In this section the complete set of differential equations and parameter values of the two models of glycolysis in *Trypanosoma brucei* is given. The model with the glycosome has been described previously (1, 2), and in these articles the references to the original kinetic studies are given. The MLAB source code is available from the authors on request.

Abbreviations

AK	adenylate kinase
ALD	fructose-1,6-bisphosphate aldolase
1,3-BPGA	1,3-bisphosphoglycerate
c	cytosolic
DHAP	dihydroxyacetone phosphate
ENO	enolase
Fru-1,6-BP	fructose 1,6-bisphosphate
Fru-6-P	fructose 6-phosphate
g	glycosomal
Glc	glucose
GA-3-P	glyceraldehyde 3-phosphate
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GDH	glycerol-3-phosphate dehydrogenase
GK	glycerol kinase
Gly-3-P	glycerol 3-phosphate
Glc-6-P	glucose 6-phosphate
GPO	glycerol-3-phosphate oxidase
HK	hexokinase
K_{eq}	equilibrium constant
PEP	phospho*enol*pyruvate
2-PGA	2-phosphoglycerate
3-PGA	3-phosphoglycerate
PGI	glucose-phosphate isomerase
PFK	phosphofructokinase
PGK	phosphoglycerate kinase
PGM	phosphoglycerate mutase
PYK	pyruvate kinase

TIM	triosephosphate isomerase
tot	total
V	volume
v	enzyme rate

Definitions

$$[\text{triose-P}] \equiv \frac{[\text{DHAP}]_c \cdot V_c + [\text{DHAP}]_g \cdot V_g + [\text{GA-3-P}]_g \cdot V_g}{V_{tot}}$$

$$[\text{N}] \equiv \frac{[\text{3-PGA}] \cdot (V_g + V_c) + [\text{2-PGA}]_c \cdot V_c + [\text{PEP}]_c \cdot V_c}{V_{tot}}$$

in which $[\text{3-PGA}] \equiv [\text{3-PGA}]_c = [\text{3-PGA}]_g$. Consequently:

$$[\text{N}] = \frac{[\text{3-PGA}] \cdot \left(1 + \frac{V_c}{V_g}\right) + [\text{2-PGA}]_c \cdot \frac{V_c}{V_g} + [\text{PEP}]_c \cdot \frac{V_c}{V_g}}{\left(1 + \frac{V_c}{V_g}\right)}$$

P_g and P_c denote the sums of high energy phosphates in the glycosome and the cytosol, respectively:

$[\text{P}]_g \equiv 2[\text{ATP}]_g + [\text{ADP}]_g$

$[\text{P}]_c \equiv 2[\text{ATP}]_c + [\text{ADP}]_c$

Model with the glycosome

The model with the glycome contains the following moiety conserved sums:

$[\text{ATP}]_g + [\text{ADP}]_g + [\text{AMP}]_g = C_1$

$[\text{ATP}]_c + [\text{ADP}]_c + [\text{AMP}]_c = C_2$

$[\text{NADH}]_g + [\text{NAD}]_g = C_3$

$$[\text{Gly-3-P}]_g + [\text{DHAP}]_g + [\text{Glc-6-P}]_g + [\text{Fru-6-P}]_g + 2[\text{Fru-1,6-BP}]_g \\ + [\text{GA-3-P}]_g + [\text{1,3-BPGA}]_g + 2[\text{ATP}]_g + [\text{ADP}]_g = C_4$$

$[\text{Gly-3-P}]_c + [\text{DHAP}]_c = C_5$

The kinetics of GPO and the transport of pyruvate across the plasma membrane were described by irreversible Michaelis-Menten kinetics:

$$v \quad = \quad V^{+} \cdot \frac{\dfrac{\mathrm{S}}{K_{\mathrm{S}}}}{1 \quad + \quad \dfrac{\mathrm{S}}{K_{\mathrm{S}}}}$$

in which S is the substrate concentration.

The kinetics of HK were described by an irreversible Michaelis-Menten type equation for two substrates:

$$v_{\mathrm{HK}} \quad = \quad V^{+} \cdot \left(\frac{\dfrac{[\mathrm{Glc}]}{K_{\mathrm{m,Glc}}}}{1 \quad + \quad \dfrac{[\mathrm{Glc}]}{K_{\mathrm{m,Glc}}} \quad + \quad \dfrac{[\mathrm{Glc\text{-}6\text{-}P}]}{K_{\mathrm{m,Glc6P}}}} \right) \cdot \left(\frac{\dfrac{[\mathrm{ATP}]}{K_{\mathrm{m,ATP}}}}{1 \quad + \quad \dfrac{[\mathrm{ATP}]}{K_{\mathrm{m,ATP}}} \quad + \quad \dfrac{[\mathrm{ADP}]}{K_{\mathrm{m,ADP}}}} \right)$$

