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FREE ENERGY CHANGES AND METABOLIC REGULATION IN STEADY-STATE PHOTOSYNTHETIC CARBON REDUCTION*

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SUMMARY

The standard physiological free energy changes of reactions of glycolysis, the reductive pentose phosphate cycle (photosynthetic carbon reduction cycle) and the oxidative pentose phosphate cycle have been calculated from available data. The concentrations of metabolites of the photosynthetic carbon reduction cycle, measured during steady-state photosynthesis in *Chlorella pyrenoidosa* in the presence of radioactive tracers, and the concentrations of some intermediates of the oxidative pentose phosphate cycle measured during a subsequent dark period, have been employed to calculate the free energy changes of each reaction of the reductive cycle and of some of the reactions of the oxidative cycle during steady-state light and dark conditions. With respect to the magnitude of the negative free energy change, at steady state, such reactions have been found to be of two types. Those with high negative free energy changes (–6 to –11 kcal) are in each case reactions from which there exists independent evidence of a role in metabolic regulation. Those with small negative free energy changes (0 to –2 kcal) are not regulated reactions and are highly reversible. Thus most of the negative free energy change occurring under steady-state conditions in this metabolic system is dissipated for purposes of control.

By the criterion of negative free energy change, ribulosediphosphate carboxylase, and fructose- and heptosediphosphatases are regulated enzymes. The activities of these enzymes are known to be high in the light and low in the dark. Phosphoribulokinase, which mediates the one reaction with an intermediate negative free energy change (–3.82 kcal) also may be a regulated enzyme with greater activity in the light than in the dark. In the oxidative cycle, the reaction mediated by glucose-6-phosphate dehydrogenase has a very high negative free energy change and appears to be active in the dark and inactive in the light. One function of these controls is thought to be the exclusive operation of the reductive cycle in the light and the oxidative cycle in the dark.

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INTRODUCTION

"Irreversibility" has been postulated as a common, if not universal or exclusive, property of biochemical reactions catalyzed by metabolically regulated enzymes[1-3]. Those biochemical steps which proceed at a much greater rate in the forward direction as compared with the reverse direction are termed "irreversible".

It is often difficult to measure the ratio of forward to back rate of a biochemical reaction *in vivo*, where metabolic regulation is of the most interest. A quantitative determination of the negative free energy change associated with a steady-state reaction *in vivo* can provide such a measure.

Reaction reversibility is related more directly to the steady-state free energy change, ΔG^s, than to the standard physiological free energy change, $\Delta G'$. A valid test of the hypothesis relating large negative ΔG^s values to reactions catalyzed by allosterically controlled enzymes requires rather complete knowledge of the thermodynamics of a sequence of reactions and of the activities of all the intermediates, reactants, and products involved. Only then can the ΔG^s values of each step be calculated for the particular chosen steady-state condition. Also, the relation between ΔG^s and regulation becomes more interesting if it can be shown that not only are all regulated steps associated with large negative ΔG^s but also that all other non-regulated steps have a small ΔG^s.

Few reported studies have been able to satisfy all of these conditions. One such study of steady-state metabolite concentrations was made by MINAKAMI AND HARUHISA[4], who followed the reactions of glycolysis in erythrocytes. Calculated "mass action" constants were compared with equilibrium constants which, in turn, were based upon published free energy data for the steps in glycolysis. Large differences between the "mass action" and equilibrium constants were found for some reactions, which were thus identified as the rate limiting and regulated steps.

The photosynthetic carbon reduction cycle[5], has been found to contain several sites of metabolic regulation[6-8]. This regulation was indicated by ^{14}C and ^{32}P tracer studies of intermediate compound levels. Enzymes catalyzing several steps of the cycle appear to be inactivated in the dark[6,7], or upon addition of chemicals which alter photoelectron transport[8], or photophosphorylation[9].

Although the indicated light–dark regulation involved activation during photosynthesis, it might be suspected that the same enzymes also would be sites of metabolic regulation in the light, since they are already equipped with a regulatory mechanism. There is some experimental indication that this is the case from studies of diffusion from isolated photosynthesizing spinach chloroplasts[10]. Intermediate compounds of the carbon reduction cycle formed just before one light-activated step (fructosediphosphatase) diffused from the chloroplasts much more rapidly than did intermediate compounds in the remainder of the cycle. This suggests a limited activity for the diphosphatase step, and a consequent accumulation of metabolites just preceding it.

Steady-state conditions are easily maintained with photosynthesizing *Chlorella pyrenoidosa*[11], when light intensity, inorganic nutrient solution, temperature, pH, and CO_2 level are kept constant. Metabolites can be labeled with ^{14}C and ^{32}P for subsequent analysis by paper chromatography and radioactivity measurement. The principal requirement is that $^{14}CO_2$ be supplied at nearly constant pressure and known constant specific radioactivity for a time sufficient to completely label all of the carbon skeletons of the metabolites to be measured. The use of $^{32}P_i$ makes possible the measurement of ATP and ADP concentrations as well[6]. These techniques have been used in the present study to obtain values of the concentration of metabolites under conditions of steady-state photosynthesis. Since these concentrations are small (1 mM or less) they have been used in place of activities to calculate ΔG^s from $\Delta G'$.

Although $\Delta G'$ values for the reactions of glycolysis have been published[13,14], these surveys do not contain the most recently available thermodynamic data, and in some cases appear to be in error by 1 kcal or more. Thus we have recalculated the free energy changes for all the reactions of glycolysis.

In the case of reactions of the carbon reduction cycle of photosynthesis (reductive pentose phosphate cycle), equilibrium data for transketolase and transaldolase reactions, as well as the interconversion of the pentose phosphates, are available and permit the calculation of the free energy changes of most of the reactions of the cycle. Free energy changes of other steps can be estimated by making justifiable assumptions.

Once the physiological free energies are calculated, and the steady-state free energies are calculated from the measured steady-state concentrations of the intermediates, it becomes possible to test the correlation between those reactions with high negative free energies and sites of metabolic regulation.

METHODS AND MATERIALS

The steady-state concentrations of intermediates of the carbon reduction cycle were determined by measuring *in vivo* the incorporation of $^{14}CO_2$ into the individual intermediate compounds. The given data were obtained from two experiments with air-adapted *C. pyrenoidosa* described previously[8].

In those experiments the algae were grown for some days on air[6], then centrifuged and resuspended in a medium consisting of 0.1 mM KH_2PO_4 and 1 mM KNO_3.

