Molecular Biology Reports **29**: 21–26, 2002.
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Flux control of the bacterial phosphoenolpyruvate:glucose phosphotransferase system and the effect of diffusion

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Abstract.

We analyzed the role of diffusion and cell size on the flux control properties of the glucose-PTS of *Escherichia coli*, in silicon cells under various metabolic conditions. To our surprise, the influence of the concentration of phosphoryl-donor PEP on the distribution of control was small. We found for cells of bacterial size that PTS-flux control was mainly located in processes taking place in the membrane and that diffusion hardly controlled the flux (< 2.8 %). Enlargement of the cells shifted the control from membrane to cytoplasm and from process rates to diffusion rates, the latter now having a total control of about 38 %. In the presence of glucose, nearly all diffusion flux control resided in the component that links the cytoplasmic processes to those in the membrane.

Abbreviations: glc: glucose; PEP: phosphoenolpyruvate; PTS: phosphoenolpyruvate: carbohydrate phosphotransferase system.

Introduction

Cellular processes that involve the movement of components from one place to another will always be affected by diffusion. Of course, the question is to what extent. Analysis of an imaginary membrane located kinase/ cytoplasmic phosphatase couple revealed that the effect of diffusion on flux and on the spatial distribution of the proteins, can be quite substantial in a eukaryotic cell (Kholodenko et al., 2000). Because average distances in bacteria are much smaller, diffusion potentially has less effect in bacteria. We decided to investigate the effect of diffusion on a real bacterial system, i.e. the phosphoenol-pyruvate(PEP):carbohydrate phosphotransferase system (PTS). The PTS combines the import and phosphorylation of carbohydrate (i.e. glucose) through protein-mediated transfer of a phosphoryl-group which derives from PEP (Postma et al., 1993). The inward flux of glucose is thus coupled to a flux of 'phosphoryl' towards the membrane, via the cycling of the PTS-proteins between phosphorylated and non-phosphorylated state (see Fig. 1).

We developed an *in silicon* replica of the glucose-PTS in *E. coli* (Blom and Peletier, 2000), combining the kinetic description of Rohwer et al. (2000) with *in vivo* diffusion data of the Green Fluorescent Protein (Elowitz et al., 1999). We calculated the flux and the spatial distribution of the different enzyme species and found that, in a bacterial cell, the effect of diffusion on the flux through the glucose-PTS and on the spatial distribution of most PTS-enzyme species is negligible. However, one of the components (non-phosphorylated IIA^{Glc}) showed significant gradients which might have implications for its regulatory function. These results will be described elsewhere in detail (Francke C., Postma P.W., Westerhoff H.V., Blom J.G. and Peletier M.A., *in preparation*). This paper is devoted to the analysis of the model using metabolic control analysis. In the main text we focus on flux control exerted by reaction and diffusion rates and in the appendix we look at the flux control exerted by enzyme concentration.

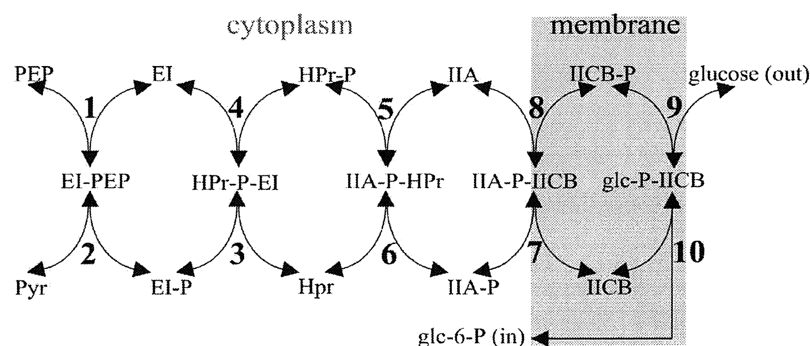

Figure 1 Reaction mechanism of the glucose-PTS of E. coli. *The system consists of three cytoplasmic proteins, Enzyme I (EI), HPr and IIA^{Glc}, and one membrane protein, $IICB^{Glc}$. These can be phosphorylated by each other and therefore can occur in non-phosphorylated, phosphorylated and complexed form. Glucose comes from the periplasm, is phosphorylated, and the resulting glucose-6-phosphate is released into the cytoplasm.*