The kinetics of GAPDH, PGK, GDH and GK were described by a reversible Michaelis-Menten equation for two non-competing product-substrate couples:

$$v \quad = \quad V^{+} \cdot \frac{\dfrac{\mathrm{S}_1}{K_{\mathrm{S1}}} \cdot \dfrac{\mathrm{S}_2}{K_{\mathrm{S2}}} \quad - \quad \dfrac{V^{-}}{V^{+}} \cdot \dfrac{\mathrm{P}_1}{K_{\mathrm{P1}}} \cdot \dfrac{\mathrm{P}_2}{K_{\mathrm{P2}}}}{\left(1 \quad + \quad \dfrac{\mathrm{S}_1}{K_{\mathrm{S1}}} \quad + \quad \dfrac{\mathrm{P}_1}{K_{\mathrm{P1}}}\right) \cdot \left(1 \quad + \quad \dfrac{\mathrm{S}_2}{K_{\mathrm{S2}}} \quad + \quad \dfrac{\mathrm{P}_2}{K_{\mathrm{P2}}}\right)}$$

The transport of glucose was described according to a 4-state model for a symmetrical facilitated diffusion carrier

$$\mathrm{v}_{\text{glucose transport}} \quad = \quad V^{+} \cdot \frac{[\mathrm{Glc}]_{\mathrm{o}} - [\mathrm{Glc}]_{\mathrm{i}}}{K_{\mathrm{Glc}} + \quad [\mathrm{Glc}]_{\mathrm{o}} + \quad [\mathrm{Glc}]_{\mathrm{i}} + \quad \alpha \cdot [\mathrm{Glc}]_{\mathrm{o}} \cdot \ [\mathrm{Glc}]_{\mathrm{i}} / K_{\mathrm{Glc}}}$$

in which $[\mathrm{Glc}]_i$ is the intracellular and $[\mathrm{Glc}]_o$ is the extracellular glucose concentration. K_{Glc} is the Michaelis constant for glucose transport. α is a symmetry index, equal to 1 in case of complete symmetry of the carrier (2).

The rate of PFK exhibits a slightly cooperative dependence on the concentration of fructose 6-phosphate:

$$v_{\mathrm{PFK}} \quad = \quad V^{+} \cdot \left(\frac{K_{\mathrm{i1}}}{[\mathrm{Fru\text{-}1,6\text{-}BP}] \quad + \quad K_{\mathrm{i1}}} \right) \cdot \left(\frac{\dfrac{[\mathrm{Fru\text{-}6\text{-}P}]}{K_{\mathrm{m,Fru6P}}}}{1 \quad + \quad \dfrac{[\mathrm{Fru\text{-}6\text{-}P}]}{K_{\mathrm{m,Fru6P}}} \quad + \quad \dfrac{[\mathrm{Fru\text{-}1,6\text{-}BP}]}{K_{\mathrm{i2}}}} \right) \cdot \left(\frac{\dfrac{[\mathrm{ATP}]}{K_{\mathrm{m,ATP}}}}{1 \quad + \quad \dfrac{[\mathrm{ATP}]}{K_{\mathrm{m,ATP}}}} \right)$$

The rate of PYK depends cooperatively on the concentration of PEP:

$$v_{\text{PYK}} = V^{+} \cdot \left(\frac{\left(\frac{[\text{PEP}]}{K_{\text{m,PEP}}}\right)^{n}}{1 + \left(\frac{[\text{PEP}]}{K_{\text{m,PEP}}}\right)^{n}} \right) \cdot \left(\frac{\left(\frac{[\text{ADP}]_{\text{c}}}{K_{\text{m,ADP}}}\right)}{1 + \left(\frac{[\text{ADP}]_{\text{c}}}{K_{\text{m,ADP}}}\right)} \right)$$

n is the cooperativity index (Hill coefficient).

$$K_{\text{m,PEP}} = 0.34 \cdot \left(1 + \frac{[\text{ATP}]_{\text{c}}}{0.57\ \text{mM}} + \frac{[\text{ADP}]_{\text{c}}}{0.64\ \text{mM}} \right) \text{mM.}$$