80 ml of this suspension (1 %, v/v, wet packed algae) were placed in the steady-state photosynthesis apparatus[12]. Then $^{32}P_i$ (10–20 mC) was introduced[6] and after 30 min photosynthesis in air, $^{14}CO_2$ was admitted into the closed gas recirculating system. Even though the concentration of CO_2 must be made somewhat higher at the start (0.08 %) of the experiment to prevent depletion, the steady-state levels of intermediate compounds were found not to change significantly as the concentration fell to 0.04 %.

The air volume of the system was 6 l. The concentration of labeled CO_2 was about 0.04 % at the time of the measurements. The specific radioactivity of the CO_2 was 50 μC/μmole. The pH was kept between 5.5 and 6.0 by adding dil. HNO_3 when necessary.

Between 12 and 20 min after the addition of $^{14}CO_2$, at a time when the pools of the intermediate compounds had been saturated completely with ^{14}C, five samples of the algae suspension were taken at intervals.

The radioactive compounds were analyzed by two-dimensional paper chromatography and radioautography, as described previously[6].

Those values listed in Table I as "*Dark*" are actually obtained from two other experiments in which conditions were similar, and the steady-state levels in the light were close to those obtained in Expts. 1 and 2. A true steady-state dark respiration may not have been obtained, but the values shown were seen after 10 min darkness, and none of the metabolite levels were changing rapidly at that time.

RESULTS

The concentrations of intermediate compounds of the photosynthetic carbon reduction cycle are given in Table I. These were calculated as follows.

TABLE I

CONCENTRATIONS AND FREE ENERGY CORRECTIONS OF METABOLITES IN PHOTOSYNTHESIZING AND RESPIRING *C. pyrenoidosa*

Compound	*Steady-state concentration (μatoms carbon per cm^3 cells)*			*Concentration (mM) Av. × 4/N*	*$\Delta\Delta G$ (kcal) $+1.363$ log concn.*
	Expt. 1	*Expt. 2*	*Av.*		
Photosynthesis					
3-P-glycerate^{3-}	0.96	1.15	1.05	1.40	−3.9
Dihydroxyacetone-P^{2-}	0.30	0.65	0.48	0.64	−4.4
Glyceraldehyde-3-P^{2-}		(a)	0.024	0.032	−6.0
Fru-1,6-P_2^{4-}	0.15	0.14	0.145	0.097	−5.5
Fru-6-P^{2-}	0.76	0.83	0.80	0.53	−4.5
Glc-6-P^{2-}	1.10	1.10	1.10	0.73	−4.3
Ery-4-P^{2-}		(a)	0.02	0.02	−6.4
Sed-7-P^{2-}	0.42	0.45	0.435	0.248	−4.9
Sed-1,7-P_2^{4-}	0.02	0.02	0.02	0.114	−5.4
Rib-5-P^{2-} + Xyl-5-P^{2-}	0.06	0.08	0.07		
Rib-5-P^{2-}		(b)	0.043	0.034	−6.1
Xyl-5-P^{2-}		(b)	0.026	0.021	−6.4
Ribul-5-P^{2-}	0.01	0.02	0.015	0.012	−6.7
Ribul-1,5-P_2^{4-}	2.3	2.8	2.55	2.04	−3.7
ADP/ATP				Ratio = 1:3.0 (d)	−0.7
Dark					
Glc-6-P^{2-}		(c)	0.5	0.33	−4.7
6-P-gluconate^{3-}		(c)	0.07	0.047	−5.9
Ribul-5-P^{2-}		(c)	0.015	0.012	−6.7
Light and dark					
CO_2	$0.4 \cdot 10^{-3}$ atm				−4.6
P_i^{2-}			(d)	1.0	−4.1
[NADPH]/[NADP$^+$]			(e)	Ratio = 1.0	0
H^+			(f)	$5 \cdot 10^{-7}$ (pH 7.7)	−1.0

Abbreviations: 3-P-glycerate^{3-}, 3-phosphoglycerate; dihydroxyacetone-P^{2-}, dihydroxyacetone phosphate; glyceraldehyde-3-P^{2-}, glyceraldehyde 3-phosphate; Fru-1,6-P_2^{4-}, fructose 1,6-diphosphate; Fru-6-P^{2-}, fructose 6-phosphate; Glc-6-P^{2-}, glucose 6-phosphate; Ery-4-P^{2-}, erythrose 4-phosphate; Sed-7-P^{2-}, sedoheptulose 7-phosphate; Sed-1,7-P_2^{4-}, sedoheptulose 1,7-diphosphate; Rib-5-P^{2-}, ribose 5-phosphate; Xyl-5-P^{2-}, xylulose 5-phosphate; Ribul-5-P^{2-}, ribulose 5-phosphate; Ribul-1,5-P_2^{4-}, ribulose 1,5-diphosphate; 6-P-gluconate^{3-}, 6-phosphogluconate; 2-P-glycerate^{3-}, 2-phosphoglycerate; 1,3-P_2-glycerate^{4-}, 1-phosphoryl-3-phosphoglycerate; P-enolpyruvate^{3-}, phosphoenolpyruvate.

(a) Measurable amounts of labeled glyceraldehyde-3-P^{2-} and Ery-4-P^{2-} were not detected at the known chromatographic positions of these compounds in these experiments. They have been detected in experiments under other conditions, but it appears that some loss occurs during chromatography, possibly due to oxidation of the free aldehyde groups. Allowing for an estimated maximum loss of 67% and a minimum detection level of 0.01 μatom of carbon per cm^3 cells, we estimate the upper limits for the concentrations of these compounds as 0.03 μatom of carbon per cm^3 cells. Lower limits of 0.02 μatom for glyceraldehyde-3-P^{2-} and 0.015 μatom for Ery-4-P^{2-} are required by the necessity for $\Delta G^s \leq 0$ for Reactions C and G, Table IV, in which concentrations of the other reactants and products were measured. Thus we estimate the concentrations of glyceraldehyde-3-P^{2-} and Ery-4-P^{2-} to be 0.024 ± 0.006 μatom/cm^3 cells and 0.020 ± 0.010 μatom/cm^3 cells, respectively. When glyceraldehyde-3-P^{2-} was detected, in other experiments, its concentration was found to be about 1/20 that of dihydroxyacetone-P^{2-}, as would be expected at equilibrium ($K' = 0.045$ for [glyceraldehyde-3-P^{2-}]/[dihydroxyacetone-P^{2-}], see Table II, Reaction 9).

