The payoff phase converts 2 GAP to 2 pyruvate, yielding 4 ATP and 2 NADH

$$2\text{GAP} + 4\text{ADP} + 2\text{P}_i + 2\text{NAD}^+$$

$$\downarrow$$

$$2\text{pyruvate} + 4\text{ATP} + 2\text{H}_2\text{O} + 2\text{NADH}$$
Step 6: GAPDH catalyzes the oxidation and phosphorylation of GAP to 1,3-BPG

- This step provides for the net synthesis of ATP in glycolysis
- The oxidation yields NADH, which can be used to make more ATP via aerobic metabolism

\[ \Delta G' = 6.3 \text{ kJ/mol} \]
GAPDH links oxidation to phosphorylation through temporary formation of a thioester
NAD+ binding to GAPDH promotes deprotonation of active-site Cys

Electrostatic catalysis

Formation of enzyme-substrate complex. The active-site Cys has a reduced $pK_a$ (5.5 instead of 8) when NAD$^+$ is bound, and is in the more reactive, thiolate form.
Deprotonated Cys is nucleophilic and attacks the electrophilic carbonyl carbon

A covalent thiohemiacetal linkage forms between the substrate and the $-S^-$ group of the Cys residue.
The tetrahedral intermediate kicks out a hydride ion, which is accepted by NAD$^+$.

**Oxidation-reduction step**

The energy that would be released on oxidation is retained through the formation of the thioester.

The enzyme-substrate intermediate is oxidized by the NAD$^+$ bound to the active site.
\(P_i\) acts as a nucleophile and releases the product

The energy of the broken thioester is retained in the mixed-anhydride product.

The covalent thioester linkage between the substrate and enzyme undergoes phosphorolysis (attack by \(P_i\)) releasing the second product, 1,3-bisphosphoglycerate.

The NADH product leaves the active site and is replaced by another molecule of \(NAD^+\).
Step 7: Phosphoglycerate kinase (PGK) catalyzes the first synthesis of ATP

This step replaces the two ATPs used in the preparatory phase

\[
\begin{align*}
1,3\text{-Bisphosphoglycerate} & \quad \text{ADP} \\
(\text{PGP})^2 & \quad \text{Mg}^{2+} \\
\text{phosphoglycerate kinase} & \\
3\text{-Phosphoglycerate} & \quad \text{ATP} \\
\Delta G^\circ & = -18.5 \text{ kJ/mol}
\end{align*}
\]
PGK undergoes a conformational change (induced fit) on binding its substrates
Step 8: PGM catalyzes the isomerization of 3PG to 2PG, in preparation for making PEP.

\[ \Delta G^\circ = 4.4 \text{ kJ/mol} \]
The active PGM enzyme contains a phospho-His in its active site

Enzyme $\text{CH}_2 \overset{\text{N}}{\text{N}} \overset{\text{PO}_3^{2-}}{\text{N}}$

Phospho-His residue
Phosphoglycerate mutase

Phosphoryl transfer occurs between an active-site His and C-2 (OH) of the substrate. A second active-site His acts as general base catalyst.

Figure 14-8 part 1
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Phosphoryl transfer from C-3 of the substrate to the first active-site His. The second active-site His acts as general acid catalyst.
Step 9: Enolase catalyzes the dehydration of 2PG to PEP, a ‘high-energy’ intermediate.

\[
\begin{align*}
\text{2-Phosphoglycerate} & \rightleftharpoons \text{Phosphoenolpyruvate} \\
\text{HO--C--OPo}_{3}^{2-} & \rightleftharpoons \text{C--OPo}_{3}^{2-} \\
\text{H--C--OPo}_{3}^{2-} & \rightleftharpoons \text{C--OPo}_{3}^{2-} \\
\end{align*}
\]

\[\Delta G^{\circ} = 7.5 \text{ kJ/mol}\]
Mg$^{2+}$ ions are essential in the enolate reaction

Lys$^{345}$ abstracts a proton by general base catalysis. Two Mg$^{2+}$ ions stabilize the resulting enolic intermediate.

2-Phosphoglycerate bound to enzyme

Enolic intermediate

Glu$^{211}$ facilitates elimination of the –OH group by general acid catalysis.

Phosphoenolpyruvate
Step 10: Pyruvate kinase catalyzes a phosphoryl transfer from PEP to ADP

Substrate-level phosphorylation

This step yields a net production of two ATPs per glucose

\[ \Delta G^{\circ} = -31.4 \text{ kJ/mol} \]
Catalysis by PK depends on Mg$^{2+}$ and K$^+$ ions
The enol-keto tautomerization of pyruvate is why PEP is a ‘high-energy phosphate’ cpd.
The payoff phase converts 2 GAP to 2 pyruvate, yielding 4 ATP and 2 NADH

\[ 2\text{GAP} + 4\text{ADP} + 2\text{P}_i + 2\text{NAD}^+ \rightarrow 2\text{pyruvate} + 4\text{ATP} + 2\text{H}_2\text{O} + 2\text{NADH} \]
Glucose → ATP → phosphorylation → G6P → isomerization → F6P → ATP → phosphorylation → FBP → cleavage → GAP ↔ DHAP

GAP → 1,3-BPG → NAD+ → NADH → 3PG → substrate-level phosphorylation → 2PG → rearrangement → PEP → substrate-level phosphorylation → Pyruvate

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