Method

Metabolic Control Analysis (Kacser and Burns, 1973; Heinrich and Rapoport, 1974) serves as a methodological framework to evaluate the control of the different pathway components and/or their properties over a pathway quantitatively. The flux control coefficient C_i^J quantifies the importance of any reaction process '*i*' for the steady state flux *J*. Its sum over all pathway processes equals one: $\sum_i C_i^J = 1$ **(Eq. 1)**. However, in a live cell the flux is not only sustained by the 'chemical' rates but also by the diffusion rate, and as a consequence the control is distributed over the two. This sharing of control leads to an extended summation theorem: $\sum_i C_i^J + \sum_j C_{Dj}{}^J = 1$ **(Eq. 2)**, as shown by Peletier M.A., Westerhoff H.V. and Kholodenko B.N.(*in preparation*). D_j indicates the diffusion coefficient of species '*j*'.

We analyzed the flux control of any process rate using the method introduced by (Kacser and Burns, 1973): The forward and the reverse rate equations of process '*i*' are both multiplied by parameter λ_i. The derivative of the system flux is then taken with respect to this parameter, which is initially set at 1, so that: $C_i^J = (\partial \ln|J| / \partial \ln \lambda_i)_{steady\ state}$ **(Eq. 3)**. Likewise, the flux control of D_j is determined by introduction of a parameter λ_j into the diffusion equation of species '*j*'.

Results and Discussion

We used the *in silicon* replica of the glucose-PTS of *E. coli* developed earlier (Blom and Peletier, 2000) to investigate the behavior of the glucose-PTS under four different physiological conditions. We varied the availability of glucose (500 and 0.5 μM) and the cellular free energy in the form of PEP (2.8 and 0.09 mM), for cells of bacterial size (r = 0.6 μm) and for mammalian sized cells (r = 10 μm). Then the PTS-mediated glucose flux and spatial distribution of the PTS-enzyme species were calculated, and the flux control coefficients of the process and diffusion rates determined. The rationale for the exact choice of these model parameters can be found in the appendix.

In case the diffusion rate was set very high the model became equivalent to the '0D' model of Rohwer et al. (2000), and the results identical. The corresponding process flux control coefficients are represented in the four left panels of Fig. 2. The flux control summation theorem of Eq. 1 can be seen to hold (compare totals). Use of realistic diffusion rates led to a distribution of control over 'chemistry' and diffusion, as indicated by the totals in the central and right-hand panels. However, for small cells the contribution of diffusion to flux control remained insignificant (< 2.8 %). Addition of the totals confirms the extended summation theorem as derived by Peletier M.A., Westerhoff H.V. and Kholodenko B.N. (*in preparation*), given in Eq. 2.