(b) After treatment of the pentose phosphates eluted from paper chromatograms with phosphatase, and rechromatography, ribose and xylulose separated from ribulose but not completely from each other, though from visual observation of the film it appears the two sugars are present in roughly comparable amounts. Reactions 20–22 of Table II, which interconvert the three pentose phosphates are highly reversible and the concentrations of Rib-5-P^{2-} and Xyl-5-P^{2-} are estimated to be about the same as in an equilibrium mixture. The concentration of Ribul-5-P^{2-}, which is measured, is close to the equilibrium value, when compared with the assumed values for Rib-5-P^{2-} and Xyl-5-P^{2-}.

(c) These concentrations were measured in the dark, 10 min after the light was turned off in experiments comparable to Expts. 1 and 2, and in which the steady-state concentrations in the light were close to those shown in this table.

(d) Although a concentration of 0.1 M P_i, *plus* added carrier-free $^{32}P_i$, was present in the suspending medium, *C. pyrenoidosa* grown in enriched media contain sizable pools of bound phosphate. From other experiments in which similar conditions were used, and similar metabolic concentrations were found, the effective P_i concentration was estimated to be 1 mM, based on the $^{32}P/^{14}C$ ratio in label-saturated 3-P-glyceric acid, the known specific radioactivity of $^{14}CO_2$ used, and the total ^{32}P radioactivity added.

(e) No determination of actual [NADPH]/[NADP$^+$] was made, and the value 1.0 is assumed as a basis for calculation of free energies both in light and in dark (see text for DISCUSSION).

(f) The optimum pH for photosynthesis in isolated chloroplasts from spinach, and the optimum for many enzymes of the carbon reduction cycle is 7.5–7.8.

The ^{14}C content of each compound was measured in two identical experiments (2nd and 3rd column). The number of μatoms of carbon per cm^3 of algae was determined by dividing the μC of ^{14}C found in each compound by the known specific radioactivity, μC/μmole[12]. The resulting values were averaged for each compound (4th column) and the average was divided by the number of carbon atoms per molecule, N, and multiplied by 4, in accordance with an estimate that the metabolites are dissolved in a "liquid" volume which is 1/4 of the total packed cell volume. Although this estimate could be incorrect by as much as a factor of 1.5, such an inaccuracy would introduce no error for most of the reactions considered and an error of only $2.3\ RT \log 1.5 = 0.2$ kcal in the computation of ΔG^s for those reactions in which one metabolite appears on one side of the equation and two appear on the other side (*e.g.* the formation of fructose 1,6-diphosphate from 3-phosphoglyceraldehyde and dihydroxyacetone phosphate).

Calculations of free energy changes

Equilibrium data are available for most of the reactions of glycolysis and for many of the interconversions of sugar phosphates involved in the pentose phosphate cycle. Other methods of calculation are required for the conversion of fructose 1,6-diphosphate to fructose 6-phosphate, the formation of sedoheptulose 1,7-diphosphate from dihydroxyacetone phosphate and erythrose phosphate, the formation of ribulose 1,5-diphosphate from ribulose 5-phosphate with ATP, and the carboxylation of ribulose 1,5-diphosphate to give two molecules of 3-phosphoglyceric acid.

TABLE II

FREE ENERGIES OF REACTIONS OF GLYCOLYSIS, AND OF OXIDATIVE AND REDUCTIVE PENTOSE PHOSPHATE CYCLES

Reaction	*ΔG′ (kcal)*	*Calculation basis*	*Ref. No. (a)*
(1) NADH + ½ O_2 + H^+ → NAD^+ + H_2O	−52.41	e' = 1.136 V (b)	13, 15, 1
(2) NADPH + ½ O_2 + H^+ → $NADP^+$ + H_2O	−52.59	e' = 1.140 V (b)	13, 15, 1
(3) α-D-Glc + ATP^{4-} → Glc-6-P^{2-} + ADP^{3-} + H^+	− 4.35	$K' = 1.55 \cdot 10^3$ (c)	17
(4) Glc-6-P^{2-} + H_2O → α-D-Glc + P_i^{2-}	− 3.29	$K' = 2.60 \cdot 10^2$	18
(5) ATP^{4-} + H_2O → ADP^{3-} + P_i^{2-} + H^+	− 7.64	Reactions 3, 4 (d)	
(6) Glc-6-P^{2-} → Fru-6-P^{2-}	+ 0.50	$K' = 0.43$	20
(7) α-L-Glycerol-P^{2-} + H_2O → glycerol + P_i^{2-} + H_2O	− 2.40	$K' = 59.6$ (e)	21
(8) α-L-Glycerol-P^{2-} + NAD^+ → NADH + dihydroxyacetone-P^{2-} + H^+	+ 5.80	$K' = 5.5 \cdot 10^{-5}$ (f)	15
(9) Dihydroxyacetone-P^{2-} → glyceraldehyde-3-P^{2-}	+ 1.82	$K' = 4.5 \cdot 10^{-2}$ (g)	22
(10) Fru-1,6-P_2^{4-} → dihydroxyacetone-P^{2-} + glyceraldehyde-3-P^{2-}	+ 5.25	$K' = 1.4 \cdot 10^{-4}$ (h)	23
(11) H_2O + Fru-1,6-P_2^{4-} → P_i^{2-} + Fru-6-P^{2-}	− 3.44	Reactions 1, 4, 6–10 (i)	
(12) Glyceraldehyde-3-P^{2-} + NAD^+ + P_i^{2-} → 1,3-P_2-glycerate^{4-} + NADH + H^+	+ 0.27	$K' = 0.63$ (j)	24
(13) 1,3-P_2-glycerate^{4-} + ADP^{3-} → 3-P-glycerate^{3-} + ATP^{4-}	− 4.77	$K' = 3.18 \cdot 10^3$	25
(14) 3-P-glycerate^{3-} → 2-P-glycerate^{3-}	+ 1.40	$K' = 0.095$ (k)	26
(15) 2-P-glycerate^{3-} → P-enolpyruvate^{3-} + H_2O	− 0.82	$K' = 4.0$ (k)	27
(16) P-enolpyruvate^{3-} + H^+ + ADP^{3-} → ATP^{4-} + pyruvate$^-$	− 5.60	$K' = 1.3 \cdot 10^4$ (l)	28
(17) Xyl-5-P^{2-} + Ery-4-P^{2-} → Fru-6-P^{2-} + glyceraldehyde-3-P^{2-}	− 1.47	$K' = 11.9$	29
(18) Xyl-5-P^{2-} + Rib-5-P^{2-} → Sed-7-P^{2-} + glyceraldehyde-3-P^{2-}	− 0.10	$K' = 1.18$	29
(19) Fru-6-P^{2-} + Ery-4-P^{2-} → Sed-7-P^{2-} + glyceraldehyde-3-P^{2-}	− 0.38	$K' = 1.90$ (m)	30
(20) Ribul-5-P^{2-} → Xyl-5-P^{2-}	− 0.24	$K' = 1.5$	31
(21) Rib-5-P^{2-} → Ribul-5-P^{2-}	+ 0.54	$\Delta H = -3.06$ kcal $\Delta S = -12.1$ e.u.	31
(22) Xyl-5-P^{2-} → Rib-5-P^{2-}	− 0.30	Reactions 20 + 21	
(23) H_2O + Sed-1,7-P_2^{4-} → P_i^{2-} + Sed-7-P^{2-}	− 3.44	Analogy with Reaction 11 (n)	
(24) H_2O + Ribul-1,5-P_2^{4-} → P_i^{2-} + Ribul-5-P^{2-}	− 2.40	Analogy with Reaction 7 (o)	
(25) Glc-6-P^{2-} + $NADP^+$ + H_2O → 6-P-gluconate^{3-} + NADPH + 2H^+	− 8.44	(p)	
(26) 6-P-Gluconate^{3-} + $NADP^+$ → Ribul-5-P^{2-} + CO_2 + NADPH	+ 1.56	(p)	

