THERMODYNAMIC ASPECTS OF GLYCOLYSIS

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The experiments of Harden and Young[1–3] in the early years of this century are all but forgotten by today's biochemists and are barely mentioned in modern textbooks. Yet they were not only crucial in the development of our understanding of the glycolytic pathway; they also provide some of the most instructive examples for understanding biochemical equilibria. Understanding them is easier for us than it was for Harden and Young because of all of the knowledge about the chemical details of glycolysis that has been gained since their time. In modern terminology, the crucial observations were these:

(1) In the presence of an ample supply of inorganic phosphate, alcoholic fermentation of glucose by yeast extract proceeds until all of the glucose is consumed.[1]

(2) Under inorganic phosphate limitation fermentation becomes very slow and fructose 1,6–bisphosphate accumulates.[2]

(3) Addition of a small amount of inorganic phosphate to yeast extract fermenting under phosphate limitation causes a rapid increase in the rate of fermentation, during which 1 mol CO_2 is produced per mol phosphate added, after which very slow fermentation resumes.[2]

(4) In the absence of inorganic phosphate, addition of a *trace* of arsenate causes a rapid increase in fermentation, which may continue until all of the glucose is consumed, and analysis shows no perceptible change in the concentration of arsenate at any time.[3]

To understand the first three of these observations it is sufficient to know the glycolytic reactions and their standard Gibbs energies $\Delta G^{o\prime}$ shown in Fig 1. To understand the fourth it is also necessary to know that glyceraldehyde 3–phosphate dehydrogenase will accept arsenate as a substrate instead of inorganic phosphate, but that the 1–arseno–3–phosphoglycerate presumed to be produced is unstable and is spontaneously hydrolysed to 3–phosphoglycerate and arsenate.

If we ignore for the moment the experiment with arsenate, it is clear that the reaction catalysed by glyceraldehyde 3–phosphate dehydrogenase cannot proceed in the absence of inorganic phosphate and that consequently the whole process must cease. But why should fructose 1,6–bisphosphate accumulate rather than any of the other four intermediates that occur before the blocked reaction? To understand this we need to examine the $\Delta G^{o\prime}$ values shown in Fig 1.

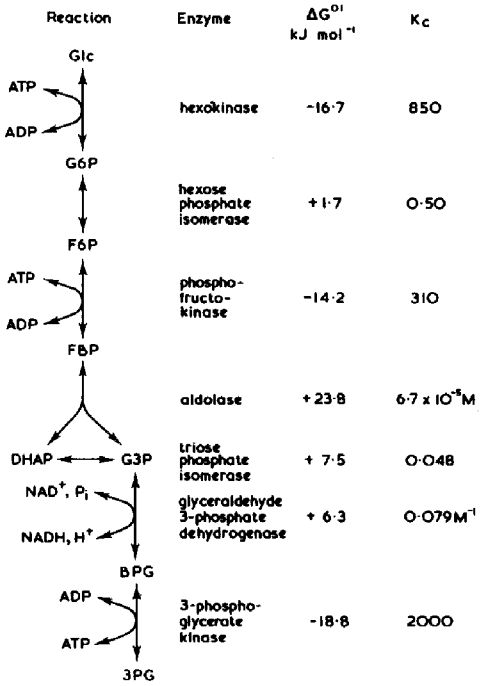

Figure 1 *The first seven reactions of glycolysis*
The following abbreviations are used: Glc, glucose; G6P, glucose 6–phosphate; F6P, fructose 6–phosphate; FBP, fructose 1,6–bisphosphate; DHAP, dihydroxyacetone phosphate; G3P, glyceraldehyde 3–phosphate; P_i, inorganic phosphate; BPG, 1,3–bisphosphoglycerate; 3PG, 3–phosphoglycerate.

