View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Evidence for the phosphorylation of enzyme $II^{glucose}$ of the phospho*enol*pyruvate–sugar phosphotransferase system of *Escherichia coli* and *Salmonella typhimurium*

Krishna G. Peri, Hans L. Kornberg* and E. Bruce Waygood

Department of Biochemistry, University of Saskatchewan, Saskatoon, Saskatchewan, Canada and **Department of Biochemistry, University of Cambridge, Cambridge, England*

Received 15 March 1984

A membrane-bound phosphoprotein, of subunit M_r 48000 by sodium dodecyl sulphate-polyacrylamide gel electrophoresis, has been found in *Escherichia coli* and *Salmonella typhimurium*. The phosphorylation of this protein is dependent upon phospho*enol*pyruvate and the proteins of the sugar phosphotransferase (PT) system: enzyme I, HPr and $III^{glucose}$. The membrane-bound phosphoprotein is identified as enzyme $II^{glucose}$. Other membrane-bound phosphoproteins, that are also dependent upon the PT system, remain to be identified.

*Phospho*enol*pyruvate–sugar phosphotransferase system* *Enzyme $II^{glucose}$* *Phosphoprotein* *Sugar transport* *Factor $III^{glucose}$* *Membrane phosphorylation*

1. INTRODUCTION

The bacterial phospho*enol*pyruvate–sugar phosphotransferase (PT) system effects the concomitant transport and phosphorylation of many carbohydrates including glucose. In *Escherichia coli* and *Salmonella typhimurium* there are two main routes by which glucose is taken up via this system; these can be readily distinguished by the specificity they exhibit towards different glucose analogues [1] and by their different protein components [2]. One such route involves a membrane-bound enzyme II^{man} that catalyses the transport of mannose, glucosamine and 2-deoxyglucose as well as that of glucose; this is specified by the gene *ptsM* [3,4]. Associated with it is a factor III^{man} that is phosphorylated by the sugar-independent components of the PT system, phospho*enol*pyruvate (PEP), enzyme I and the small histidine-containing carrier protein HPr [2]. Similarly, the other (and main [4]) route for glucose uptake via the PT-system involves a membrane-bound enzyme II^{glc} that catalyses the transport of methyl α-glucoside as well as that of glucose; this is specified by the gene *ptsG* [3,5]. Associated with it is a factor III^{glc} that is also phoshorylated by PEP in the presence of enzyme I and HPr [6]. However, although the phosphorylation of membrane-bound phosphoproteins, identified as enzymes II for mannitol (II^{mtl}) and for *N*-acetylglucosamine (II^{nag}), was shown to occur and to be dependent on PEP and the components of the PT system, no unambiguous evidence for the phosphorylation of enzyme II^{man} or of enzyme II^{glc} had been obtained.

It is the purpose of this paper to show that there is a phosphoprotein, of subunit M_r 48000, in the membranes of *E. coli* and *S. typhimurium* whose phosphorylation is dependent on the PT system and whose properties suggest its identity as enzyme II^{glc}.

2. MATERIALS AND METHODS

The bacteria used, listed in table 1, were grown in 100 ml of the appropriate growth medium to late log phase (540 nm = 1.0). The cells were

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00 © 1984 Federation of European Biochemical Societies

Table 1

Bacterial strains used in this work

Strain	Genotype	Relevant phenotype	Source
Salmonella typhimurium			
SB3507	trpB223	Wild-type	[8]
Escherichia coli			
HK951	*metB ilv umgC*	Wild-type; constitutive for enzyme II^{glc}	[9,10]
HK952	*metB ilv umgC gsr*	Lacks III^{glc}; constitutive for enzyme II^{glc}	[9,10]
ZSC17[a]	*glk*	Wild-type	[3]
ZSC103	*glk7 ptsG2*	Lacks enzyme II^{glc}	[3]
ZSC112	*glk7 ptsG2 ptsM1*	Lacks enzyme II^{glc} and enzyme II^{man}	[3]
ZSC114	*glk7 ptsM1*	Lacks enzyme II^{man}	[3]

[a] Equivalent to ZSC13

harvested [2], and were resuspended in 1 ml of 0.01 M *N*-2-hydroxymethylpiperazine-*N'*-2-ethanesulphonic acid buffer (pH 7.5) containing 1 mM EDTA and 0.2 mM dithiothreitol. The cell suspensions were sonicated with a microprobe (3 × 10 s bursts with 1 min cooling inbetween bursts) and the membranes and supernatant solutions were separated by centrifugation (200000 × *g*, 2 h). The membranes were resuspended in 10 ml of the above buffer and recentrifuged. The pellet was then resuspended in 0.2 ml of the buffer. Subsequent procedures were as described in the legend to fig.1 and as described elsewhere [2,7].