Abbreviations: see Table I.

(a) Reference is to data from which K' or e' is obtained. In some cases, K' has been calculated from data and is not the constant given by original authors. Explanations are given in subsequent footnotes. Values are carried to two decimal places in this table to avoid increasing errors by calculation. In Reactions 1–24 the values are believed to be accurate ± 0.2 kcal, but those in Reactions 25 and 26 may be no better than ± 1.5 kcal.

(b) From the oxidation potential of $O_2 \rightarrow H_2O$ at pH 7, which is +0.816 V[16], and the reduction potential of NADH → NAD^+ at pH 7, which is +0.320 V[15], or of NADPH → $NADP^+$ at pH 7, which is +0.324 V[15].

(c) K' given at 30°. We assume it to be the same at 25°.

(d) A value of −7.69 kcal is obtained by using the data reviewed by ATKINSON *et al.*[19] for Reaction 4 (this table), and the conversion of glucose 1-phosphate to glucose 6-phosphate (−1.68 kcal), the conversion of galactose 1-phosphate to glucose 1-phosphate (+0.70 kcal), the galactokinase reaction forming galactose 1-phosphate (−1.91 kcal), and the conversion of α-D-glucose to α-D-galactose (−1.51 kcal), from the free energies of formation of glucose and galactose from the elements[13]. The data of BENZINGER *et al.*[20] on glutamine synthetase and glutamine hydrolase lead to a value of $\Delta G' = -7.91$ for Reaction 5, when total ATP and ADP species (the convention used throughout this paper) in the presence of Mg^{2+}, rather than $ATP \cdot Mg^{2-}$ and $ADP \cdot Mg^{-}$, are considered. The value obtained from glucokinase and glucose-6-phosphatase reactions is the most direct and is chosen in this report.

(e) Measured at 38°. At pH 8.5, $K_{8.5} = 38.5$ (selected value); at pH 5.8, $K_{5.8} = 82.5$. Interpolation of the logarithms gave $\log K' = 1.761$, $K' = 59.6$; this value was used for calculation of $\Delta G'$.

(f) The standard equilibrium constant was given as $5.5 \cdot 10^{-12}$, so $K' = 5.5 \cdot 10^{-5}$.

(g) At 25°, found 4.3% glyceraldehyde-3-P^{2-} in equilibrium with dihydroxyacetone-P^{2-} (95.7%).

(h) At 10 mM Mg^{2+}, $K' = 1.4 \cdot 10^{-4}$. Equilibrium constant is strongly dependent on Mg^{2+} concentration.

(i) From the ΔG of formation of α-D-glucose, 1 M, aq. (−219.22 kcal)[13], ΔG formation of Fru-6-P^{2-} is calculated *via* Reactions 4, 6, and 7 to be P −215.43 (see also Table III). From the ΔG of formation of glycerol, 1 M, aq. (−116.76 kcal)[13], ΔG formation of Fru-1,6-P_2^{4-} is calculated *via* Reactions 1, 7, 8, 9, and 10 to be 2P−211.99 kcal (Table III), the $\Delta G'$ of Reaction 11 is determined as the difference, the P represents the difference in the ΔG of formation of phosphate and water (see text).

(j) Choosing values measured at relatively low ionic strengths and averaging values at each pH, we got $K_{6.0} = 0.072$, $K_{6.95} = 0.68$, $K_{7.15} = 0.81$, $K_{8.2} = 8.3$. When corrected to pH = 7.0, log K's were −0.242, −0.142, −0.117, −0.280; av. $\log K' = -0.195$, $K' = 0.63$.

(k) In order to avoid reliance on determinations of [2-*P*-glycerate]/[3-*P*-glycerate] by measurement of optical rotation, which have given widely varying results, we chose to use [*P*-enolpyruvate]/[3-*P*-glycerate] ratios and the accurately determined K' for 2-*P*-glycerate → *P*-enolpyruvate. From the data of WOLD AND BALLOU[27], K' at 10 mM Mg^{2+} is 4.0 for Reaction 15, and $\Delta G' = -0.82$. UTTER AND WERKMAN[26] found in an equilibrium mixture of 2-*P*-glycerate, 3-*P*-glycerate, and *P*-enolpyruvate at 24°, 29% *P*-enolpyruvate at pH 7.4 and 16.4% at pH 5.8. The same authors found about 10% as much 2-*P*-glycerate as 3-*P*-glycerate, so we calculate $K_{7.4}$ for [3-*P*-glycerate]/[*P*-enolpyruvate] = 64:29 = 2.21 and $K_{5.8} = 75:16 = 4.6$. Interpolating the logarithms, $\log K' = 0.424$ and $\Delta G' = +0.58$ kcal for 3-*P*-glycerate → *P*-enolpyruvate. It then follows that for Reaction 14, $\Delta G' = +0.58 - (-0.82) = +1.40$ kcal. This is larger than most published values, but is reasonable for transfer of phosphate from primary to secondary position, a transfer which in the case of glucose 6-phosphate → glucose 1-phosphate has a $\Delta G' = +1.68$ kcal (see Footnote d).