The rate equation for aldolase reads:

$$v_{\text{ALD}} = V^{+} \cdot \frac{\frac{[\text{Fru-1,6-BP}]}{K_{\text{m,Fru16BP}}} - \frac{V^{-}}{V^{+}} \cdot \frac{[\text{GA-3-P}][\text{DHAP}]}{K_{\text{m,GA3P}}\, K_{\text{m,DHAP}}}}{1 + \frac{[\text{Fru-1,6-BP}]}{K_{\text{m,Fru16BP}}} + \frac{[\text{GA-3-P}]}{K_{\text{m,GA3P}}} + \frac{[\text{DHAP}]}{K_{\text{m,DHAP}}} + \frac{[\text{Fru-1,6-BP}][\text{GA-3-P}]}{K_{\text{m,Fru16BP}}\, K_{\text{i,GA3P}}} + \frac{[\text{GA-3-P}][\text{DHAP}]}{K_{\text{m,GA3P}}\, K_{\text{m,DHAP}}}}$$

in which $K_{\text{m,Fru16BP}} = 9 \cdot 10^{-3} \cdot \left(1 + \frac{[\text{ATP}]_{\text{g}}}{0.68\ \text{mM}} + \frac{[\text{ADP}]_{\text{g}}}{1.51\ \text{mM}} + \frac{[\text{AMP}]_{\text{g}}}{3.65\ \text{mM}} \right) \text{mM.}$

The hydrolysis of ATP for free-energy-dissipating processes was described by:

$$v_{\text{ATP utilization}} = k \cdot \frac{[\text{ATP}]}{[\text{ADP}]}$$

The differential equations used were the following:

$$\frac{d[\text{Glc}]_{\text{i}}}{dt} = \frac{v_{\text{glucose transport}} - v_{\text{HK}}}{V_{\text{tot}}}$$

$$\frac{d[\text{hexose-P}]_{\text{g}}}{dt} = \frac{v_{\text{HK}} - v_{\text{PFK}}}{V_{\text{g}}}$$

$$\frac{d[\text{Fru-1,6-BP}]_{\text{g}}}{dt} = \frac{v_{\text{PFK}} - v_{\text{ALD}}}{V_{\text{g}}}$$

$$\frac{d[\text{triose-P}]}{dt} = \frac{2v_{\text{ALD}} - v_{\text{GAPDH}} - v_{\text{GDH}} + v_{\text{GPO}}}{V_{\text{tot}}}$$

$$\frac{d[\text{1,3-BPGA}]_{\text{g}}}{dt} = \frac{v_{\text{GAPDH}} - v_{\text{PGK}}}{V_{\text{g}}}$$

$$\frac{d[\text{N}]}{dt} = \frac{v_{\text{PGK}} - v_{\text{PYK}}}{V_{\text{tot}}}$$

$$\frac{d[\text{PYR}]_{\text{c}}}{dt} = \frac{v_{\text{PYK}} - v_{\text{pyruvate transport}}}{V_{\text{c}}}$$

$$\frac{d[\mathrm{NADH}]_{\mathrm{g}}}{dt} = \frac{v_{\mathrm{GAPDH}} - v_{\mathrm{GDH}}}{V_{\mathrm{g}}}$$

$$\frac{d[\mathrm{P}]_{\mathrm{g}}}{dt} = \frac{-v_{\mathrm{HK}} - v_{\mathrm{PFK}} + v_{\mathrm{PGK}} + v_{\mathrm{GK}}}{V_{\mathrm{g}}}$$

$$\frac{d[\mathrm{P}]_{\mathrm{c}}}{dt} = \frac{v_{\mathrm{PYK}} - v_{\mathrm{ATP\ utilization}}}{V_{\mathrm{c}}}$$

From the above variables the other variables were calculated:

$$[\text{Glc-6-P}]_{\mathrm{g}} = \frac{[\text{hexose-P}]_{\mathrm{g}}}{1 + K_{\mathrm{eq,PGI}}}$$

$$[\text{Fru-6-P}]_{\mathrm{g}} = [\text{hexose-P}]_{\mathrm{g}} - [\text{Glc-6-P}]_{\mathrm{g}}$$

$$[\mathrm{DHAP}]_{\mathrm{c}} = \frac{-b + \sqrt{b^2 - 4ac}}{2a}$$

in which:

$$a = K_{\mathrm{eq,TIM}} \cdot \frac{V_{\mathrm{c}}}{V_{\mathrm{g}}}$$

$$b = [\mathrm{Q}] \cdot \left(1 + K_{\mathrm{eq,TIM}}\right) + \frac{V_{\mathrm{c}}}{V_{\mathrm{g}}} \cdot \mathrm{C}_5 - [\text{triose-P}] \cdot \left(1 + \frac{V_{\mathrm{c}}}{V_{\mathrm{g}}}\right) \cdot K_{\mathrm{eq,TIM}}$$