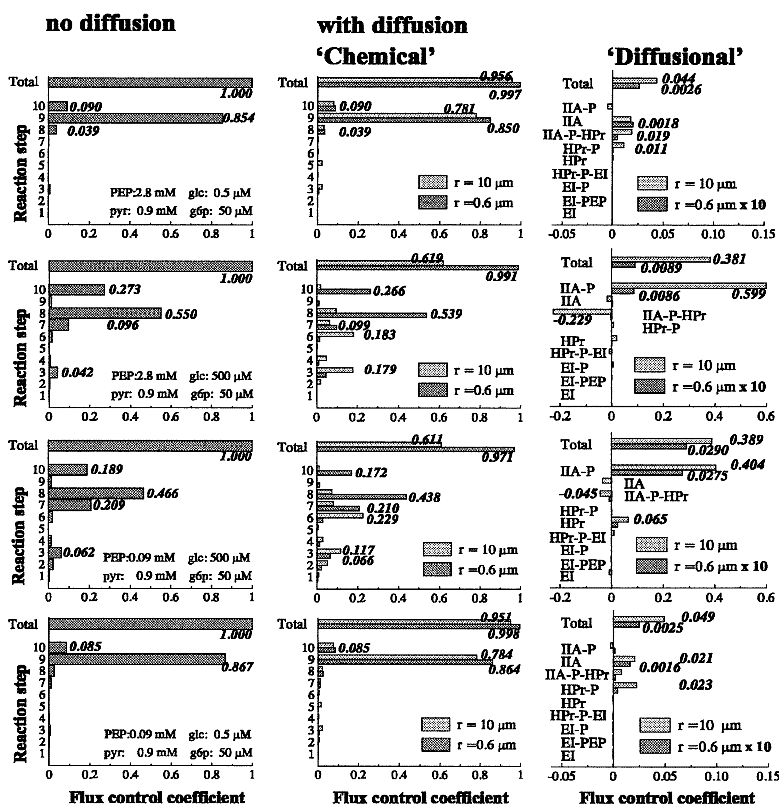

Figure 2 ***Flux control of the glucose-PTS under various metabolic conditions in model cells of different sizes.*** *The panels on the left present the control of the process rates over the influx of glucose in case diffusion is infinitely fast. The central panels give the flux control of the process rates for spherical cells with a 0.6 μm radius (dark gray) and a 10 μm radius (light gray). The panels on the right give the diffusion flux control in the same cells. The dark bars connected to the diffusion control in the small cells are enlarged 10 times, as indicated in the legend. Note however, that the associated numbers list the real value.*

The introduction of realistic diffusion hardly affected the distribution of control over the different processes in small cells, only a slight shift towards processes taking place in the cytoplasm was observed. Then upon increasing the cell size (light gray bars), larger shifts of the control from membrane to the cytoplasm occurred. Surprisingly, changes in the concentration of phosphoryl-donor PEP also sorted little effect on the overall distribution of control. This means that the distribution of the control over the glucose flux is rather insensitive to the cellular free energy state, which can vary dramatically during glucose uptake (Hogema et al., 1998).

Changes in the glucose concentration affected the flux and the distribution of control. In the absence of glucose (upper and lower panels), reaction 9, the binding of glucose to the membrane carrier, had about 85% of the control and even in large cells with diffusion it was not lower than 78%. In the presence of glucose, in small cells, the processes in the membrane controlled the flux, i.e. the transfer of phosphoryl from cytoplasmic IIA^{Glc} to the membrane carrier $IICB^{Glc}$ (reaction 7 and 8) and the release of glucose-6-phosphate (reaction 10). There was some control in the binding of phosphorylated EI to HPr (reaction 3). In large cells control shifted to the production of phosphorylated IIA^{Glc} (reaction 6) and for about 40% to diffusion. Most of the diffusion control over the flux lay with phosphorylated IIA^{Glc}, so that in fact only hindering the movement of phosphorylated IIA^{Glc} will influence the glucose influx. Thus, nearly all diffusion flux control is exerted by the component that links the processes in the cytoplasm to those in the membrane.

Acknowledgments : Last April Pieter W. Postma passed away. His work forms the solid basis of this project. The authors wish to thank F. Bruggeman for useful comments. The work was supported in part via the ICES-KIS II program and by NWO.

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Appendix

Model parameters

Details of the model can be found in Blom and Peletier (2000). The parameters used were based solely on experimental data (see also Rohwer et al., 2000). For our modelling we assumed the cell to be a sphere with radius 0.6 μm for a bacterial cell (Woldringh and Nanninga, 1985), or 10 μm for a mammalian cell. The diffusion constant of the Green Fluorescent Protein (27 kDa) in live *E. coli* (Elowitz et al., 1999) was used to calculate that of the PTS 'enzyme species'. The diffusion constants were calculated assuming that: i) all enzyme species were spheres, ii) the diffusion coefficient varied linearly with the inverse of the radius of that sphere, and iii) the volume of the sphere varied proportionally with the mass of the protein species. We thus arrived at a diffusion coefficient of: 3.15 $\mu m^2\ s^{-1}$ for HPr-P-EI; 3.30 $\mu m^2\ s^{-1}$ for EI, EI-P, EI-PEP; 4.37 $\mu m^2\ s^{-1}$ for IIA^{Glc}-P-HPr; 5.00 $\mu m^2\ s^{-1}$ for IIA^{Glc}, IIA^{Glc}-P; and 6.30 $\mu m^2\ s^{-1}$ for HPr, HPr-P.

