Laura (June 2019)

In vitro enzyme reactions

Hexokinase (HXK, EC 2.7.1.1)

i) GLC* + ATP $\overset{\text{HXK}}{\Longleftrightarrow}$ G6P + ADP

ii) G6P + NADP $\overset{\text{Glucose 6-phosphate dehydrogenase}}{\Longleftrightarrow}$ 6P Gluconolactone + NADPH

(1,2) D-Glucose: 10 mM; ATP: 1 mM; NADP: 1 mM; G6PDH (EC 1.1.1.49): 1.8 U/mL

Phosphoglucose isomerase (PGI, EC 5.3.1.9)

i) G6P* $\overset{\text{PGI}}{\Longleftrightarrow}$ F6P

ii) F6P + ATP $\overset{\text{PFK}}{\Longleftrightarrow}$ FBP + ADP

iii) FBP $\overset{\text{ALD}}{\Longleftrightarrow}$ DHAP + G3P

iv) DHAP $\overset{\text{TPI}}{\Longleftrightarrow}$ G3P

v) 2 DHAP + 2 NADH $\overset{\text{(G3PDH, EC 1.1.1.8)}}{\Longleftrightarrow}$ 2 GLY3P + 2 NAD^+

(3) G6P: 5 mM; ATP: 1 mM; NADH: 0.15 mM; PFK: not mentioned; ALD: 0.45 U/mL; TPI: 2.5 U/mL; G3PDH: 0.86 U/mL

(4) G6P: 5 mM; ATP: 1 mM; NADH: 0.15 mM; PFK: 2.5 U/mL; ALD: 0.45 U/mL; TPI: 5.5 U/mL; G3PDH: 0.94 U/mL

Notes: the forward reaction is not included in the papers.

Phosphofructokinase (PFK, EC 2.7.1.11)

F6P* + ATP* $\overset{\text{PFK}}{\Longleftrightarrow}$ F16BP + ADP

F16BP $\overset{\text{ALD}}{\Longleftrightarrow}$ DHAP + G3P

DHAP $\overset{\text{TPI}}{\Longleftrightarrow}$ G3P

2 DHAP + 2 NADH $\overset{\text{G3PDH}}{\Longleftrightarrow}$ 2 GLY3P + 2 NAD^+

Notes: F26BP is also added. It allosterically regulates the activity of PFK

(1) F6P: 0.25 mM; ATP: 0.5 mM; F26BP: 0.1 mM; NADH: 0.15 mM; ALD (EC 4.1.2.13): 0.45 U/mL TPI (EC 5.3.1.1): 1.8 U/mL G3PDH (EC 1.1.1.8): 0.6 U/mL

(2) **F6P: 10 mM;** ATP: 0.5 mM; F26BP: 0.1 mM; NADH: 0.15 mM; ALD: 0.45 U/mL TPI: 1.8 U/mL G3PDH (EC 1.1.1.8): 0.6 U/mL

Aldolase (ALD, EC 4.1.2.13)

F16BP* $\overset{\text{ALD}}{\Longleftrightarrow}$ G3P + DHAP

G3P $\overset{\text{TPI}}{\Longleftrightarrow}$ DHAP

2 DHAP + 2 NADH $\overset{\text{G3PDH}}{\Longleftrightarrow}$ 2 GLY3P + 2 NAD^+

(1,2) NADH: 0.15 mM; F16BP: 2 mM; G3PDH: 0.6 U/mL; TPI: 1.8 U/mL

Triosephosphate isomerase (TPI, EC 5.3.1.1)

G3P* $\overset{\text{TPI}}{\Longleftrightarrow}$ DHAP

DHAP + NADH $\overset{\text{G3PDH}}{\Longleftrightarrow}$ + NAD^+

(1) NADH: 0.15 mM; G3P: 5.8 mM; G3PDH: 8.5 U/mL;

Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH forward direction, EC 1.2.1.12)

G3P* + NAD^+ $\overset{\text{PGK}}{\Longleftrightarrow}$ 1,3-BPG + NADH

1,3-BPG + ADP $\overset{\text{G3PDH}}{\Longleftrightarrow}$ 3PGA + ATP

(2) ADP: 10 mM; NAD: 1 mM; G3P: 5.8 mM; PGK: 22.5 U/mL

Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH reverse direction, EC 1.2.1.12)

