense and regulate the utilisation of carbon sources (Chater & Hopwood 1983).

Streptomycetes control the preferential use of readily metabolisable carbon sources via a mechanism termed carbon catabolite repression (CCR) (Chater & Hopwood 1983). The phenomenon of CCR has been studied extensively in the low-GC Gram-positive bacterium *Bacillus subtilis* and in the Gram-negative organism *Escherichia coli*. The proposed models show that the responsible mechanisms are complex and diverse (Stülke & Hillen 1999; Mahr et al. 2000). Nevertheless, proteins of the phosphoenolpyruvate-

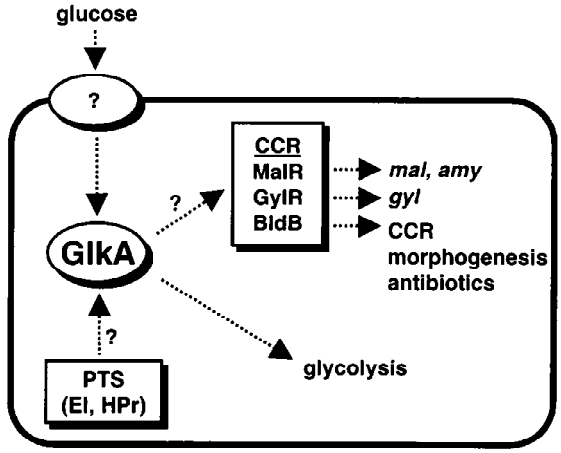

Figure 1. Model of carbon catabolite repression in *S. coelicolor*. The signal transduction pathway of CCR begins with the sensing and uptake of carbohydrates. A putative permease for glucose uptake is depicted. Glucose kinase GlkA may integrate these signals and may transmit them to transcriptional regulators such as MalR, GylR, or BldB. Further candidates to fill in the pathway are the general PTS components EI and HPr (see Parche et al. 2000 this issue). *amy* (amylase), *mal* (maltose operon), *gyl* (glycerol operon). For further explanation see text.

dependent sugar:phosphotransferase system (PTS) play a key role in each case (Saier 1989; Postma 1993). The existence of a PTS has been shown biochemically in three *Streptomyces* species (Titgemeyer et al. 1994b; 1995). Recently, a histidine phosphocarrier protein HPr of the *S. coelicolor* PTS complement has been described in molecular detail (Butler et al. 1999; Parche et al. 1999). But unlike in *B. subtilis* or *E. coli* a link between the PTS and CCR could not yet be established.

In *S. coelicolor* the enzyme glucose kinase (gene *glkA*), which is crucial for glucose utilisation, seems to be of central importance for CCR (Figure 1) (Angell et al. 1992a; 1994; Kwakman & Postma 1994). Mutants in *glkA* are unable to grow on glucose and were shown to be deregulated in glucose repression of catabolite-controlled genes including those for the utilisation of agar, glycerol, maltose, and galactose (Hodgson 1982; Angell et al. 1992; Mattern et al. 1993; Hindle & Smith 1994; van Wezel et al. 1997). These mutants could no longer confer CCR even if the repressing carbon source was not metabolised via GlkA (Kwakman & Postma 1994). Introduction of the corresponding gene from *Zymomonas mobilis* resulted in restoration of glucose utilisation but failed to restore CCR (Angell et al. 1994). Therefore, it was suggested that CCR is mediated by glucose kinase through a regulatory site that is different from the catalytic site. Analysis of the evolutionary relationship of GlkA showed that it belongs to the ROK-family, which comprises sugar kinases and transcription factors (Angell et al. 1992; Titgemeyer et al. 1994a). Since glucose kinase contains no obvious DNA-binding motif and is therefore unlikely to directly regulate transcription, it has been proposed that it eventually transmits CCR-signals via interaction with transcription factors (Angell et al. 1992). These may include pathway-specific regulators like MalR and GylR, repressors of the maltose and glycerol operons, respectively, or pleiotropic regulators like CcrA and BldB (Hindle & Smith 1994; Ingram et al. 1995; Pope et al. 1996; van Wezel et al. 1997).

