Cloning, Heterologous Expression, and Characterization of Three Aquaglyceroporins from Trypanosoma brucei* 

Nestor L. Uzcategui‡‡, Alexander Szallies‡, Slavica Pavlovic-Djuradovic‡, Monica Palmada‡, Katherine Figarella‡‡*, Christoph Boeher†, Florian Lang‡, Eric Beitz‡, and Michael Duszenko‡‡

From the ‡Biochemical Institute, †Department of Pharmaceutical Biochemistry, and ‡‡Institute of Physiology, University of Tübingen, 72076 Tübingen Germany

The protozoan parasite Trypanosoma brucei undergoes a complex life cycle including two dividing stages, i.e. the long slender form in blood, lymphatic fluid, and cerebrospinal fluid of its mammalian host and the procyclic form in the mid-gut of the tsetse fly. Due to the lack of a functional Krebs cycle and oxidative phosphorylation capabilities, the bloodstream forms depend on glycolysis for ATP production, with glycerol as an alternative substrate (1, 2). Glycolysis in trypanosomes differs markedly from the corresponding pathway in higher eukaryotes. Most strikingly, the first seven glycolytic reactions occur in a peroxisome-like organelle called glycosome (3).

Under aerobic conditions, glucose is converted into pyruvate, which is disposed by facilitated diffusion (4). The concurrently formed NADH cannot leave the glycosome and is reoxidized during the formation of glycerol-3-phosphate from dihydroxyacetone phosphate. Glycerol-3-phosphate enters the mitochondrion, in which it is oxidized by glycerophosphate oxidase. This enzyme uses molecular oxygen (alternative respiration) and is inhibited by salicylhydroxamic acid. Finally, the newly formed dihydroxyacetone phosphate re-enters the glycosome and can be glycolytically converted. Obviously, suppression of glycolysis by blocking the release of pyruvate or glycerol is a potential therapeutic approach against sleeping sickness (2, 4–6).

Under anaerobic conditions or in the presence of salicylhydroxamic acid, glucose degradation results in equimolar amounts of pyruvate and glycerol to maintain the redox balance within the glycosome (7). In this case, glycerol is formed from glycerol-3-phosphate by glycerokinase, which in trypanosomes leads to the formation of ATP (8). To drive this reaction to ATP formation, glycerol has to be readily released. Consequently, in the presence of salicylhydroxamic acid, the addition of 5 mM glycerol to the medium reverses the glycerol diffusion gradient across the membrane and results in cell death. A therapeutic exploitation of the glycerol sensitivity of the parasite, however, has not yet been successful in situ (5, 9). Hence, more information is required to understand how the parasite copes with non-aerobic conditions, regarding metabolism and glycerol transport.

We have shown previously that glycerol uptake is consistent with facilitated diffusion through a transporter protein (10). This investigation gave clear evidence for an additional non-saturable channel, which remained active after inhibition of the transporter. As we now know, glycerol facilitators are members of the superfAMILY of major intrinsic protein and fall into the aquaporin branch. Aquaporins facilitate permeation of water (orthodox aquaporins) or of small non-ionic solutes, such as glycerol and urea (aquaglyceroporins). Aquaporins of both types are present throughout all kingdoms of nature and participate in diverse biological processes (11). Here, we report the identification and cloning of three aquaglyceroporins from...
**T. brucei** (TbAQP1, TbAQP2, and TbAQP3) and describe their functional expression and their biochemical characterization in *Saccharomyces cerevisiae* and in *Xenopus laevis* oocytes.

**MATERIALS AND METHODS**

*Strains and Media—T. brucei* bloodstream forms (strain 427; clone MITat 1.2) were either propagated in Sprague-Dawley rats and purified from blood using a DEAE-cellulose column (12) or cultured at 37 °C in a 5% CO₂ atmosphere using modified minimal essential medium as described previously (13). Procyelic forms, obtained from MITat 1.2 using a standard transformation protocol (14), were also grown in a modified minimal essential medium (15).

Yeast strains used in this study were W303-1A (MATa Leu2-3/112 ura3-1 trp1-1 his3-1115 ade2-1 can1-100 gal SCC2) and YSH295 (MATa Leu2-3/112 ura3-1 trp1-1 his3-1115 ade2-1 can1-100 gal SCC2 fps1 LEU2), kindly provided by Dr. Stefan Hohmann (Goteborg University, Goteborg, Sweden). Cells were grown on medium containing 2% peptone and 1% yeast extract (YPE) supplemented with 2% glucose or on synthetic medium for the selection of transformants (16). The phenotype was analyzed by a plate growth assay (see Fig. 4).