A naïve (and wrong) interpretation of $\Delta G^{\circ\prime}$ values commonly encountered states that large negative values indicate that the forward reaction is strongly favoured, whereas large positive values indicate that the reverse reaction is strongly favoured. (To make matters more precise I should say that 'large' means 'larger than about 11 kJ mol^{-1}' and 'strongly favoured' means 'favoured by at least a factor of 100'). This seems to explain the result in the absence of inorganic phosphate, because it indicates that the hexokinase and phosphofructokinase equilibria favour the forward reactions (which is true), that the hexose phosphate isomerase equilibrium does not strongly favour either direction (which is also true), but the aldolase equilibrium strongly favours the reverse reaction, ie accumulation of fructose 1,6–bisphosphate. This 'explanation' creates a worse problem than the one it solves, however, because it suggests that the aldolase reaction should never proceed forwards at all, whether inorganic phosphate is available or not — yet fermentation *does* proceed readily when phosphate is available.

To understand properly what is happening we must examine the relationship between $\Delta G^{\circ\prime}$ and the equilibrium constant K for a reaction:

$$\Delta G^{\circ\prime} = -RT\ln K \simeq -5.7\log K \text{ (in kJ mol}^{-1}\text{) at 25°C}$$

where $R = 8.314$ J mol^{-1} K^{-1} is the gas constant and T is the absolute temperature. Thus at 25°C each -5.7 kJ mol^{-1} in the value of $\Delta G^{\circ\prime}$ corresponds to a factor of 10 in the equilibrium constant in favour of product formation. If we apply this relationship for the first three reactions of glycolysis we have: $K = 850$ for hexokinase, $K = 0.50$ for hexose phosphate isomerase, and $K = 310$ for phosphofructokinase, in good agreement with the interpretation of the standard Gibbs energies given above. For aldolase, however, we find that $K = 6.7 \times 10^{-5}$, which quite wrongly suggests that the reaction will not readily proceed forwards, even though we know that it does. What has gone wrong? Why should a calculation that works with the first three enzymes not give a sensible result for aldolase?

The explanation lies in the fact that if we calculate an equilibrium constant K from the definition of $\Delta G^{\circ\prime}$ above we must get a dimensionless result, because the expression contains $\ln K$ (or $\log K$), and only dimensionless numbers have logarithms. So each K given above is a dimensionless number. This presents no problem for the first three, because we would never expect them to have dimensions, as each refers to a reaction in which the number of reactants is the same as the number of products. For aldolase, however, we might expect to interpret the equilibrium constant as

$$K_c = \frac{[\text{G3P}]_{\text{eqm}}[\text{DHAP}]_{\text{eqm}}}{[\text{FBP}]_{\text{eqm}}}$$

in which the subscripts eqm indicate concentrations at equilibrium. Now this quantity K_c cannot be the same as K, because it is not a dimensionless number but a concentration.

What is the relationship between K_c and K? The convention that we use to make K dimensionless so that we can take its logarithm and relate it to the thermodynamic quantity $\Delta G^{\circ\prime}$ is to say that we are not defining K in terms of real concentrations measured in mol l^{-1}, but concentrations relative to a set of standards, thus:

$$K = \frac{([\text{G3P}]_{\text{eqm}}/[\text{G3P}]^{\circ})([\text{DHAP}]_{\text{eqm}}/[\text{DHAP}]^{\circ})}{([\text{FBP}]_{\text{eqm}}/[\text{FBP}]^{\circ})}$$

where the superscripts (°) indicate these standards. In principle we could choose any values we liked for the standard concentrations, and they could be different for each chemical species if we wished. But a chaotic set of standards would be very difficult to remember and so, with a very few exceptions such as the proton (see below) and water, we choose the same standard concentration of 1 M for every species. Thus we have, for the aldolase equilibrium,

$$K = K_c/(1\text{ M})$$

or, from the value we calculated for K,

$$K_c = 6.7 \times 10^{-5}\text{ M}$$

The appearance of *units* in this equation provides the key to understanding why aldolase is special: it is certainly true that a mixture of the three reactants *in their standard concentrations* of 1 M will tend to react in the reverse direction, i.e. from triose to hexose. But why should a biochemist care what happens at 1 M? Much more interesting is what happens at a physiologically realistic concentration, such as 50 μM. If we put both [G3P] and [DHAP] to this value (ignoring for the moment the complication that in the presence of triose phosphate isomerase they will equilibrate to unequal concentrations) we can readily calculate the concentration of fructose 1,6–bisphosphate at equilibrium with them as

$$[\text{FBP}] = 5 \times 10^{-5} \times 5 \times 10^{-5}/(6.7 \times 10^{-5}) = 3.7 \times 10^{-5}\text{M}$$

which is a little smaller than the concentrations of the two triose phosphates, despite the large positive value of $\Delta G^{\circ\prime}$.