In this communication, we report on an approach to improve our understanding of the role of glucose kinase. We show that glucose kinase is present in the cell at similar levels under repressing and non-repressing growth conditions, and that the *S. coelicolor* and *Streptomyces lividans* GlkA homologues are identical. Glucose kinase was purified to homogeneity and antibodies were raised against the histidine-tagged protein. Finally, a possible cross-communication of glucose kinase with proteins of the PTS was also investigated.

Materials and methods

Bacterial strains

S. coelicolor A3(2) M145 (wild-type, SCP1$^-$, SCP2$^-$) and *S. coelicolor* M480 (M145 Δ*glkA*) were used for monitoring *glkA* synthesis and for surface plasmon resonance experiments (Hopwood et al. 1985; Angell et al. 1994). *S. coelicolor* A3(2) M145 and *S. lividans* TK24 were used for the isolation of chromosomal DNA. *E. coli* DH5α was used for standard cloning procedures (Sambrook et al. 1989). Overexpression of histidine-tagged GlkA (His$_6$-GlkA) was performed in *E. coli* FT1 (Δ*ptsHIcrr* Km^R,pLysS) (Parche et al. 1999).

Glucose kinase activity assay

S. coelicolor strains M145 and M480 were pre-grown on 300 ml mineral medium (MM) supplemented with 50 mm mannitol under vigorous shaking at 28 °C for 36 h. MM had the following composition (initial pH 7.2): K_2HPO_4, 10 mM; KH_2PO_4, 10 mM; NH_4SO_4, 10 mM; $CaCl_2$, 0.5 mM; $MgSO_4$, 0.4 mM; casamino acids, 0.1%; PEG_{6000}, 1%; and trace element solution,

1 ml/l (stock solution consisting of $FeSO_4$, 50 mM; $ZnSO_4$, 40 mM; $MnCl_2$, 2 mM; $CaCl_2$, 2 mM). Washed cells were used to inoculate 50 ml MM supplemented with 50 mM glucose, fructose, glycerol, or mannitol. Cells were harvested after 2 h, washed two times in cold 150 mM NaCl and resuspended in a buffer containing 75 mM KH_2PO_4 (pH 7.0), 2 mM dithiothreitol, and 1 mM EDTA. Crude extracts were prepared by sonification at 50 W (Labsonic U, Braun; five times for 30 s) and subsequent removal of cell debris by centrifugation. Glucose kinase activity in cell extracts was assayed using 300 μg of protein as described previously (Skarlatos & Dahl 1998).

Preparation of antibodies and immunoblotting

New Zealand White rabbits were immunised with 100 μg of purified His_6-GlkA to generate polyclonal antibodies. For Western blot experiments *S. coelicolor* strains M145 and M480 were grown as described for the glucose kinase activity assay. Proteins of cell extracts were separated by SDS-polyacrylamide gel electrophoresis on a 7.5% polyacrylamide gel and transferred to a polyvinylidene difluoride membrane (Fluorotrans) by electroblotting. GlkA was detected with the obtained rabbit polyclonal antiserum (1:5000 dilution). GlkA antibodies were visualized by using the ECL Western blot analysis system (Amersham).

Cloning procedures and plasmid construction

Chromosomal DNA of *S. coelicolor* M145 and *S. lividans* TK24 was isolated as described previously (Parche et al. 1999). The *glkA* genes of both organisms were cloned as follows: DNA fragments of 981 bp were amplified by PCR from 500 ng chromosomal DNA using oligonucleotides GLK1 (ACTCTAGAACATATGGGTCTGACCATCGGCGTCGACATCGG) and GLK2 (GTCGCTCGAGGATCCTCACATGATCGGGTCGGGTTC) introducing *Xba*I/*Nde*I and *Xho*I/*Bam*HI restriction sites, respectively (restriction sites are underlined). GLK1 and GLK2 were designed based on *glkA* of *S. coelicolor* and are complementary to nucleotide positions – 12 to +29 (GLK1) and +969 to +934 (GLK2). The second and third codons of the *glkA* coding region in GLK1 were adapted to the codon bias of *E. coli*. PCRs were performed with *Taq* polymerase in the presence of Q-solution (Qiagen). The amplification products were digested with *Nde*I and *Bam*HI and cloned into expression plasmid pET15b (Novagen), which was cut with the same restriction endonucleases giving plasmids pFT61 (*glkA* of *S. coelicolor*) and pFT67 (*glkA* of *S. lividans*). Sequencing of DNA was performed on an ABI PRISM 310 Genetic Analyzer (Perkin-Elmer) with fluorescence-labelled dideoxyribonucleoside triphosphates provided in the BigDye Terminator Mix (Perkin-Elmer). The *glkA* sequence of *S. lividans* TK24 has been submitted to GenBank under accession no. AF228048.