**Cloning of Aquaglyceroporin Genes from T. brucei and Heterologous Expression in *S. cerevisiae*—**The complete cDNAs of TbAQP1–3 were cloned by reverse transcriptase-PCR using RNA obtained from bloodstream form and primers against the spliced leader sequence (5'-GATCAG-3'). The PCR products were purified by agarose gel electrophoresis, desalted and cloned by reverse transcriptase-PCR using RNA obtained from bloodstream forms (strain 427; clone MITat 1.2) into the standard transformation protocol (14), were also grown in a modified minimal essential medium (15).

Yeast strains used in this study were W303-1A (MATa Leu2-3/112 ura3-1 trp1-1 his3-1115 ade2-1 can1-100 gal SCC2) and YSH295 (MATa Leu2-3/112 ura3-1 trp1-1 his3-1115 ade2-1 can1-100 gal SCC2 fps1 LEU2), kindly provided by Dr. Stefan Hohmann (Goteborg University, Goteborg, Sweden). Cells were grown on medium containing 2% peptone and 1% yeast extract (YPE) supplemented with 2% glucose or on synthetic medium for the selection of transformants (16). The phenotype was analyzed by a plate growth assay (see Fig. 4).

**Expression of TbAQP1, TbAQP2, and TbAQP3 in*** S. cerevisiae **under Iso-osmotic and Hyperosmotic Conditions—**Cells were grown at iso-osmotic conditions overnight in synthetic medium *(S. cerevisiae)* and sequenced (GATC GmbH, Konstanz, Germany). A list of primers used is available upon request. PCR products were also cloned into Smal-cut pRS416 with an egfp gene for which the stop codon was replaced by a HindIII site.

Glycerol Efflux Experiments of TbAQP1, TbAQP2, and TbAQP3 in *S. cerevisiae* under Iso-osmotic and Hyperosmotic Conditions—Cells were grown at iso-osmotic conditions overnight in synthetic medium until an optical density of 1 OD at a = 600 nm was obtained. Part of this culture was harvested by centrifugation (4 °C, 3,500 g, 10 min), resuspended in the same medium containing 5% NaCl (hyperosmotic stress), and further incubated at room temperature and 160 rpm for 2 h. Determination of intracellular glycerol was performed by filtration (18).

For the determination of extracellular glycerol, an aliquot of the cell-free supernatant was used. To determine the dry weight of yeast, aliquots of cells were collected on Whatman GF/C filters and dried at 50 °C in the presence of 25 nM rNTPs, 40 units of RNase inhibitor, and 100 mM unlabeled glycerol (20, 21).

**Kinetic Glycerol Uptake of TbAQPs in Oocytes—**For transport assays, 8–10 oocytes were washed and placed in 0.5 ml of fresh ND96 buffer. Glycerol uptake was started by replacement of the ND96 solution by ND96 containing 1 mg unlabeled glycerol and 1 µCi/ml of [U-14C] glycerol. Uptake of radiolabeled glycerol was stopped at defined time points by washing the cells three times in ND96 solution (at about −3 °C) containing 100 µM unlabeled glycerol (20, 21).

Individual oocytes were dissolved in 1 ml of 10% SDS and subject to scintillation counting.

**Functional Expression of TbAQPs in *S. cerevisiae*—**To characterize channel activity of TbAQPs, the corresponding proteins were N-terminally fused with GFP and functionally expressed in an *S. cerevisiae* fps1Δ mutant, which lacks a functional glycerol channel. Expression of GFP alone served as a control. As shown by fluorescence microscopy, GFP-TbAQPs were clearly located within the cellular membrane of the yeast (Fig. 2). For a functional characterization, TbAQP-expressing cells were grown overnight in isotonic medium. Subsequently, intra- and extracellular glycerol concentrations were determined. As shown in Fig. 3, control cells produced very little glycerol, which was equally distributed between the cells and the surrounding medium, whereas TbAQP1- and TbAQP3-transformed fps1Δ mutant cells showed a dramatic increase of glycerol production, which was almost exclusively secreted into the medium. Notably, yeast TbAQP2-transformants produced twice as much glycerol as compared with TbAQP1 and -3, which was also mainly secreted, although in this case, some glycerol was retained intracellularly (note the different scales between TbAQP1/3 and TbAQP2 in Fig. 3).