To determine the direction in which any particular mixture of reactants will be able to react the most convenient quantity to consider is not $\Delta G^{\circ\prime}$ but ΔG, ie not the standard Gibbs energy but the Gibbs energy, which is defined as

$$\Delta G = \Delta G^{\circ\prime} + RT\ln \frac{([G3P]/[G3P]^{\circ})([DHAP]/[DHAP]^{\circ})}{([FBP]/[FBP]^{\circ})}$$

(As in the definition of K above, we obtain a dimensionless number before taking logarithms by dividing each concentration by the corresponding standard. In practice we usually omit the standard concentrations from such expressions, which is not strictly correct but acceptable provided we remember that it is these ratios that we mean when we write the concentrations. No problems arise if all concentrations are measured in M, but if other units are used the concentrations must always be converted to M before calculating ΔG.) The value of ΔG directly answers the question, how far is the system from equilibrium and in which direction? If ΔG is negative the reaction must proceed forwards to reach equilibrium; if it is positive it must proceed backwards; if it is zero the system is at equilibrium. If we put all three of the concentrations in the aldolase equilibrium to 50 μM, we have

$$\Delta G = 23.8 + 5.7\log(5 \times 10^{-5}) = -0.72 \text{ kJ mol}^{-1}$$

This small negative value shows that such a mixture is close to equilibrium but needs to react forwards to a small degree to reach equilibrium, a result in good agreement with our calculation above that the equilibrium concentration of fructose 1,6–bisphosphate is 37 μM when the two triose phosphates are present at concentrations of 50 μM.

This calculation, then, allows us to understand why fermentation can proceed under normal conditions, but it leaves open the question of why fructose 1,6–bisphosphate (rather than, for example, dihydroxyacetone phosphate or glyceraldehyde 3–phosphate) accumulates when the supply of inorganic phosphate is cut off. When the reaction catalysed by glyceraldehyde 3–phosphate dehydrogenase is blocked, the pool of all three aldolase reactants must increase, so that 50 μM ceases to be a realistic concentration to consider. As the concentrations increase, ΔG must become positive and then increase steadily, unless the equilibrium shifts in favour of fructose 1,6–bisphosphate, and the more the concentration increases the more the reverse reaction is favoured. Thus it is fructose 1,6–bisphosphate that accumulates, not the two triose phosphates.

Actually the situation in the cell is a little more complicated than I have indicated, because dihydroxyacetone phosphate and glyceraldehyde 3–phosphate are not at equal concentrations at equilibrium, because they are interconvertible by a reaction catalysed by triose phosphate dehydrogenase, which has $\Delta G^{\circ\prime} = 7.5$ kJ mol^{-1}. This corresponds to the ratio [G3P]/[DHAP] = 0.048 at equilibrium and so if we consider a concentration of 50 μM for glyceraldehyde 3–phosphate we must assume a concentration of 1.04 mM for dihydroxyacetone phosphate before calculating the equilibrium concentration of fructose 1,6–bisphosphate as described above. The results of two such calculations are illustrated schematically in Fig 2. When the concentration of glyceraldehyde 3–phosphate is 20 μM (a realistic value under ordinary conditions *in vivo*[4]), the equilibrium concentration of fructose 1,6–bisphosphate is 0.12 mM, and although this is greater than the concentration of glyceraldehyde 3–phosphate it is still not the predominant species. But if the concentrations of the triose phosphates are increased 10-fold there is a 100-fold increase in the concentration of fructose 1,6–bisphosphate and it becomes the predominant component of the equilibrium mixture. This is effectively what happens in the fermentation by yeast extract when the supply of inorganic phosphate is cut off and the reaction catalysed by glyceraldehyde 3–phosphate dehydrogenase is blocked.