Purification of His_6-GlkA

For the purification of recombinant His_6-GlkA of *S. coelicolor* M145, *E. coli* FT1(pLysS) was transformed with pFT61. Growth of cells and purification of protein was performed as described with the exception that gene expression was induced for 16 h (Parche et al. 1999). His_6-GlkA was purified from soluble cell extract on a Ni^{2+} HiTrap chelating column using the ÄKTA purifier (Pharmacia). His_6-GlkA eluted at approximately 300 mM imidazole from the column.

Dynamic Light Scattering

Experiments were performed on a DynaProMS instrument and data analysed with the DynaLS software. The protein was filtered through a Whatman Anodisc13 filter (0.1 μm). Experiments were done both at room temperature and at 4 °C, for comparison.

Circular dichroism

Experiments were performed on a Jasco J-715 Circular Dichroism Spectropolarimeter. Typically, measurements were done with a 1 nm band width at 22 °C and data points were collected every 0.1 nm. His_6-GlkA was used at 5 μm in 1 mm path length cuvettes in a buffer containing 10 mM NaH_2PO_4, pH 7.5.

Surface plasmon resonance analysis

Interactions were detected by surface plasmon resonance analysis using the BIAcore X optical biosensor (Pharmacia Biosensor AB). Mycelia of *S. coelicolor* strains M145 and M480 were grown on tryptic soy broth without dextran (TSB, Difco) supplemented with either 50 mM glycerol or glucose. His_6-GlkA and the tetracycline repressor His_6-TetR of *E. coli* (control for unspecific binding) were routinely immobilised on the surface of an NTA sensor chip (BIACore) to a surface concentration of 4.5 to 5 ng mm^{-2}. 200 μg protein of *S. coelicolor* cell extract were introduced at

a flow rate of 5 μl min^{-1} for 20 min. The standard running buffer was eluent buffer containing 10 mm HEPES (pH 7.4), 150 mM NaCl, 50 μM EDTA, and 0.005% (v/v) polysorbate 20.

Protein phosphorylation

[^{32}P]PEP was prepared from [γ^{32}P]ATP as described previously (Roossien et al. 1983). Reactions were carried out at 37 °C for 10 min in a total volume of 30 μl in 50 mM Tris/HCl (pH 7.5) and 10 mM $MgCl_2$ in the presence of either [^{32}P]PEP (0.1 μM) with a specific radioactivity of 4 mCi mmol^{-1} or [γ^{32}P]ATP as described (Reizer et al. 1998; Parche et al. 1999). The reactions were stopped by addition of 5 μl protein gel loading buffer. An aliquot was subjected to SDS-PAGE. Radiolabelled proteins were detected by radioluminography on a phosphoimager (Fuji).

Computer analyses

The LaserGene workstation software (DNASTAR, Inc.) was used to process DNA and protein sequence data. Secondary structure predictions were performed using the SOPMA secondary structure prediction method of the Network Protein Sequence Analysis server at the Pôle Bio-Informatique Lyonnais, Lyon, France (URL http://pbil.ibcp.fr) (Geourjon & Deléage 1995).