(l) The average values of K in forward and reverse directions at pH 7.4, 8.0, 8.4, and 9.0, when corrected to pH 7 in accordance with Reaction 16, gave values whose logarithms were 4.210, 4.336, 4.361, and 4.565, respectively. When these are plotted *vs.* pH, they fall on a straight line, which extrapolates to 4.11 at pH 7.0, giving $K' = 1.3 \cdot 10^4$ and $\Delta G' = -5.60$ kcal.

(m) Authors[30] state that $K' = 0.95$, but from their data we calculate $K' = 1.90$.

(n) In order to evaluate the free energy of formation of sedoheptulose 1,7-diphosphate we have had to make an assumption regarding the free energy for the esterification of the hydroxyl on C-1 of sedoheptulose 7-phosphate. We estimate this free energy to be the same as that which we calculated for the esterification of the C-1 of fructose 6-phosphate, namely, +3.44 kcal. This is also quite close to the value +3.29 kcal calculated from equilibrium data for the esterification of glucose to give glucose 6-phosphate. The energy of esterification of fructose to give fructose 6-phosphate can be calculated from the data in Tables II and III as +3.35 kcal. Apparently, destabilization of pyranose and furanose rings by the presence of attached phosphate groups increases the negative free energy of hydrolysis by approx. 1.0 kcal per phosphate group, since for α-glycerol phosphate hydrolysis, $\Delta G' = +2.40$ kcal.

(o) Since ribulose 1,5-diphosphate is assumed to be in the form of a straight chain (noncyclized) sugar, hydrolysis of the C-1 phosphate group should be accompanied by about the same negative free energy change as in the case of glycerol phosphate.

(p) Complete data on Reactions 25 and 26 appear to be not available. From the data and calculations of STRECKER AND KORKES[32], the $\Delta G'$ for conversion of glucose, water, and NAD^{+} to gluconate, 2 H^{+}, and NADH was calculated to be −7.93 kcal. Using this value and Reaction 1, $\Delta G'$ of formation of gluconate ion was calculated to be −270.01 kcal. The phosphate hydrolysis energy of 6-*P*-gluconate^{3-} is estimated to be −2.60 kcal, intermediate between −2.40 for α-glycerol phosphate[21] and −3.1 for 3-*P*-glycerate^{3-} (ref. 33). Thus $\Delta G'$ of formation of 6-*P*-gluconate^{3-} is calculated to be P−267.41 kcal. This value, *plus* Reaction 2 and $\Delta G'$ formation of Ribul-5-*P*, CO_2, and H_2O (Table III) permit calculation of the $\Delta G'$ values for Reactions 25 and 26. Reaction 26 has been found to be reversible[34], although seemingly not with a positive $\Delta G'$. However, high ionic strength in the reaction mixture makes precise calculation of the equilibrium constant from published data uncertain.

Calculated $\Delta G'$ values for all reactions of glycolysis and for the reductive and oxidative pentose phosphate cycle are given in Table II. Also shown are the bases for the calculations, references to the original data, and by means of footnotes the assumptions involved in the calculations. The standard physiological state is taken as pH 7, and all other activities were taken to be unity unless otherwise indicated. In most of the original data, concentrations were used rather than activities. A concentration of 10 mM Mg^{2+} is assumed and data from the original literature were chosen to approximate that condition whenever available. Free energy data are indicated for total phosphate ester species rather than for only the magnesium chelated species. Thus the $\Delta G'$ for the hydrolysis of ATP is listed as -7.64 kcal rather than -7.0, as it would be for the hydrolysis of ATP-Mg^- to ADP-Mg^{2-}.

In order to obtain the free energy change for conversion of fructose 6-phosphate to fructose 1,6-diphosphate, the free energies of formation of each of these compounds were calculated starting with glycerol and glucose, together with the free energies of the reactions converting those compounds to the sugar phosphates.

The free energy of formation of 3-phosphoglyceric acid was calculated by two methods. In the first method the free energy of formation of glycerol was used, together with the free energies of the reactions catalyzed by glycerolphosphatase, α-glycerolphosphate dehydrogenase, triosephosphate isomerase, 3-phosphoglyceral-

TABLE III

FREE ENERGIES OF FORMATION FROM THE ELEMENTS OF METABOLITES

Compound	*$\Delta G'$ formation (1 M aq., kcal)*	*$\Delta\Delta G$ (from Table I) (kcal)*	*ΔG^s formation conc. under exptl. conds. (kcal)*
P_i^{2-} ($-H_2O$)	P	−4.1	P− 4.1
α-D-Glucose	−219.22	(−4.1)	(−223.2)
Glc-6-P^{2-} (in light)	P−215.93	−4.3	P−220.2
Glc-6-P^{2-} (in dark)	P−215.93	−4.7	P−220.7
Fru-6-P^{2-}	P−215.43	−4.5	P−219.9
Fru-1,6-P_2^{4-}	2P−211.99	−5.5	2P−217.5
Dihydroxyacetone-P^{2-}	P−104.28	−4.4	P−108.6
Glyceraldehyde-3-P^{2-}	P−102.46	−6.0	−108.5
Glycerol	−116.76		
α-Glycerol-P^{2-}	P−114.36		
3-*P*-Glycerate^{3-}	P−157.46	−3.9	P−161.4
2-*P*-Glycerate^{3-}	P−156.06		
P-Enolpyruvate^{3-}	P−100.20		
Pyruvate$^-$	−113.44		
Ery-4-P^{2-}	P−139.14	−6.4	P−145.6
Sed-7-P^{2-}	P−252.49	−4.9	P−257.4
Sed-1,7-P_2^{4-}	2P−249.05	−5.4	P−254.4
Rib-5-P^{2-}	P−177.58	−6.1	P−183.7
Ribul-5-P^{2-}	P−177.04	−6.70	P−183.7
Ribul-1,5-P_2^{4-}	2P−174.64	−3.7	2P−178.3
Xyl-5-P^{2-}	P−177.28	−6.4	P−183.7
6-*P*-Gluconate^{3-} (in dark)	P−267.41	−5.9	P−273.3
CO_2	− 94.26	−4.6	− 98.9
H_2O	− 56.69	0	− 56.7
H^+	− 9.55	−1.0	− 10.5

Abbreviations: see Table I.

dehyde dehydrogenase, and 3-phosphoglycerate kinase. In the second method the free energy of formation of pyruvate was used together with the free energies of the reactions catalyzed by pyruvate kinase, enolase, and phosphoglycerate mutase. In both calculations the free energies of the hydrolysis of ATP and of the oxidation of NADH with O_2 were employed as needed. Both methods gave identical results for the free energy of formation of 3-phosphoglycerate.