To further characterize this phenomenon, the experiment was repeated under hypertonic conditions (5% NaCl for 2 h), which was described to produce a very high accumulation of...
glycerol in *S. cerevisiae* (18). Due to the absence of a functional glycerol channel, control cells showed indeed a high retention of glycerol, although some glycerol appeared also in the medium, indicating that glycerol leaked out through the membrane. TbAQP1- and TbAQP3-transformed fps1*/H9004* mutant cells, however, showed similar results as those obtained under iso-osmotic conditions, i.e., these cells were unable to accumulate glycerol and released it constitutively into the medium. This behavior is similar to that described for fps1*/H9004* yeast cells expressing the glycerol facilitator from *Escherichia coli* (*GlpF*) and indicates a non-regulated high glycerol channel activity (26, 27).

TbAQP2-transformed fps1*/H9004* mutant cells showed a similar phenotype as fps1Δ mutant cells expressing TbAQP1 and TbAQP3. However, a significant amount of glycerol was retained within the cytosol, regardless of the ionic strength of the medium (Fig. 3C). In summary, the results are consistent with a role of TbAQPs in glycerol efflux. In addition, TbAQP2 appears to affect glycerol production and its intra- and extracellular distribution in *S. cerevisiae* in a different way as compared with TbAQP1 and -3.

**Phenotypes of TbAQPs**

To analyze the phenotype of TbAQP1, -2, and -3, respective transformants were grown and then spotted in serial dilutions

---

**Fig. 1. Sequence analysis.** A, amino acid sequence alignment of TbAQPs. Identical amino acids are in white on a black background; putative transmembrane regions are overlined. Dots label the highly conserved NPA motifs or their modifications in TbAQP2, and the asterisk highlights the unusual leucine residue, which replaces the arginine residue usually found in this position. B, phylogenetic tree analysis of TbAQPs.
Fig. 2. GFP-TbAQPs expression in S. cerevisiae. A, GFP transformants (control); B, GFP-TbAQP1 transformants; C, GFP-TbAQP2 transformants; D, GFP-TbAQP3 transformants.

Aquaglyceroporins from T. brucei

Fig. 3. Glycerol efflux experiments of yeast fps1Δ mutant cells transformed with an empty vector (A), with TbAQP1 (B), with TbAQP2 (C), or with TbAQP3 (D). Intra- and extracellular glycerol concentrations were determined under iso-osmotic and hyperosmotic conditions. The values given are mean ± S.D. of three independents experiments.
onto agar plates with different osmolytes (Fig. 4). Fps1 is involved in glycerol transport in *S. cerevisiae*. This regulated glycerol channel is active under hypotonic conditions but inactive under hypertonic conditions (28). Thus, growth of fps1/H9004 mutant cells was limited under hypoosmotic conditions but did not show any phenotype under hyperosmotic conditions (27). TbAQPs expressed in fps1/H9004 mutant cells suppressed hypo-osmosensitivity. In addition, these cells grew unaffected under hypertonic conditions if the respective osmolyte was 1 M glycerol. Growth was drastically reduced, however, if 1 M sorbitol or 5% NaCl was applied instead of glycerol. This phenotype of hyper-osmosensitivity for other osmolytes than glycerol was also described for yeast mutants lacking either the N- or the C-terminal regulatory domains of Fps1, indicating that TbAQPs are non-regulated glycerol channels (26, 27).

**Functional Expression of TbAQPs in Xenopus Oocytes**

For further functional characterization, TbAQPs were heterologously expressed in *Xenopus* oocytes.

**Glycerol Uptake**—Glycerol uptake was measured by incuba-
tion of oocytes expressing the different TbAQPs with 1 mM glycerol (1 μCi/ml [14C]glycerol). As shown in Fig. 5, control oocytes mediated only little glycerol uptake (57.9 pmol/oocyte within 40 min), consistent with simple membrane diffusion. In contrast, oocytes injected with 10 ng of any of the TbAQPs accumulated glycerol (about 240 pmol in 40 min) rapidly, with very similar transport kinetics. To confirm these results and for comparisons with other aquaglyceroporins, glycerol permeability was tested using the oocyte swelling assay.