Results and discussion

Analysis of glkA *expression*

Since glucose kinase (GlkA) is known to be involved in CCR exerted by glucose, but also by carbohydrates that do not require the presence of a catalytically active GlkA (Figure 1), we addressed the question as to how much GlkA is present in the cell. To assess this, we have monitored glucose kinase activity and protein levels in *S. coelicolor* A3(2) M145 wild-type grown on liquid mineral medium under repressing and non-repressing conditions. GlkA activities of mycelia that were pre-grown on mannitol (neutral carbon source) and then exposed for 2 h to glucose, fructose, glycerol, or mannitol were compared (Figure 2). Glucose kinase activities between 814±24 and 962±13 nmol min^{-1} mg protein^{-1} were observed (Figure 2a). Similar activities were also detected when cells were exclusively grown on each of these carbon sources. As expected, the congenic *glkA* mutant strain M480 showed only residual glucose kinase activity, which can most likely be attributed to a second, minor glucose kinase activity (Angell et al. 1994).

To substantiate this result, the amounts of GlkA protein in cells were determined by Western blot analysis (Figure 2b). Immunosignals of GlkA of similar intensities were observed in extracts of mycelia that were grown on mineral medium with glucose, mannitol, or fructose. Thus, it appeared that *glkA* is constitutively expressed, independent of the carbon source used.

Glucose kinase activities have been studied in *Bacillus subtilis*, *Bacillus megaterium*, and *E. coli* (Meyer et al. 1997; Späth et al. 1997; Skarlatos & Dahl 1998). Cellular activities of the glucose kinases of *B. subtilis* and *B. megaterium* were shown to be carbon source-independent. Similar results were reported for glucose kinase of *E. coli*, with the exception that the activity was reduced about two-fold when cells were grown in the presence of glucose as the sole carbon source.

DNA sequence of S. lividans glkA

The role of glucose kinase in CCR was recently studied in the closely related *Streptomyces lividans* (Saito et al. 1998). The authors demonstrated by mutant analysis that a functional glucose kinase was required for glucose repression of chitinase. The *S. lividans* mutants could be complemented with respect to glucose utilisation and CCR by *glkA* of *S. coelicolor*. However, the sequence of *glkA* of *S. lividans* has not yet been determined. To find out how similar the *glkA* genes are in the two *Streptomyces* species, we have resolved the *glkA* sequence of *S. lividans* strain TK24 as described in Materials and methods. DNA sequence analysis of the respective plasmid pFT67 revealed 904 bp that matched with 99.8% to *glkA* of *S. coelicolor* giving identical gene products of 317 amino acid residues with a calculated molecular weight of 33,061 (accession nos. P40184 and AF228048). This strongly indicates that *glkA* of *S. lividans* has the same function as in *S. coelicolor*.

Overexpression and purification of GlkA

To study glucose kinase function and structure and to search for proteins that may communicate with GlkA, it is essential to establish an expression system that allows large-scale purification. Earlier attempts had failed to overproduce GlkA and often the high-GC gene content prevents heterologous expression of