For convenience in expressing free energies of formation of phosphate esters we have adopted the convention of letting the letter P represent the free energy of formation from the elements of phosphate, HPO_4^{2-}, *minus* the free energy of formation from the elements of water. Thus, the free energy of formation from the elements of 1 M aqueous glycerol is −116.76 kcal, and the free energy of the conversion of glycerol *plus* P_i to α-glycerol phosphate and water is calculated to be −2.40 kcal. We therefore give the free energy of formation of α-glycerol phosphate, 1 M aqueous, as P − 114.36 kcal.

The free energies of formation of a number of sugar phosphates and sugar acid phosphate esters of interest are given in Table III. Also in Table III we have calculated the free energies of formation of the various compounds at the actual measured steady-state concentrations (from Table I). These free energies of formation, ΔG^s, are of course valid for only one set of physiological conditions which gave the concentrations that were listed in Table I.

The $\Delta G'$ and ΔG^s values for reactions of the photosynthetic carbon reduction

TABLE IV

FREE ENERGY CHANGES OF THE PENTOSE PHOSPHATE CYCLES IN *C. pyrenoidosa*

Reaction	$\Delta G'$ *(kcal)*	ΔG^s *(kcal)*	
Reductive cycle			
(A) $CO_2 + \text{Ribul-1,5-}P_2^{4-} + H_2O \rightarrow 2\ \text{3-}P\text{-glycerate}^{3-} + 2\ H^+$	−8.4	−9.8	R
(B) $H^+ + \text{3-}P\text{-glycerate}^{3-} + ATP^{4-} + NADPH$ $\rightarrow ADP^{3-} + \text{glyceraldehyde-3-}P^{2-} + NADP^+ + P_i^{2-}$	+4.3	−1.6	
(C) $\text{Glyceraldehyde-3-}P^{2-} \rightarrow \text{dihydroxyacetone-}P^{2-}$	−1.8	−0.2	
(D) $\text{Glyceraldehyde-3-}P^{2-} + \text{dihydroxyacetone-}P^{2-} \rightarrow \text{Fru-1,6-}P_2^{4-}$	−5.2	−0.4	
(E) $\text{Fru-1,6-}P_2^{4-} + H_2O \rightarrow \text{Fru-6-}P^{2-} + P_i^{2-}$	−3.4	−6.5	R
(F) $\text{Fru-6-}P^{2-} + \text{glyceraldehyde-3-}P^{2-} \rightarrow \text{Ery-4-}P^{2-} + \text{Xyl-5-}P^{2-}$	+1.5	−0.9	
(G) $\text{Ery-4-}P^{2-} + \text{dihydroxyacetone-}P^{2-} \rightarrow \text{Sed-1,7-}P_2^{4-}$	−5.6	−0.2	
(H) $\text{Sed-1,7-}P_2^{4-} + H_2O \rightarrow \text{Sed-7-}P^{2-} + P_i^{2-}$	−3.4	−7.1	R
(I) $\text{Sed-7-}P^{2-} + \text{glyceraldehyde-3-}P^{2-} \rightarrow \text{Rib-5-}P^{2-} + \text{Xyl-5-}P^{2-}$	+0.1	−1.4	
(J) $\text{Rib-5-}P^{2-} \rightarrow \text{Ribul-5-}P^{2-}$	+0.5	−0.1	
(K) $\text{Xyl-5-}P^{2-} \rightarrow \text{Ribul-5-}P^{2-}$	+0.2	−0.1	
(L) $\text{Ribul-5-}P^{2-} + ATP^{4-} \rightarrow \text{Ribul-1,5-}P_2^{4-} + ADP^{3-} + H^+$	−5.2	−3.8	R′
(M) $\text{Fru-6-}P^{2-} \rightarrow \text{Glc-6-}P^{2-}$	−0.5	−0.3	
(N) $\text{Glc-6-}P^{2-} + H_2O \rightarrow \alpha\text{-D-Glc} + P_i^{2-}$	−3.3	(−7.2)*	
Oxidative cycle			
(X) $\text{Glc-6-}P^{2-} + NADP^+ + H_2O \rightarrow \text{6-}P\text{-gluconate}^{3-} + NADPH + 2\ H^+$	−8.4	−11.4	R
(Y) $\text{6-}P\text{-Gluconate}^{3-} + NADP^+ \rightarrow CO_2 + \text{Ribul-5-}P^{2-} + NADPH$	+1.6	−2.1	

Abbreviations: see Table I; R, probable sites of metabolic regulation; R′, possible site of metabolic regulation. All other sites are probably unregulated and freely "reversible".

* Not a reaction that occurs to any significant extent in photosynthesis. It is included for purposes of energy balance (see Table V).

cycle (reductive pentose phosphate cycle) are given in Table IV. Also shown are those reactions of the oxidative pentose phosphate cycle by which glucose 6-phosphate is oxidized to ribulose 5-phosphate and CO_2.

Large differences between the standard physiological free energy changes ($\Delta G'$) and the free energy changes of reactions under experimental conditions, ΔG^s, are readily apparent. These differences are the consequences of the adjustments in the steady-state concentrations of the metabolites of the reactions. Note particularly the differences between $\Delta G'$ and ΔG^s in the cases of the aldolase reactions, Reactions D and G, and in the case of the diphosphatase reactions, Reactions E and H. Possible regulatory sites suggested by kinetic tracer studies of the light–dark transients are indicated by R, while another possible site suggested by kinetic tracer studies upon the addition of vitamin K_5 is indicated by R′.

The hydrolysis of glucose 6-phosphate to give D-glucose is not prominent in photosynthetic metabolism and is shown here simply to permit a balance between

TABLE V

ENERGY BALANCE SUMMARY—PHOTOSYNTHETIC CARBON REDUCTION CYCLE

Reaction (from Table IV)	*E (number of times per cycle)*	$\Delta G'$ *(kcal)*	ΔG^s *(kcal)*	$E\Delta G'$ *(kcal)*	$E\Delta G^s$ *(kcal)*	
(A) Ribulosediphosphate carboxylase	3	−8.43	−9.8	−25.3	−29.5	(R)
(B) 3-Phosphoglycerate reduction	6	+4.32	−1.6	+25.9	− 9.4	
(C) Triosephosphate isomerase	2.5*	−1.82	−0.2	− 4.5	− 0.4	
(D) Fructosediphosphate aldolase	1.5*	−5.25	−0.4	− 7.9	− 0.5	
(E) Fructosediphosphatase	1.5*	−3.44	−6.5	− 5.2	− 9.8	(R)
(F) Transketolase	1	+1.47	−0.9	+ 1.5	− 0.9	
(G) Sedoheptulosediphosphate aldolase	1	−5.63	−0.2	− 5.6	− 0.2	
(H) Sedoheptulosediphosphatase	1	−3.44	−7.1	− 3.4	− 7.1	(R)
(I) Transketolase	1	+0.10	−1.4	+ 0.1	− 1.4	
(J) Ribosephosphate isomerase	1	+0.54	−0.1	+ 0.5	− 0.1	
(K) Pentosephosphate epimerase	2	+0.24	−0.1	+ 0.5	− 0.2	
(L) Phosphoribulokinase	3	−5.24	−3.8	−15.7	−11.5	(R′)
(M) Hexosephosphate isomerase	0.5*	−0.50	−0.3	− 0.3	− 0.2	
(N) Glucosephosphatase	0.5*	−3.29	−7.2	− 1.7	− 3.6	
Totals				−41.1	−74.8	