$$c = -[\text{triose-P}] \cdot \left(1 + \frac{V_{\mathrm{c}}}{V_{\mathrm{g}}}\right) \cdot \mathrm{C}_5$$

and:

$$\begin{aligned}[\mathrm{Q}] &= [\mathrm{DHAP}]_{\mathrm{g}} + [\text{GA-3-P}]_{\mathrm{g}} + [\text{Gly-3-P}]_{\mathrm{g}} \\ &= \mathrm{C}_{4,\mathrm{antiporter}} - [\text{Glc-6-P}]_{\mathrm{g}} - [\text{Fru-6-P}]_{\mathrm{g}} \\ &\quad - 2[\text{F-1,6-BP}]_{\mathrm{g}} - [\text{1,3-PGA}]_{\mathrm{g}} - 2[\mathrm{ATP}]_{\mathrm{g}} - [\mathrm{ADP}]_{\mathrm{g}}\end{aligned}$$

$$[\mathrm{DHAP}]_{\mathrm{g}} = \frac{[\mathrm{Q}] \cdot [\mathrm{DHAP}]_{\mathrm{c}}}{\mathrm{C}_5 + K_{\mathrm{eq,TIM}} \cdot [\mathrm{DHAP}]_{\mathrm{c}}}$$

$$[\text{Gly-3-P}]_{\mathrm{c}} = \mathrm{C}_5 - [\mathrm{DHAP}]_{\mathrm{c}}$$

$$[\text{GA-3-P}]_{\mathrm{g}} = K_{\mathrm{eq,TIM}} \cdot [\mathrm{DHAP}]_{\mathrm{g}}$$

$$[\text{Gly-3-P}]_{\mathrm{g}} = [\mathrm{Q}] - [\mathrm{DHAP}]_{\mathrm{g}} - [\text{GA-3-P}]_{\mathrm{g}}$$

$$[\text{3-PGA}] = \frac{[\mathrm{N}] \cdot \left(1 + \frac{V_{\mathrm{c}}}{V_{\mathrm{g}}}\right)}{1 + (1 + K_{\mathrm{eq,PGM}} + K_{\mathrm{eq,PGM}} K_{\mathrm{eq,ENO}}) \cdot \frac{V_{\mathrm{c}}}{V_{\mathrm{g}}}}$$

$$[\text{2-PGA}]_\text{c} = K_\text{eq,PGM}\cdot[\text{3-PGA}]$$

$$[\text{PEP}]_\text{c} = K_\text{eq,ENO}\cdot[\text{2-PGA}]_\text{c}$$

$$[\text{ATP}]_\text{g} = \frac{-b_\text{g} + \sqrt{b_\text{g}^2 - 4a_\text{g}c_\text{g}}}{2a_\text{g}}$$

in which:

$$a_g = 1 - 4K_\text{eq}$$

$$b_\text{g} = \text{C}_1 - [\text{P}]_g\cdot(1 - 4K_\text{eq,AK})$$

$$c_g = -K_\text{eq}\cdot[\text{P}]_\text{g}^2$$

$$[\text{ADP}]_\text{g} = [\text{P}]_\text{g} - 2[\text{ATP}]_\text{g}$$

$$[\text{AMP}]_\text{g} = \text{C}_1 - [\text{ATP}]_\text{g} - [\text{ADP}]_\text{g}$$

To obtain the cytosolic concentrations $[\text{ATP}]_c$, $[\text{ADP}]_c$, and $[\text{AMP}]_c$, P_c was substituted for P_g, and C_2 for C_1.

$$[\text{NAD}^+]_\text{g} = \text{C}_3 - [\text{NADH}]_\text{g}$$

Parameter values

Glucose transport	V^+	106.2	nmol· min^{-1}·(mg cell protein)$^{-1}$
	K_Glc	2	mM
	α	0.75	
HK	V^+	625	nmol·min^{-1}·(mg cell protein)$^{-1}$
	$K_{m,\text{ Glc}}$	0.1	mM
	$K_{m,\text{ Glc6P}}$	12	mM
	$K_{m,ATP}$	0.116	mM
	$K_{m,ADP}$	0.126	mM
PGI	K_eq	0.29	
PFK	V^+	780	nmol·min^{-1}·(mg cell protein)$^{-1}$
	K_i1	15.8	mM
	K_i2	10.7	mM
	$K_\text{m,F6P}$	0.82	mM