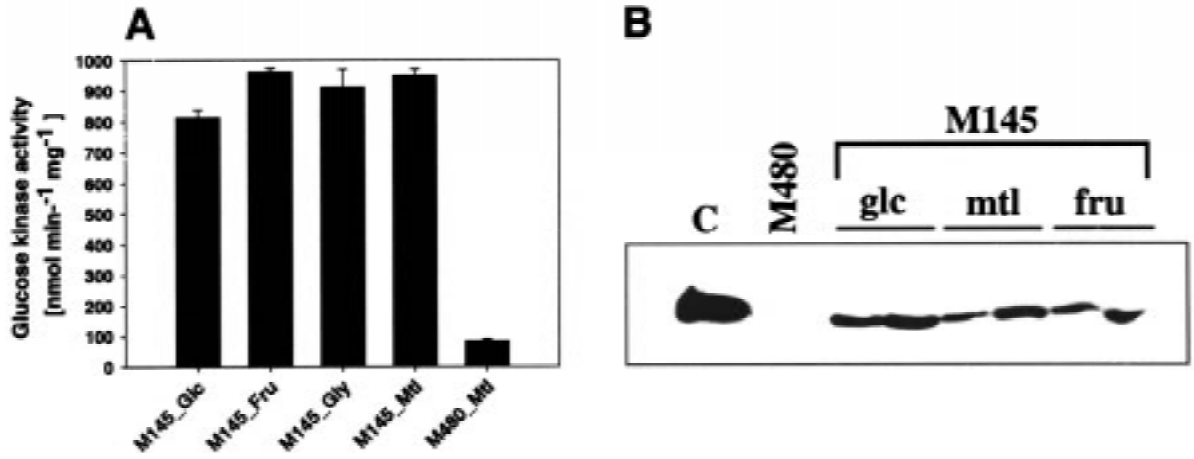

Figure 2. Carbon source dependent expression of *glkA* of *S. coelicolor*. (a) Glucose kinase activities of cell extracts of *S. coelicolor* strains M145 A3(2) (wt) and M480 (Δ*glkA*) are displayed. Activities were determined at least in triplicate. Error bars indicate standard deviations. (b) A Western blot of a 7.5% SDS-polyacrylamide gel is shown after incubation with rabbit polyclonal antibodies raised against His_6-GlkA of *S. coelicolor*. Lane His_6-GlkA: 100 ng of purified *S. coelicolor* His_6-GlkA. Lane M480: 50 μg of cell extract of strain M480 (Δ*glkA*) grown on MM with 50 mM mannitol. Lanes glc, mtl, and fru: 20 μg and 50 μg of cell extracts of strain M145 (wt) grown on MM with 50 mM glucose, mannitol, or fructose, respectively.

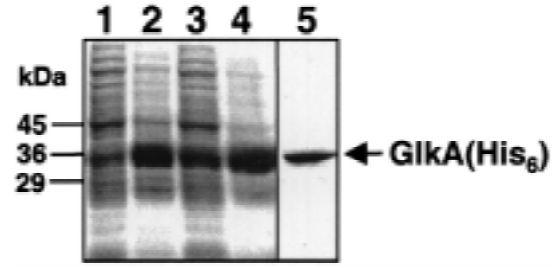

Figure 3. Overexpression and purification of His_6-GlkA. A 7.5% Coomassie stained SDS-polyacrylamide gel showing *E. coli* extract (20 μg) with overexpressed His_6-GlkA harvested after 4 h and 16 h, respectively, is shown. Lanes 1 and 2: soluble fraction and insoluble fraction after 4 h induction, respectively. Lanes 3 and 4: soluble fraction and insoluble fraction after 16 h induction, respectively. Lane 5: purified His_6-GlkA after affinity chromatography. Molecular weights of standard proteins are indicated in kDa.

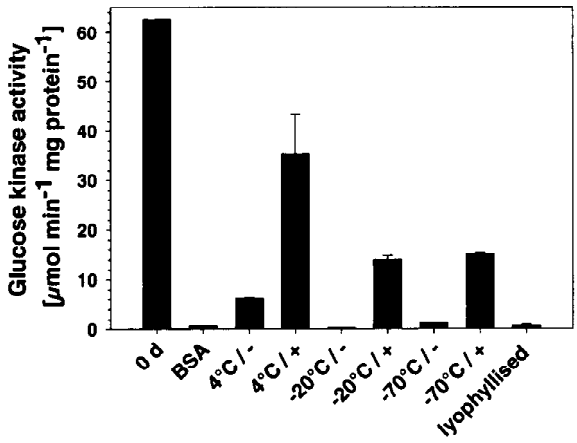

Figure 4. Activity of purified His_6-GlkA measured directly after purification (0 d), after lyophilisation (lyophillised), and after storage for 34 days at different temperatures. BSA was used as a negative control (BSA). +/− indicates the presence or absence of 15% glycerol, respectively.

Streptomyces genes (Angell et al. 1992; Parche et al. 1999). To achieve *glkA* overexpression, we used a previously successful strategy, which is based on gene fusion and heterologous expression in *E. coli* (Parche et al. 1999). The first codons of the gene fusion contain an optimal codon usage for *E. coli* allowing efficient initiation of translation. The gene product comprises six histidines (His_6) at the N-terminus for convenient one-step purification. A *glkA* production plasmid (pFT61) was constructed as described in Materials and methods and transformed to *E. coli* FT1(pLysS). Cells of the exponential growth phase of FT1 (pLysS, pFT61) were supplemented with IPTG to induce production of His_6-GlkA (Figure 3). Within the first 4 h after induction, almost all His_6-GlkA protein was overproduced in inclusion bodies, while after 16 h significant amounts of His_6-GlkA appeared in the soluble fraction. Glucose kinase assays of soluble cell extract exhibited an activity of 2713 nmol mg^{-1} min^{-1}. An activity of 9 nmol mg^{-1} min^{-1} was detected in *E. coli* control extracts, which reflected intrinsic glucose kinase activity. His_6-GlkA was purified from the soluble fraction by affinity chromatography yielding

about 10 mg protein/l of *E. coli* culture (Figure 3, lane 5). The purified protein migrated on the SDS-protein gel at a molecular size corresponding to 36 kDa, which is in good agreement with the calculated mass of 35,225 Da.