The swelling rate in a 130 mM glycerol gradient was similar among all three TbAQPs (Fig. 6) and about 10-fold higher as compared with water-injected control oocytes. This was in good agreement with the result of [14C]glycerol uptake in the same time interval of 1 min (Fig. 5). These results were comparable with those obtained using aquaglyceroporins from other protzoa, e.g. *Plasmodium falciparum* and *Toxoplasma gondii* (22, 29).

**Water Permeability**—To analyze water transport, oocytes expressing TbAQPs were exposed to hypotonic shock using a 1:3 diluted medium, which generates an osmotic gradient of 140 mosM/kg across the oocyte membrane (22). Expression of TbAQPs in *Xenopus* oocytes equally led to a 6–7-fold increase of the swelling rate as compared with control oocytes (Fig. 7). From the calculated *P* values (150–180 μm/s), TbAQPs can be characterized as channels expressing an intermediate water permeability between typical aquaglyceroporins, e.g. AQP3 (*P* ~ 70 μm/s), and orthodox aquaporins, e.g. AQP1 (*P* ~ 290 μm/s).

**Selectivity Profile for Other Solutes**—Beside water, aquaglyceroporins usually facilitate permeation of other small uncharged solutes. We thus measured the swelling rates of TbAQP-expressing oocytes in the presence of a variety of polyols such as urea, dihydroxyacetone, alanine, and pyruvate. Although permeability for polyols was restricted, erythritol and ribitol led to about half the swelling rate of glycerol in TbAQP3-expressing oocytes (Fig. 6). Urea passed the pores of TbAQP2 and -3 but was hardly transported by TbAQP1 (Fig. 6). Surprisingly, dihydroxyacetone (DHA) was excellently transported by all TbAQPs; when compared with glycerol, the transport rate was about 2-fold higher for TbAQP2, 1.5-fold higher for TbAQP1, and about equal for TbAQP3.

Interestingly, a concentration of 0.2 M DHA is toxic for yeast cells (30). In glucose-containing medium, 0.2 M DHA led to a slightly decreased growth rate of the fps1Δ deletion strain, whereas growth of TbAQP-transformed fps1Δ was drastically reduced (Fig. 7). These results are consistent with a role of TbAQPs for DHA permeation and stress the importance of aquaglyceroporins for secretion of toxic metabolites.

**Transcription of the TbAQP Genes from T. brucei**

Northern blot analysis using total RNA isolated from different life cycle stages and *TbAQP* genes as probe showed a stage-specific regulation of their transcripts. *TbAQP1* was slightly expressed in log phase, highly expressed in stationary phase, and virtually the only one expressed in procyclic trypanosomes. *TbAQP2* was scarcely expressed throughout the life cycle, whereas transcripts of *TbAQP3* were only detectable in bloodstream form parasites (Fig. 8).

**DISCUSSION**

The importance of aquaporins can be inferred from their wide distribution throughout nature from bacteria to human. Single cell organisms may possess only one type, like *P. falciparum* (22), or several of them, e.g. *S. cerevisiae*. Metazoa
usually express numerous isoforms, e.g. 11 AQP isoforms in mammalians (30).

Due to the presence of glycosomes in *T. brucei*, the energy metabolism of this parasite is unique. Especially important is the sensitivity for glycerol under anaerobic conditions, which can be mimicked by salicylhydroxamic acid. We have cloned TbAQP1, TbAQP2, and TbAQP3. Experimental evidence for the function of TbAQPs as glycerol facilitators was obtained by complementation studies of functionally expressed TbAQPs in an *S. cerevisiae* fps1Δ mutant, comparable with AQP3 and AQP9 (31).

TbAQPs stress the role for glycerol release depending on oxygen supply. This may not only be important under anaerobic conditions, but also under a diminution of oxygen, because glycerol production was observed even under aerobic conditions (6).

Participation of TbAQPs in glycerol catabolism was inferred from glycerol influx experiments. As judged from both uptake of radiolabeled glycerol and standard oocyte swelling assays, TbAQPs expressed in *Xenopus* oocytes transport glycerol efficiently, comparable with AQP3 and AQP9 (31).

Under aerobic conditions, glycerol serves as an alternative substrate (6) and inhibits glucose uptake efficiently (50% at 0.8 mM glycerol (2)); it also competes for ATP (32). Therefore, the glycerol blood concentration of about 50 μM could be relevant as an energy source for trypanosomes.