3. RESULTS AND DISCUSSION

There are, in *S. typhimurium* and *E. coli*, a number of phosphoproteins whose phosphorylation is dependent upon PEP, enzyme I and HPr [2]. Such phosphoproteins (factors III for glucose, for mannose, for fructose and for sorbitol, as well as enzyme II^{mtl} and enzyme II^{nag}) all have properties indicating that they contain a N^3-phosphohistidine. In addition, a phosphoprotein of subunit M_r 48000 was found in membranes whose phosphorylation was dependent on PEP and the protein components of the PT system, but the labelled phosphoamino acid formed was more labile than was N^3-phosphohistidine.

As shown in fig.1, membranes from *E. coli* strain HK952 did not incorporate labelled phosphorus into any membrane-bound phosphoprotein with a subunit M_r between 33000 (factor III^{man}) and 65000 (added enzyme I) when they were incubated with [^{32}P]PEP, enzyme I and HPr. However, when factor III^{glc}, which has a subunit M_r of 20000, was added, ^{32}P was incorporated also into a phosphoprotein of subunit M_r 48000; the phosphorus-containing bond was labile in hot SDS-sample buffer. This protein is not the same as the soluble phosphoprotein of similar subunit mass described in [2], as the phosphorylation of that material was not dependent upon the PT system and was detected only in the presence of fluoride. In contrast, the phosphoprotein of subunit M_r 48000 found in membranes is readily seen in the absence of fluoride, both in crude extracts and in the separated membranes (fig.1, lane 6).

In *S. typhimurium* SB3507 and a variety of *E. coli* strains, the phosphorylation of this phosphoprotein was often observed in membranes although no factor III^{glc} had been added (fig.2, lane 2). This is due to the fact that even washed membranes from wild-type bacteria have some factor III^{glc} associated with them; this could be

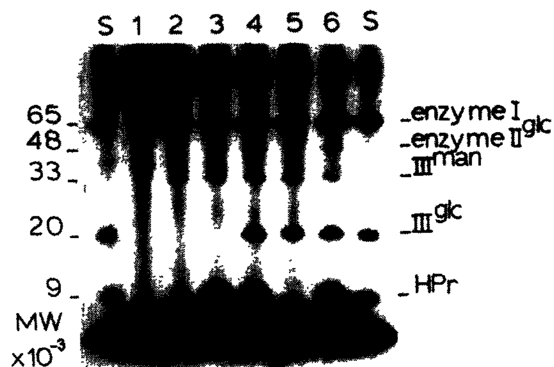

Fig.1. Phosphorylation of a membrane-bound protein of subunit M_r 48000 in *E. coli* strain HK952. *E. coli* strain HK952 was grown to mid log phase in nutrient broth medium containing 0.2% (w/v) glucose. The cells were harvested and membranes were prepared from crude extracts as described in section 2. Incubation mixtures, in a total volume of 0.03 ml, contained: 0.02 ml membrane preparation (10 mg protein·ml^{-1}), 0.1 mM [^{32}P]PEP (specific activity 10^5 counts·min^{-1}·$nmol^{-1}$), 5 mM $MgCl_2$, 12.5 mM NaF and added pure HPr, enzyme I and factor III^{glc} where indicated. The mixtures were incubated at 22°C for 5 min, after which the reactions were stopped by the addition of an equal volume of SDS-sample buffer (pH 8.0). The mixtures were boiled only where indicated, otherwise they were kept at room temperature for about 30 min, during which time 0.04 ml samples were loaded onto a 0.75 cm thick SDS–12% PAGE gel prepared as in [14]. The gel was run in a Borad Protean gel electrophoresis apparatus at 15 ma per 16 × 16 cm plate, maintained at 10°C during the 6–7 h of electrophoresis. Thereafter, the gel was frozen and autoradiographed for 60 h at −70°C [2]. The samples applied were: S, standard proteins of the PT system; enzyme I (M_r 65000), factor III^{glc} (M_r 20000) and HPr (M_r 9000); lane 1, membranes (140 µg); lane 2, membranes, with enzyme I (1.6 µg); lane 3, membranes with enzyme I and HPr (0.8 µg); lane 4, membranes with enzyme I, HPr and factor III^{glc} (0.2 µg); lane 5, the same as lane 4, but boiled for 1 min; lane 6, the same as lane 4, but NaF omitted from the incubation mixture.

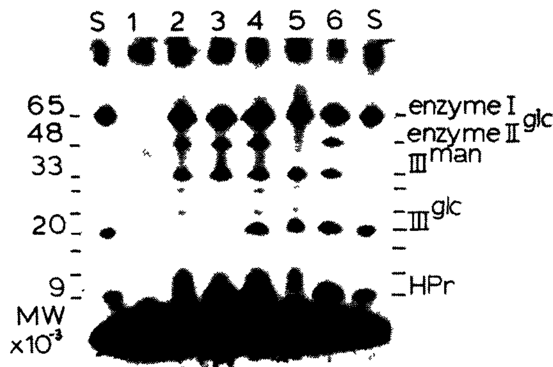

Fig.2. Phosphoproteins in the membranes of glucose-grown *S. typhimurium* strain SB3507. Membranes from *S. typhimurium* strain SB3507 were isolated from cells grown to late log phase on minimal salts and 0.2% (w/v) glucose. Samples were incubated as described in fig.1 and similarly run on SDS–PAGE gels.

detected as a phosphoprotein of subunit M_r 20000 when the autoradiographs of the SDS–PAGE gels were exposed for longer periods. Only with the *E. coli* strain HK952, which produces no factor III^{glc} as evidenced either by assay or by the failure to detect a phosphoprotein of M_r 20000, was the incorporation of ^{32}P into the membrane-bound phosphoprotein of subunit M_r 48000 consistently dependent on factor III^{glc}.