We have seen from this discussion that the quantity to be examined is not $\Delta G^{\circ\prime}$ but ΔG if we want to know which direction of reaction will be possible under any set of conditions. Why, then, do we seem to get the right answer if we look at $\Delta G^{\circ\prime}$ for the first three reactions of glycolysis? This is because we have, by implication, assumed that we are dealing with equal (though not necessarily standard) concentrations of reactants and products, eg for hexose phosphate isomerase:

$$\Delta G = \Delta G^{\circ\prime} + 5.7\log \frac{[F6P]}{[G6P]} = \Delta G^{\circ\prime} = 1.7 \text{ kJ mol}^{-1} \text{ if } [F6P] = [G6P]$$

Under cellular conditions, however, these concentrations are not likely to be equal (and if they were glycolysis could not proceed because this value is, though small, positive) and so ΔG is not exactly equal to $\Delta G^{\circ\prime}$. In fact, under glycolytic conditions in the human erythrocyte Minakami and Yoshikawa[4] found [G6P] = 83 μM, [F6P] = 14 μM, so

$$\Delta G = \Delta G^{\circ\prime} + 5.7\log(14/83) = 1.7 - 4.4 = -2.7 \text{ kJ mol}^{-1}$$

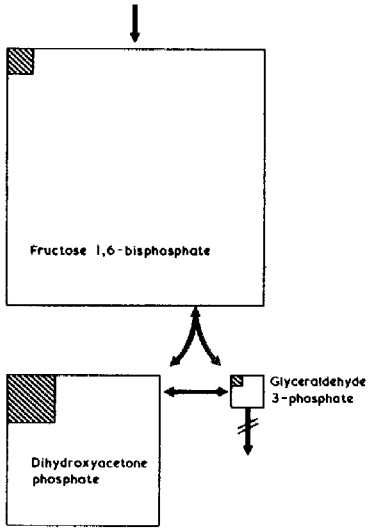

Figure 2 *The pool of aldolase reactants*
The figure illustrates schematically how the relative proportions of fructose 1,6-bisphosphate, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate change when the reaction catalysed by glyceraldehyde 3-phosphate dehydrogenase is blocked but the early steps of glycolysis continue. A realistic initial state is indicated by the shaded squares, with areas proportional to the concentrations, [G3P] = 20 μM, [DHAP] = 0.42 mM, [FBP] = 0.12 mM. If equilibrium is maintained, both between fructose 1,6-bisphosphate and the two triose phosphates (catalysed by aldolase) and between the triose phosphates (triose phosphate isomerase), a 10-fold increase in the concentration of glyceraldehyde 3-phosphate is accompanied by a 10-fold increase in the concentration of dihydroxyacetone phosphate, but a 100-fold increase in the concentration of fructose 1,6-bisphosphate. This second state is indicated by the areas of the complete squares.

Although this correction is not large it is crucial because it shows that under glycolytic conditions ΔG is negative, as it must be if glycolysis is to be possible.

Most of the other glycolytic reactions are free from the sort of complication that had to be considered for aldolase, because they have equal numbers of molecules participating in the forward and reverse directions. Glyceraldehyde 3-phosphate dehydrogenase does need to be considered, however: if we include H^+ as a product we do have equal numbers of reactant and product molecules, but if we omit H^+ from consideration we have an excess of reactants over products. In principle, we could treat H^+ just like any other reactant, and that is what chemists typically do. But a standard state of 1M for H^+ (corresponding to pH 0) is extremely inconvenient for most biochemical purposes, and biochemists customarily use a standard concentration of 0.1 μM for H^+ (corresponding to pH 7); they write $\Delta G^{\circ\prime}$ (rather than ΔG°) to indicate this, and they work with buffered solutions and consequently do not have to worry about changes in the H^+ concentration as a reaction proceeds.