TbAQPs are aquaglyceroporins, which are able to transport water and other small solutes. The *P* values of oocytes expressing TbAQPs are in the range of aquaporins with intermediate water permeability, such as *T. gondii* (29). It is generally assumed that *T. brucei* live under constant conditions in blood and are thus not exposed to osmotic stress. However, it is well known that erythrocytes possess a high density of AQP1 in their plasma membrane, which shows a high capacity for water transport. This aquaporin seems to be fundamental to respond to abrupt changes in extracellular osmolarity, e.g. when blood cells travel through the renal medulla (33). This may also be important for trypanosomes within their mammalian host. However, osmoregulation may become pivotal for survival of trypanosomes also during the course of transmission and during their life within the insect vector.

Transport of other solutes by TbAQPs was restricted (Fig. 6). Surprisingly, uptake of DHA in oocytes expressing TbAQPs was similar to or even better than glycerol. This result was confirmed by experiments with TbAQP-transformed fps1Δ mutant yeast. So far, the physiological relevance of the DHA transport is unknown, but a simple explanation would be that DHA may be used as energy source. However, this seems not to be the case since detailed searches of the trypanosome gene data bank (TIGR and Sanger) for enzymes related to DHA consumption (i.e. glycerol dehydrogenase and dihydroxyacetone kinase) gave negative results. Moreover, trypanosomes

---

**Fig. 7. Growth of TbAQP-transformed fps1Δ mutant cells.** Growth is characterized as increase in optical density at 600 nm in the absence (filled symbols) or presence (open symbols) of 200 mM DHA. Squares, control cells without TbAQPs; circles, TbAQP1-transformed cells; triangles, TbAQP2-transformed cells; diamonds, TbAQP3-transformed cells.

**Fig. 8. Northern blot analysis of bloodstream form (BSF) and procyclic form trypanosomes.** Total RNA was isolated from the logarithmically growing bloodstream form, the stationary phase bloodstream form, and logarithmically growing procyclic forms and then separated on an agarose gel. After blotting, membranes were first hybridized with probes corresponding to the different TbAQPs, stripped thereafter, and reprobed with a β-tubulin sequence as a control for loading.

Aquaglyceroporins from *T. brucei*
incubated in buffer containing different DHA concentrations as the sole energy source did not survive (data not shown). In literature, DHA has been related to anaerobic glycerol metabolism and osmoregulation, but cell toxicity has also been described (30, 34, 35).

The expression profile of TbAQPs transcripts suggests a distinct importance of the respective proteins throughout the life cycle. We interpret these data as follows. Although TbAQPS seems to be the main AQP in the logarithmically growing slender bloodstream form, the procyclic form relies on the expression of TbAQPI. Stationary phase trypanosomes, equivalent to stumpy bloodstream forms, still express some TbAQPs but also a huge amount of TbAQPI. This may reflect either the onset of the differentiation process or, more likely, the specific need for aquaglyceroporins from T. brucei strains.

These proteins constitute channels with a high glycerol and an intermediate water permeability, which may be mainly involved in glycerol uptake and release and in osmoregulation. In addition, their transcripts seem to be regulated in a stage-specific fashion. We also studied permeability of TbAQPs for DHA, which was in the same range (TbAQPS) or even more prominent as the glycerol transport mediating glycerol permeability of TbAQPs for DHA, which was in the same range (TbAQPS) or even more prominent as the glycerol transport (TbAQPI and -2). Since the metabolic function of DHA in TbAQPs has not been experimentally addressed yet, experiments are underway to improve our understanding of the relevance of this metabolite in the biology of trypanosomes.

Acknowledgment—We are most grateful to Dr. Stefan Hohmann (Göteborg University, Göteborg, Sweden) for providing the yeast strains.