As a precondition for protein work, parameters were evaluated to characterise the quality of purified His_6-GlkA (Figure 4). Storage of the protein at 4 °C in the presence of either 15% glycerol, 1 mM ATP, or 1 mM glucose was found to maintain the protein active over a period of several weeks (Figure 4 and data not shown). Dynamic light scattering analysis demonstrated that His_6-GlkA produced in this way gave homogeneous pure protein (data not shown).

Analysis of secondary structure elements

Circular dichroism and *in silico* secondary structure prediction were used to obtain information on secondary structure. His_6-GlkA exhibited a circular dichroism spectrum typical for an α-helical protein (Figure 5a). This was reflected by minima at 209 nm and 220 nm, respectively. A computer-based analysis of secondary structure elements of GlkA predicted a composition of 32% α-helical content, 26% extended ß-strand structure, 13% ß-turn, and 29% random coil (Geourjon & Deléage 1995).

Interaction between glucose kinase and (an)other cellular factor(s)

As outlined in the introduction section, it was postulated that GlkA may interact with other proteins in signal transduction of CCR (Figure 1). We performed surface plasmon resonance experiments to address this hypothesis. A similar approach was previously successful to detect an interaction between HPr and glycogen phosphorylase in *E. coli* (Seok et al. 1997). His_6-GlkA was coupled to an NTA-sensor chip and cell extracts of *S. coelicolor* grown on TSB medium with either glucose or glycerol were allowed to flow over the immobilised protein. Binding signals of 850 and 270 resonance units were detected with extracts of mycelia grown on glucose or glycerol, respectively (Figure 6). No binding activity was detected with immobilised control protein His_6-TetR (tetracycline repressor). To rule out the possibility that the binding signal was obtained due to oligomerisation of GlkA, we demonstrated that a comparable binding signal was detected with cell extract of the *glkA* deletion mutant *S. coelicolor* M480 (data not shown). To validate the specificity, we demonstrated that application of *E. coli* cell extract did not yield any binding to His_6-GlkA. Since low molecular weight metabolites like ATP are too small to obtain binding signals in a range observed in these experiments, it can be concluded that the binding activity to glucose kinase resulted from an interaction with one or several factors, which were most likely of protein nature.

PEP-dependent phosphorylation of GlkA

To address the question whether glucose kinase is able to communicate with proteins via protein phosphorylation, we conducted ATP- and PEP-dependent phosphorylation assays. His_6-GlkA was not phosphorylated by either ATP or PEP (Figure 7, lanes 1 and 2). Then His_6-GlkA was incubated with radioactive labelled PEP in the presence of the PTS proteins enzyme I (EI) and HPr, which exhibit multiple regulatory functions in many other bacteria with respect to carbon control. When His_6-GlkA, EI, and PEP were incubated simultaneously, phosphorylation was observed solely for EI (lane 3). Addition of HPr resulted in the transfer of the phosphoryl group from EI to HPr (lane 4). No detectable phosphorylation at His_6-GlkA was observed. Thus, a cross-talk between GlkA and the general components of the PTS complement, with HPr as the most likely candidate, could not be demonstrated under the applied experimental conditions.