		$\Delta G'$	ΔG^s
Energy input:	6 ($NADPH + H^+ + \frac{1}{2} O_2 \rightarrow NADP^+ + H_2O$)	−315.5	−306.9
	9 ($ATP^{4-} + H_2O \rightarrow ADP^{3-} + P_i^{2-} + H^+$)	− 68.8	−120.1
	Totals	−384.3	−427.0
Energy stored:	3 ($CO_2 + H_2O \rightarrow [CH_2O] + O_2$)	+343.2	+352.21
Energy expended in cycle		− 41.1	− 74.8
Energy expended in postulated regulated steps (R)			− 46.4 (62 %)
Energy expended in postulated regulated steps (R) + (R′)			− 57.9 (77 %)

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* 0.5 represents formation of 0.5 mole of glucose as end product from 1 mole of 3-phosphoglyceraldehyde. Actually, glucose is not an end product (glucose 6-phosphate is converted to polysaccharides), but its formation is included for "bookkeeping" purposes.

free energy input in the form of cofactors from photosynthesis and storage in the form of reduced carbon compounds. For the most part, glucose 6-phosphate is converted to polysaccharides and oligosaccharides. However, we have not attempted to include this and other secondary pathways in this report.

While we have included only the oxidative reactions of the oxidative pentose phosphate cycle, we have a general impression from our studies of the labeling patterns in the dark that the remaining reactions of this cycle which involve isomerases, transketolase, and transaldolase, are highly reversible, just as the isomerase, epimerase, and transketolase reactions are highly reversible in the light, as indicated in this study.

The balance of energy supplied to the cycle in the form of NADPH and ATP as compared with the energy stored in the formation of glucose moieties is shown in Table V. In Table V E indicates the number of times each reaction occurs for each complete turn of the cycle, in which three molecules of CO_2 are reduced to one molecule of triose phosphate. Also included are the necessary reactions for the conversion of one triose phosphate molecule to one-half molecule of glucose. When E is multiplied by $\Delta G'$ and ΔG^s, the standard physiological energy expenditure and steady-state free energy expenditure used in running the cycle are the totals of such products. These totals of course must equal the difference between the energy input in the form of cofactors and the energy stored in the reduction of CO_2 and liberation of O_2 from water, and this is shown at the bottom of Table V. Also indicated is the part of the expended energy dissipated in reactions believed to be regulated.

DISCUSSION

Inherent in determination of ΔG^s values is the assumption that the reactions are proceeding *in vivo* under conditions sufficiently close to the conditions of thermodynamic reversibility to permit the laws of equilibrium to apply to a steady-state situation. This assumption is justified on the basis that even though the specific rate constants (at pH 7), k_f', k_b', for forward and back reactions, respectively, may change between equilibrium and steady state for an enzyme-catalyzed reaction, the ratio k_f'/k_b' should remain constant. Thus, if k_f' is diminished under steady-state conditions owing to a high level of substrate relative to the binding constant of the enzyme and a consequent decreased free enzyme concentration, the k_b', which is also proportional to the free enzyme content, will be decreased proportionately. Thus the ratio is unchanged.

For the generalized equation $v\ \mathrm{A} + w\ \mathrm{B} \leftrightarrows x\ \mathrm{C} + y\ \mathrm{D}$, the steady-state free energy change is given by $\Delta G^s = \Delta G' - RT \ln ([\mathrm{A}]^v\ [\mathrm{B}]^w/[\mathrm{C}]^x\ [\mathrm{D}]^y)$.

Since $\Delta G' = -RT \ln K' = -RT \ln k_f'/k_b'$, where $\Delta G'$ is the standard free energy change at pH 7.0, $\Delta G^s = -RT \ln k_f'/k_b' - RT \ln ([\mathrm{A}]^v\ [\mathrm{B}]^w/[\mathrm{C}]^x\ [\mathrm{D}]^y)$, $\Delta G^s = -RT \ln f/b$, where f and b are, respectively, forward and back rates at steady state.

It should be noted that when the ratio of forward to back reaction rates are measured *in vitro*, the ratio of the measured "specific" rate constants k_f'/k_b' can be much different from the above thermodynamic ratio (generally much smaller for a reaction with large negative $\Delta G'$) since different concentrations of reactants and products must be used in the two directions and the concentration of available enzyme will be very different in the two directions.

Since the ratio, k_f'/k_b', should remain constant even if k_f' and k_b' change proportionately together, this expression will hold for any set of concentrations of reactants, products and enzyme. Thus, when ΔG^s is -2.7 kcal, f/b = 100 and the reaction might be considered "reversible"; at ΔG^s = -4.1 kcal, f/b = 1000 and the reaction might be termed "irreversible".

The data shown in Table IV clearly demonstrate that under steady-state conditions only a limited number of reactions are accompanied by high negative free energy changes. All of the other reactions are highly reversible, with most having negative free energy changes under steady-state conditions of less than 2 kcal/mole. 2 kcal/mole corresponds to a ratio of f/b of approx. 30.

In contrast, steps implicated in light-dark regulation (Reactions A, E, and H) have negative free energy changes of 6-10 kcal. A negative free energy change of 6 kcal corresponds to an f/b ratio of 25000.

Reaction A, ΔG^s = -9.8 kcal, is mediated by ribulosediphosphate carboxylase (EC 4.1.1.39). It might be argued that this enzyme, aside from any regulatory function, catalyzes a relatively complex biochemical reaction and also has to utilize in nature a substrate (CO_2) which occurs at a rather low concentration. However, it is interesting to note that despite the relatively low substrate concentration, the ΔG^s for this reaction is even more negative than the $\Delta G'$. Also the reaction seems inherently not more complicated than the two-step activation-reduction reaction indicated as Reaction B.