With this convention we can ignore H^+ as a reactant and treat the reaction catalysed by glyceraldehyde 3-phosphate dehydrogenase as one in which there are three reactants but only two products. The practical equilibrium constant is consequently not

$$K = 7.9 \times 10^{-2}$$

but

$$K_c = 7.9 \times 10^{-2}\ \mathrm{M}^{-1}$$

which is a reciprocal concentration. The effect of dilution thus works in the opposite direction for this reaction from the way it works for the aldolase reaction: the aldolase equilibrium is by no means as unfavourable to the forward reaction as it appears at first sight; this reaction is much more unfavourable to the forward direction than it appears at first sight. How, then, is it able to proceed when cells undergo glycolysis? The explanation is partly that the $[NAD^+]/[NADH]$ ratio is typically maintained at a value much greater than unity, and partly that the reaction is followed in glycolysis by a reaction with $\Delta G^{\circ\prime} = -18.8$ kJ mol^{-1}, a large negative value that ensures a very low concentration of 1,3-bisphosphoglycerate.

If we put $[NAD^+]/[NADH] = 240$, [G3P] = 19 μM, [BPG] = 0.6 μM and $[P_i]$ = 1 μM (as given by Minakami and Yoshikawa[4]), we obtain

$$\Delta G = 6.3 \times 5.7 \log \frac{0.6 \times 10^{-6}}{240 \times 0.001 \times 19 \times 10^{-6}} = +1.3\ \mathrm{kJ\ mol^{-1}}$$

As this value is positive it cannot be quite right, but it is close enough to zero for us to believe that the discrepancy can be accounted for by errors in measuring the concentrations of the various metabolites in the cell.

This last result raises one further question: if a very favourable reaction can overcome a very unfavourable equilibrium by ensuring that the concentration of the product of

the unfavourable reaction is very low, why can this solution not be used to overcome many other unfavourable equilibria? From the thermodynamic point of view there is no objection to this: any unfavourable equilibrium can indeed be pulled over by removing the product. But kinetically there is a serious objection. Any species that is present in very low concentrations must react slowly in second-order reactions (such as binding to an enzyme) because it will take a finite time for the other participant in the reaction to 'find' a reactant in low concentration. No matter how efficient an enzyme may be as a catalyst it cannot react faster than the diffusion limit, which corresponds to a second-order rate constant of about 10^8 M^{-1} s^{-1}. So if both enzyme and substrate are present at concentrations less than 1 μM there is no way in which a rate greater than about 10^{-4} M s^{-1} or 0.1 mM s^{-1} can be achieved, and even this presupposes a perfectly efficient enzyme. Thus in general it is not desirable to have intermediates in major pathways such as glycolysis present at extremely low concentrations.

It remains to consider the effect of arsenate discovered by Harden and Young. As I have suggested, this is a consequence of the ability of glyceraldehyde 3–phosphate dehydrogenase to accept arsenate and the extreme lability of the product, 1–arseno–3–phosphoglycerate. This presumed species is hydrolysed to 3–phosphoglycerate and arsenate as soon as it is formed. The former continues through the later stages of fermentation whereas the arsenate is immediately available for a further cycle of the glyceraldehyde 3–phosphate dehydrogenase reaction. Thus even though fermentation requires reagent quantities of inorganic phosphate, it can proceed in the presence of catalytic amounts of arsenate.

Study questions

(1) The 'irreversible' reactions of glycolysis are bypassed by hydrolytic reactions in gluconeogenesis, but there is no bypass for the reaction catalysed by 3–phosphoglycerate kinase, even though its value of $\Delta G^{\circ\prime} = -18.8$ kJ mol^{-1} is one of the most negative in glycolysis. How is it possible for this reaction to proceed in the direction of gluconeogenesis?

(2) In the absence of inorganic phosphate, Harden and Young observed a low rate of fermentation, not a zero rate? How can this low rate be explained?

(3) The usual experimental practice is to assay glyceraldehyde 3–phosphate dehydrogenase with arsenate as substrate in place of phosphate. Why is this advantageous?

References

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