Conclusions

In this communication we report on an approach to further analyse the role of glucose kinase in *S. coelicolor*. We showed that *glkA* appears to be expressed constitutively. This finding supports previously reported data that demonstrated that GlkA is essential for CCR even if the repressive carbon source is not metabolized via GlkA (Angell et al. 1994; Kwakman & Postma 1994). Sequencing of *glkA* of *S. lividans* revealed that its gene product is identical to that of *S. coelicolor*, suggesting that CCR is regulated in similar ways in these organisms. An expression system for GlkA was established that allowed purification in amounts sufficient for protein chemical studies. The purified protein was catalytically active and its properties regarding stability and homogeneity indicated that it is perfectly suited for structure-function analysis. As an ultimate goal, it would be of interest to determine the three-dimensional structure of GlkA, in

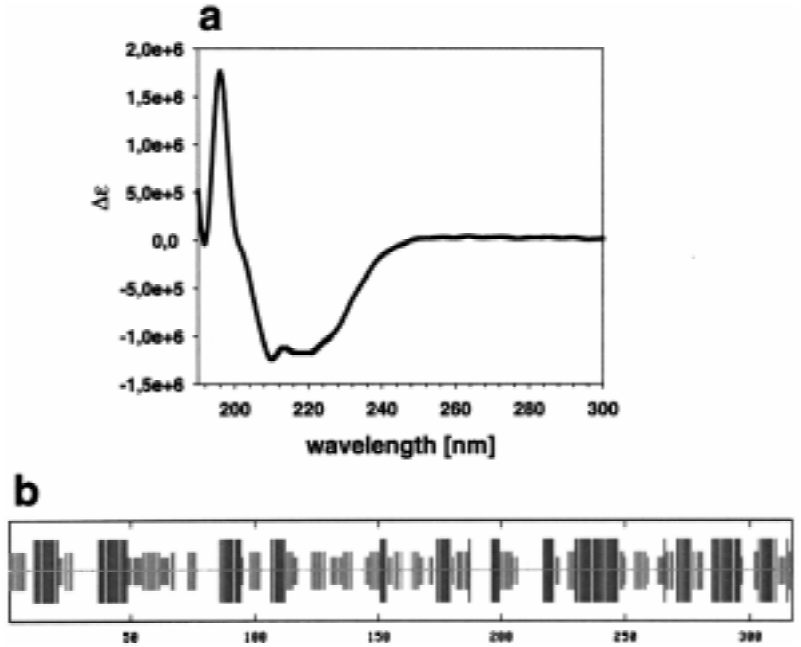

Figure 5. Circular dichroism and secondary structure prediction. (a) CD-spectrum of His_6-GlkA. The molar ellipticity ($\Delta\epsilon$) is presented as a function of wavelength. (b) Secondary structure prediction for GlkA. Amino acid positions of GlkA are depicted by numbers. Long dashes indicate α-helical regions, whereas medium dashes determine extended β-sheet regions. Short dashes show regions containing β-turns and regions of random coil structure are displayed by dots. One dash or dot represents one amino acid residue.

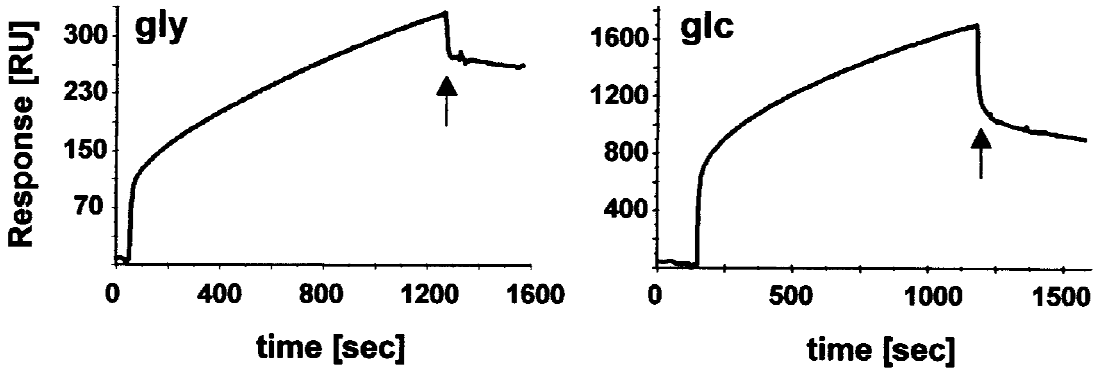

Figure 6. Surface plasmon resonance analysis. A real-time interaction analysis of His_6-GlkA with factor(s) present in extracts of *S. coelicolor* cells grown under repressing (glc) and non-repressing (gly) conditions is shown. The sensorgram represents the binding response in resonance units (RU) as a function of time. The end of injection is indicated by an arrow.