REFERENCES
1. Michels, P. A., Hanaaert, V., and Brinzaud, F. (2000) Parasitol. Today 16, 482–489
2. Bakker, B., Michels, P., Oppedo, F., and Westerhoff, H. (1997) J. Biol. Chem. 272, 3207–3215
3. Oppedo, F., and Borst, P. (1977) FEBS Lett. 80, 360–364
4. Wiemar, E. A., Michels, P. A., and Oppedo, F. R. (1995) Biochem. J. 312, 479–484
5. Bakker, B. M., Westerhoff, H. V., Oppedo, F. R., and Michels, P. A. (2000) Mol. Biochem. Parasitol. 106, 1–10
6. Eisenhal, R., and Cornish-Bowden, A. (1998) J. Biol. Chem. 273, 5500–5505
7. Clayton, C., and Michels, P. (1996) Parasitol. Today 12, 465–471
8. Steinborn, K., Szallies, A., Mecke, D., and Duszenko, M. (2000) Biol. Chem. 381, 1071–1077
9. Brech, F. J., and Clarkson, A. B. (1978) Acta Trop. 35, 23–33
10. Wille, U., Schade, B., and Duszenko, M. (1998) Eur. J. Biochem. 264, 245–250
11. Agre, P., King, L., Yasui, M., Guggino, W. B., Ottersen, O. P., Fujiiyoshi, Y., Engel, A., and Nielsen, S. (2002) J. Physiol. (Lond.) 542, 3–16
12. Cross, G. A. M. (1975) Parasitology 71, 395–417
13. Hamm, B., Schindler, A., Muhlstedt, K., and Duszenko, M. (1995) Mol. Biochem. Parasitol. 60, 13–22
14. Overath, P., Caracas, J., and Haas, C. (1986) Eur. J. Biochem. 160, 175–182
15. Hesse, F., Selzer, P. M., Muhlstedt, K., and Duszenko, M. (1995) Mol. Biochem. Parasitol. 70, 157–166
16. Ausebel, F., Brent, R., Kingston, R., Moore, D., Seidman, J., and Struhl, K. (2000) Current Protocols in Molecular Biology, pp. 13.1.2–13.1.7, John Wiley & Sons, New York
17. Sikorski, R. S., and Hieter, P. (1989) Genetics 122, 23–27
18. Albertyn, J., Hahn, S., and Prior, B. (1994) Curr. Genet. 25, 12–18
19. Wagner, C. A., Friedrich, B., Setzian, L., Long, F., and Broer, S. (2000) Cell Physiol. Biochem. 10, 1–12
20. Ma, T., Frigeri, A., Hasegawa, H., and Verkman, A. S. (1994) J. Biol. Chem. 269, 21845–21849
21. Walsh, M., SMtis, H., Schlote, M., and van Dam, K. (1994) J. Bacteriol. 176, 953–958
22. Haneeen, M., Kun, J., Schultz, J., and Beitz, E. (2002) J. Biol. Chem. 277, 4874–4882
23. Jungwirth, H., Bergler, H., and Hoegenauer, G. (2001) J. Biol. Chem. 276, 36419–36424
24. Bermeyer, H. U. (1984) Methods of Enzymatic Analysis, Metabolites 1: Carbohydrates, 3rd Ed., Vol. 6, pp. 504–506, Chemie GmbH, Weinheim, Germany
25. Bradfords, M. (1976) Anal. Biochem. 22, 246–254
26. Hedfalk, K., Bill, R., Mullins, J., Karlsson, S., Filipsson, C., Bergstrom, J., Tamas, M., Rydstrom, J., and Hohmann, S. (2004) J. Biol. Chem. 279, 14954–14960
27. Tamas, M., Luyten, K., Sutherland, F., Hernandez, A., Albertyn, J., Valadi, H., Li, H., Prior, B., Kilian, S., Ramos, J., Gastafson, L., Thevelein, J., and Hohmann, S. (1999) Mol. Microbiol. 31, 1087–1104
28. Luyten, K., Albertyn, J., Skikbe, W., Prior, B., Ramos, J., Thevelein, J., and Hohmann, S. (1995) EMBO J. 14, 1360–1371
29. Pavlovic-Djurmanovic, S., Schultz, E., and Beitz, E. (2003) FEBS Lett. 555, 500–504
30. Molin, M., Norbeck, J., and Blomberg, A. (2003) J. Biol. Chem. 278, 1415–1423
31. Tankeaguchi, H., Shayaakul, C., Berget, U. V., Mackenzie, B., Devidas, S., Guggino, W. B., and van Hoek, A. N., Hediger, M. A. (1998) J. Biol. Chem. 273, 24737–24743
32. Bakker, B. (1998) Control and regulation of glycolysis in Trypanosoma brucei. Ph.D. thesis, the University of Amsterdam, Amsterdam
33. Elsenhal, R., and Cornish-Bowden, A. (1998) J. Biol. Chem. 273, 5500–5505
34. Westerhoff, H. V., Oppedo, F. R., and Michels, P. (2000) Mol. Biochem. Parasitol. 106, 1–10
35. Norbeck, J., and Blomberg, A. (1997) J. Biol. Chem. 272, 5544–5554