The concentrations of the PTS enzymes were taken from Rohwer et al. (2000): 5 μM for EI; 50 μM for HPr; 40 μM for IIA^{Glc}; and 10 μM (volume averaged concentration) for $IICB^{Glc}$. To evaluate the influence of glucose and the cellular free energy state we chose the following metabolite concentrations: 2.8 or 0.09 mM for PEP; 0.9 mM for pyruvate; 500 or 0.5 μM for glucose; and 50 μM for glucose-6-phosphate. The PEP and pyruvate concentrations were taken from Hogema et al. (1998), who determined these in glucose grown cells. The lower PEP concentration was below the K_m ($K_{m\ PEP}$ = 20 μM, Rohwer et al., 2000). The glucose concentration was chosen such to be either at saturation or below the K_m ($K_{m\ glucose}$ = 20 μM, Rohwer et al., 2000). The above parameters were used to calculate the steady state influx of glucose and the distribution of the PTS-enzyme species throughout the cell. Control coefficients were obtained as mentioned in the method section. The flux response coefficients towards changes in the enzyme (E_k) concentration: R_{Ek}^{J}, or towards changes in the metabolite concentration: $R^J_{PEP+pyr}$ and $R^J_{glc+glc\text{-}6\text{-}P}$, were obtained in a similar way after multiplication of the enzyme concentration with parameter λ_k and of the metabolite concentration with $\lambda_{metabolite}$.

Enzyme flux response coefficients

In most pathways the response to a change in enzyme concentration is equivalent to the response to a change in the rate of the process the enzyme is involved in, but not in group transfer pathways. Whereas the enzyme flux response coefficients normally add up to one, van Dam et al. (1993) showed that in a group transfer pathway the sum could be as high as two. Kholodenko and Westerhoff (1995) derived an exact relationship for this sum, i.e.:

$$\sum_m R^J_{metabolite} + \sum_k R_{Ek}^{J}(1 + (Q_{k-1}+Q_k)/E_k) = 2\sum_i C_i^J \quad \textbf{(Eq. 4)}$$

Here, Q_{k-1} and Q_k indicate the heterodimeric complexes containing enzyme E_k. Equation 4 shows that the extent to which the enzymes control the flux is determined by the presence of these complexes. It also shows that increasing the complex concentration in general will reduce the flux control of the enzymes. In table 1 it is shown that in case diffusion had no control Eq. 4 was valid.

However, when we took limited diffusion into account the relationship broke down (see table 1 b and c). In a live cell, changes in the concentration of an enzyme species depend on both process rate as well as on diffusion rate. Because the import and phosphorylation of glucose proceed only at the membrane, PTS-enzyme species specific concentration gradients arise. Consequently, process and diffusion rates, their ratio and the total rate will diversify depending on the position inside the cell and on the enzyme species under consideration. Eq. 4 relates 'global' elements, like membrane flux (J), R_{Ek}^{J} and C_i^J, to 'local' elements, like

the concentration of enzyme species Q_{k-1} and Q_k. The addition of diffusion related parameters would introduce other 'local' elements into Eq. 4. The introduction of spatiality thus makes it difficult to derive a general enzyme flux response summation theorem for this/a group transfer pathway.

Table 1 Total glucose flux response coefficients for the glucose-PTS in the presence and absence of glucose and phosphoryl-donor PEP. *The calculations were made: a) assuming infinite diffusion rates, and b/c) for bacterial and mammalian sized cells assuming diffusion rates found in bacteria (Elowitz et al., 1999). For the calculation of the 'correction factor' from Eq. 4 the concentrations of the heterodimeric enzyme species (Q_{k-1} and Q_k) near the membrane were taken.*

	PEP: 2.8 mM		PEP:0.09mM	
a: Diffusion infinite glc:	0.5 μM	500 μM		0.5μM
$\sum_i R^J_{metabolite}$	0.917	-0.017	0.023	0.9398
$\sum_k R^J_{Ek}$	0.9557	1.1142	1.1467	0.8715
$\sum_k R^J_{Ek} \cdot (Q_{k-1} + Q_k)/[E_k]$	0.1273	0.9025	0.8301	0.1886
total	2	2	1.9999	1.9999
b: Diffusion finite (r= 0.6μm)				
$\sum_i R^J_{metabolite}$	0.9134	-0.019	0.023	0.9365
$\sum_k R^J_{Ek}$	0.9538	1.1162	1.1341	0.8704
$\sum_k R^J_{Ek} \cdot (Q_{k-1} + Q_k)/[E_k]$	0.1305	0.8752	0.8089	0.1906
total	1.9977	1.9727	1.966	1.9975
c: Diffusion finite (r= 10μm)				
$\sum_i R^J_{metabolite}$	0.8282	-0.1244	0.026	0.851
$\sum_k R^J_{Ek}$	0.9431	1.0372	0.939	0.871
$\sum_k R^J_{Ek} \cdot (Q_{k-1} + Q_k)/[E_k]$	0.2032	0.2864	0.6524	0.231
total	1.9745	1.1992	1.6171	1.9529