Analysis [1] of *E. coli* strains with lesions in their glucose phosphotransferases [3] confirmed that these strains lacked the enzymes II specified by *ptsG* and *ptsM*. Membranes were isolated from the *E. coli* ZSC strains (table 1) that had been grown in nutrient broth with 0.2% (w/v) glucose. When incubated with [^{32}P]PEP and the components of the PT system, membranes derived from the *ptsG* strains ZSC103 and ZSC112 did not incorporate labelled phosphorus into the phosphoprotein of subunit M_r 48000 whereas those from the wild-type cells and from the *ptsM* strains ZSC17 and ZSC114 did.

The dependence of the phosphorylation of this phosphoprotein on factor III^{glc}, its location in the membrane, and the results with these ZSC strains, suggest that this protein is enzyme II^{glc}. It is unlikely that it is a regulatory protein as originally proposed [11] as it is the unphosphorylated factor III^{glc} that acts as a regulator [12,13]. Enzyme II^{mtl} and enzyme II^{nag} have been shown to contain a N^3-phosphohistidine. The great lability, to heat and to acid, of the phosphorus-containing linkage in the phosphoprotein now identified as enzyme II^{glc} suggests that it does not contain a N^3-phosphohistidine but possibly a N^1-phosphohistidine or an acyl phosphate.

Both the ping-pong bi bi kinetics of the enzyme

II^{glc} [15] and stereochemical considerations [16] indicate that the membrane-bound enzyme II^{glc} should be phosphorylated in the course of translocating sugars via the PT system. Our present findings support these suggestions.

The autoradiograph (fig.2) of phosphorylated membranes isolated from *S. typhimurium* strain SB3507 shows that ^{32}P can be incorporated into a number of membrane phosphoproteins, and that this is dependent on the functioning of the PT system. These phosphoproteins, of subunit M_r 29000, 24000, 14000, 13500 and 10000, are possibly enzyme II^{man} and other enzyme II^{sugars}

ACKNOWLEDGEMENTS

We thank E. Erickson for technical assistance. K.G.P. is a recipient of a Saskatchewan Health Research Board Graduate Scholarship. This work was supported by the Medical Research Council of Canada and by the Science and Engineering Research Council, U.K.

REFERENCES

[1] Stock, J., Waygood, E.B., Meadow, N.D., Postma, P.W. and Roseman, S. (1982) J. Biol. Chem. 257, 14543–14552.

[2] Waygood, E.B., Mattoo, R.L. and Peri, K.G. (1984) J. Cell. Biochem., in press.

[3] Curtis, S.J. and Epstein, W. (1975) J. Bacteriol. 122, 1189–1199.

[4] Kornberg, H.L. and Jones-Mortimer, M.C. (1975) FEBS Lett. 51, 1–4.

[5] Kornberg, H.L. and Smith, J. (1972) FEBS Lett. 20, 270–272.

[6] Waygood, E.B., Meadow, N. and Roseman, S. (1979) Anal. Biochem. 95, 293–304.

[7] Mattoo, R.L., Khandelwal, R. and Waygood, E.B. (1984) Anal. Biochem., in press.

[8] Cordaro, J.C. and Roseman, S. (1972) J. Bacteriol. 112, 17–29.

[9] Parra, F., Jones-Mortimer, M.C. and Kornberg, H.L. (1983) J. Gen. Microbiol. 129, 349–358.

[10] Britton, P., Boronat, A., Hartley, D.A., Jones-Mortimer, M.C., Kornberg, H.L. and Parra, F. (1983) J. Gen. Microbiol. 129, 349–358.

[11] Dills, S.S., Apperson, A., Schmidt, M.R. and Saier, M.H. jr (1980) Microbiol. Rev. 44, 385–418.

[12] Osumi, T. and Saier, M.H. jr (1982) Proc. Natl. Acad. Sci. USA 79, 1457–1461.

[13] Nelson, S.O., Wright, J.K. and Postma, P.W. (1983) EMBO J. 2, 715–720.

[14] Laemmli, U.K. (1970) Nature 227, 680–685.

[15] Misset, O., Blaauw, M., Postma, P.W. and Robillard, G.T. (1983) Biochemistry 22, 6163–6170.

[16] Begley, G.S., Hansen, D.E., Jacobson, G.R. and Knowles, J.R. (1972) Biochemistry 21, 5552–5556.