We have shown in Reaction B the sum of reactions which are presumably mediated by phosphoglycerate kinase (EC 2.7.2.3) and triosephosphate dehydrogenase (EC 1.2.1.13). We are unable to detect the presumed intermediate, 1-phosphoryl-3-phosphoglyceric acid, due possibly to both its instability and its low concentration. Nevertheless, including these two steps as a single reaction does not invalidate our conclusions since the total negative free energy change is only -1.6 kcal under steady-state conditions, and the individual steps therefore must have somewhat smaller negative free energy changes than that. We are forced to assume for the basis of calculations an [NADPH]/[NADP$^+$] ratio of 1, since this ratio is not measured in our studies. Should this ratio be as high as 10:1, the true negative free energy changes for the two steps in Reaction B would be -2.9 kcal.

The other assumption mentioned in footnotes to Tables I and II contain less possibility for error than the assumption that [NADPH]/[NADP$^+$] = 1.0. In general, deviation from the true ΔG^s values that can result from possible errors in these assumptions is likely to be less than ±0.5 kcal.

Reaction E, ΔG^s = -6.5, is mediated by hexosediphosphatase (EC 3.1.3.11) while Reaction H, ΔG^s = -7.1 kcal, is mediated by a similar, if not identical enzyme. In this case, there is clearly no question of the mediated reaction being a complex one, since it involves only the replacement of an O-P bond by a O-H bond. It is possible that the activity of water is diminished in the environment of the enzymes *in vivo*. If it were decreased to 0.1, the ΔG^s values of these reactions would be reduced by 1.36 kcal, and the reactions would still have negative free energy changes of more than 5 kcal.

Both the ribulosediphosphate carboxylase step and the two diphosphatase steps have been implicated in activation of the photosynthetic carbon reduction cycle in the light by kinetic tracer studies of the light-dark transients in Chlorella[6,35] and in isolated spinach chloroplasts[7]. Other tracer studies[9] showed that the carboxylase and the diphosphatases were inhibited on the addition of certain fatty acids which disrupt both electron transport and photophosphorylation in photosynthesizing Chlorella. As mentioned earlier, further evidence for the possible role of the diphosphatases in regulation in the light, is found in the selective diffusions from the chloroplast of those intermediates just preceding the diphosphatase step in the reductive cycle[10].

The high degree of reversibility of the Reactions D and G, mediated by ketose-1-phosphate aldolase (EC 4.1.2.7), has been often indicated in kinetic tracer studies with photosynthesizing Chlorella, and was especially well demonstrated in light–dark transient studies[35] in which the transient light–dark behavior of dihydroxyacetone phosphate precisely mirrored the behavior of fructose 1,6-diphosphate and sedoheptulose 1,7-diphosphate. In contrast, the transient changes in the monophosphates of fructose and sedoheptulose were completely out of phase with that of the diphosphates, in accordance with the virtually irreversible nature of the diphosphatase reaction.

The one somewhat intermediate case is Reaction L, mediated by phosphoribulokinase (EC 2.7.1.19), and having a negative free energy change of –3.8 kcal. Kinetic studies of photosynthesizing *C. pyrenoidosa* to which vitamin K_5 was added[8] showed immediate inactivation of this enzyme along with the inactivation of the diphosphatase reaction and activation of glucose-6-phosphate dehydrogenase (see below) leading to formation of 6-phosphogluconic acid. The light–dark transient studies had not clearly indicated an immediate inactivation of this enzyme in the dark, but suggest that it becomes inactivated after the first minute of darkness, at least in isolated spinach chloroplasts[7]. Addition of ATP to these chloroplasts just after the light was turned off apparently resulted in the formation of some ribulose 1,5-diphosphate, but this formation then stopped even though excess ATP and pentose monophosphates were still present.

Turning to the oxidative pentose phosphate cycle, it seems evident that glucose-6-phosphate dehydrogenase is another site of metabolic regulation, which is in this case activated in the dark and inactive in the light. In tracer studies with *C. pyrenoidosa*[35], or spinach chloroplasts[8], 6-phosphogluconic acid is not seen until the light is turned off, at which time it appears immediately in a substantial amount.

For the oxidation of glucose 6-phosphate, mediated by glucose-6-phosphate dehydrogenase (EC 1.1.1.49) to be prevented by a high ratio of $[\mathrm{NADPH}]/[\mathrm{NADP}^+]$ the ratio would have to be $1 \cdot 10^8$, given the concentrations of the glucose 6-phosphate in the light and atmospheric CO_2. This seems to be impossible, and light inactivation and dark activation of this enzyme in photosynthetic cells is thus indicated on the basis of the calculated ΔG^s. While the accuracy of the calculations for Reactions X and Y may be somewhat less than for other reactions reported here (see footnote (p) of Table II) we believe the $\Delta G'$ and ΔG^s values to be correct *plus* or *minus* 1.5 kcal. An error of 1.5 kcal in either direction would not alter the conclusions just made.

This indication of dark activation of glucose-6-phosphate dehydrogenase in Chlorella adds to our general concept of the regulation in light and dark of photosynthetic metabolism. As discussed elsewhere[8,35], it appears that the reductive pentose phosphate cycle operates in the chloroplasts during photosynthesis and that an oxidative pentose phosphate cycle operates in the chloroplasts in the dark. The suggested function of the oxidative cycle is to provide NADPH for biosynthesis in the chloroplasts, since ATP, but not NADPH, can be imported from the cytoplasm.

We propose that inactivation of glucose-6-phosphate dehydrogenase prevents the operation of the oxidative cycle in the light, while photosynthesis is going on. Activation of this enzyme in the dark, together with inactivation of phosphoribulokinase, and ribulosediphosphate carboxylase, prevents the operation of the photosynthetic or reductive pentose phosphate cycle in the dark and permits the oxidative pentose phosphate cycle to operate. Hexosediphosphatase activity is not needed for the oxidative cycle, which bypasses the hexose and heptose diphosphates by means of the transaldolase reaction (EC 2.2.2.1).

It may be concluded that in green cells, at least at the site of photosynthetic metabolism, the chloroplasts, in both the reductive cycle in the light and the oxidative cycle in the dark, only those reactions mediated by enzymes involved in metabolic regulation have high negative steady-state free energy changes. All other reactions, irrespective of their complexity, are characterized by low negative free energy changes and a very high degree of reversibility. Thus the greater part of the energy dissipated by this network is released in reactions which provide metabolic control.

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