	$K_{m,ATP}$	$2.6 \cdot 10^{-2}$	mM
	n	1.2	
ALD	V^+	184.5	$nmol \cdot min^{-1} \cdot (mg\ cell\ protein)^{-1}$
	V^-/V^+	1.19	
	$K_{m,GA3P}$	$6.7 \cdot 10^{-2}$	mM
	$K_{m,DHAP}$	$1.5 \cdot 10^{-2}$	mM
	$K_{i,GA3P}$	$9.8 \cdot 10^{-2}$	mM
TIM	K_{eq}	0.045	
GAPDH	V^+	1470	$nmol \cdot min^{-1} \cdot (mg\ cell\ protein)^{-1}$
	V^-/V^+	0.67	
	K_{S1} (GA-3-P)	0.15	
	K_{S2} (NAD^+)	0.45	
	K_{P1} (1,3-BPGA)	0.1	
	K_{P2} (NADH)	0.02	
PGK	V^+	640	$nmol \cdot min^{-1} \cdot (mg\ cell\ protein)^{-1}$
	V^-/V^+	0.029	
	K_{S1} (1,3-BPGA)	0.05	mM
	K_{S2} (ADP)	0.1	mM
	K_{P1} (3-PGA)	1.62	mM
	K_{P2} (ATP)	0.29	mM
PGM	K_{eq}	0.187	
ENO	K_{eq}	6.7	
PYK	V^+	$2.6 \cdot 10^{3}$	$nmol \cdot min^{-1} \cdot (mg\ cell\ protein)^{-1}$
	$K_{m,\ ADP}$	0.114	mM

	n	2.5	
pyruvate transport	V^+	200	nmol min^{-1}·(mg cell protein)$^{-1}$
	K_S (pyruvate)	1.96	mM
GDH	V^+	533	nmol min^{-1}·(mg cell protein)$^{-1}$
	V^-/V^+	0.28	
	K_{S1} (DHAP)	0.1	mM
	K_{S2} (NADH)	0.01	mM
	K_{P1} (Gly-3-P)	2	mM
	K_{P2} (NAD^+)	0.4	mM
GK	V^+	0	nmol·min^{-1}·(mg cell protein)$^{-1}$
	V^-/V^+	167	
	K_{S1} (Gly-3-P_g)	5.1	mM
	K_{S2} (ADP)	0.12	mM
	K_{P1} (glycerol)	0.12	mM
	K_{P2} (ATP)	0.19	mM
GPO	V^+	200	nmol·min^{-1}·(mg cell protein)$^{-1}$
	K_S (Gly-3-P_c)	1.7	mM
ATP utilization	k	50	nmol·min^{-1}·mg cell protein^{-1}
AK	K_{eq}	0.442	
	V_{tot}	5.7	µl·(mg cell protein)$^{-1}$
	V_c/V_g	22.3	
	C_1	4	mM
	C_2	4	mM
	C_3	4	mM

C_4	45	mM
C_5	5	mM

Model without glycosome

The differential equations have been given in *Materials and Methods*. Rate equations and parameter values are the same as those of the model with the glycosome. The moiety conserved sums are:

$[ATP] + [ADP] + [AMP] = C_6$

$[NADH] + [NAD] = C_7$

in which C_6 is 4 mM and C_7 is 4 mM. From the independent variables of the differential equations the dependent variables were calculated as follows:

$$[\text{Glc-6-P}] = \frac{[\text{hexose-P}]}{1 + K_{\text{eq,PGI}}}$$

$[\text{Fru-6-P}] = [\text{hexose-P}] - [\text{Glc-6-P}]$

$[\text{DHAP}] = [\text{Triose-P}]/(1 + K_{\text{eq,TIM}})$

$[\text{GA-3-P}] = K_{\text{eq,TIM}} \cdot [\text{DHAP}]$

$$[\text{3-PGA}] = \frac{[\text{N}]}{1 + K_{\text{eq,PGM}} + K_{\text{eq,PGM}} \cdot K_{\text{eq,ENO}}}$$

$[\text{2-PGA}] = K_{\text{eq,PGM}} \cdot [\text{3-PGA}]$

$[\text{PEP}] = K_{\text{eq,ENO}} \cdot [\text{2-PGA}]$

ATP, ADP and AMP were calculated from variable P as in the model with the glycosome, but P is substituted for P_g and C_6 for C_1

$[NAD^+] = C_7 - [NADH]$.

1. Bakker B.M., Michels P.A.M., Opperdoes, F.R. & Westerhoff, H.V. (1997) *J. Biol.Chem.* **272**, 3207-3215.
2. Bakker B.M., Michels P.A.M., Opperdoes, F.R. & Westerhoff, H.V. (1999) *J. Biol. Chem.* **274**, 14551-14559.