3PGA* + ATP + $\overset{\text{PGK}}{\Longleftrightarrow}$ 1,3-BPG + ADP

1,3-BPG + NADH $\overset{\text{G3PDH}}{\Longleftrightarrow}$ G3P + NAD^+

(1,2) ATP: 1 mM; NADH: 0.15 mM; 3PGA: 5 mM; PGK (EC 2.7.2.3): 22.5 U/mL

Phosphoglycerate-mutase (PGM, EC 5.4.2.1)

3PGA* + 2,3-BPG $\overset{\text{PGM}}{\Longleftrightarrow}$ 2PGA + 2,3-BPG

2PGA $\overset{\text{ENO}}{\Longleftrightarrow}$ PEP

PEP + ADP $\overset{\text{PYK}}{\Longleftrightarrow}$ PYR + ATP

PYR + NADH $\overset{\text{LDH}}{\Longleftrightarrow}$ LAC + NAD^+

(1,2) ADP: 10 mM; NADH: 0.15 mM; 2,3-BPG: 1.25 mM; 3PGA: 5 mM; ENO: 2 U/mL; PYK: 13 U/mL;

LDH: 11.3 U/mL

Enolase (ENO, EC 4.2.1.11)

2PGA* $\overset{\text{ENO}}{\Longleftrightarrow}$ PEP

PEP + ADP $\overset{\text{PYK}}{\Longleftrightarrow}$ PYR + ATP

PYR + NADH $\overset{\text{LDH}}{\Longleftrightarrow}$ LAC + NAD^+

(1) ADP: 10 mM; 2PGA: 1 mM; PYK: 9 U/mL; LDH: 13.8 U/mL

Enolase can also be measured by the production rate of PEP (240 nm)

2PGA* $\overset{\text{ENO}}{\Longleftrightarrow}$ PEP $+H_2O$

(2) 2PGA: 6 mM

Note: use UV-plates

Pyruvate kinase (EC 2.7.1.40)

PEP* + ADP $\overset{\text{PYK}}{\Longleftrightarrow}$ PYR + ATP

PYR + NADH $\overset{\text{LDH}}{\Longleftrightarrow}$ LAC + NAD^+

Note: F16BP is used in the assay

(1,2) ADP: 10 mM; NADH: 0.15 mM; F16BP: 1 mM; PEP: 2 mM: LDH: 13.8 U/mL

Pyruvate decarboxylase (PDC, EC 4.1.1.1)

PYR* $\overset{\text{PDC}}{\Longleftrightarrow}$ Acetaldehyde + CO2

Acetaldehyde + NADH $\overset{\text{ADH}}{\Longleftrightarrow}$ ETHO + NAD^+

Note: TTP is used in the 1st reaction

(1,2) TTP: 0.2 mM; NADH: 0.15 mM; PYR: 50 mM; ADH: 88 U/mL

Phosphoglycerate kinase (PGK, EC 2.7.2.3) and Alcohol dehydrogenase (ADH, EC 1.1.1.10) are just measured in the reverse direction.

* start chemical

References

(1) M. Bakker *et all.* Measuring enzyme activities under standardized *in vivo*-like conditions for systems biology. The FEBS Journal (2009).

(2) M. Bakker *et all.* The Use of in vivo-like Enzyme Kinetics in a Computational Model of Yeast Glycolysis. Beilstein-Institut (2010).

(3) VU protocols

List of abbreviations

GLC	D-Glucose
G6P	D-Glucose 6-Phosphate
F6P	D-Fructose 6-Phosphate
FBP	D-Fructose 1,6-Biphosphate
DHAP	Dihydroxyacetone Phosphate
G3P	Glyceraldehyde 3-Phosphate
GLY3P	Glycerol-3P
1,2-BPG	1,3-Biphosphoglycerate
2,3-BPG	2,3-Biphosphoglycerate
3PGA	3-Phosphoglycerate
2PGA	2-Phosphoglycerate
PEP	Phosphoenolpyruvate
PYR	Pyruvate
LAC	Lactate
ETHO	Ethanol
TTP	Cocarboxylase