particular because so far no structure of a protein of the ROK-family has been solved. Crystallisation experiments are under way and have already yielded small crystals of His_6-GlkA (C. Svensson & U. Krengel, unpublished results). Using surface plasmon resonance analysis, we detected a GlkA binding activity, which is most likely of protein nature. This binding activity could result from one or several binding partners. Identification of the factor(s) by purification and N-terminal sequencing may ultimately reveal the relevance of interactions for the function of GlkA in glucose metabolism and CCR. Phosphorylation of His_6-GlkA by ATP, PEP, and by proteins of the PTS could not be demonstrated. The catalytic activity does not rule out the possibility that the recombinant protein (synthesised in *E. coli*) is not in the correct conformation to successfully participate in CCR, for example because posttranslational modifications are required but not applied in *E. coli* cells. In fact, the question whether catalytic and CCR functions of GlkA can be

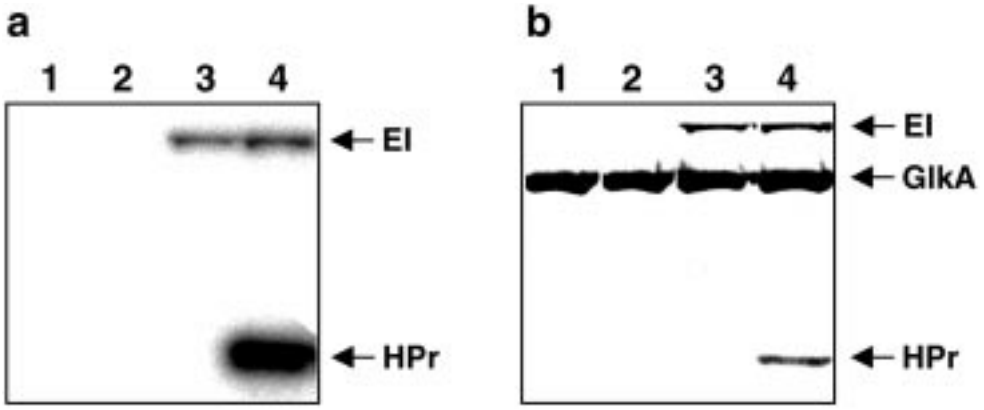

Figure 7. [γ^{32}P]ATP- and [^{32}P]PEP-dependent phosphorylation of His_6-GlkA. (a) A radioluminogram of a 15% SDS-polyacrylamide gel is shown. Lane 1: ATP and His_6-GlkA. Lane 2: PEP and His_6-GlkA. Lane 3: PEP, His_6-EI of *B. subtilis*, and His_6-GlkA. Lane 4: PEP, His_6-EI of *B. subtilis*, His_6-HPr of *S. coelicolor*, and His_6-GlkA. Pure proteins were present in the following amounts: His_6-GlkA, 150 pmol; His_6-EI, 30 pmol; His_6-HPr, 80 pmol. (b) The respective 15% Coomassie-stained SDS-polyacrylamide gel is shown. Proteins present in the different reactions are indicated.

assigned to separate domains is a very intriguing one, which we hope to answer by functional, structural, and mutational analysis of the protein.

Acknowledgements

This work was carried out in the laboratories of Wolfgang Hillen, whose support is greatly appreciated. We would like to thank Oliver Scholz for the gift of His_6-TetR. *S. lividans* TK24 was kindly provided by Andreas Schlösser. Marie Anderson at Astra Zeneca (Mölndal) is acknowleged for help with the dynamic light scattering experiments and Stephan Parche for critical reading of the manuscript.

This work was funded by the SFB473 of the Deutsche Forschungsgemeinschaft and by a grant to K.M. from the Graduiertenkolleg *Kontrolle der RNA-Synthese*. This project was further supported by a grant from the programme *Glycoconjugates in Biological Systems* sponsored by the Swedish Foundation for Strategic Research, providing funding for both C.S. and U.K.

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