Model of 2,3-bisphosphoglycerate metabolism in the human erythrocyte based on detailed enzyme kinetic equations[1]: computer simulation and Metabolic Control Analysis

Peter J. MULQUINEY and Philip W. KUCHEL[2]
Department of Biochemistry, University of Sydney, NSW 2006, Australia

This is the third of three papers [see also Mulquiney, Bubb and Kuchel (1999) Biochem. J. **342**, 565–578; Mulquiney and Kuchel (1999) Biochem. J. **342**, 579–594] for which the general goal was to explain the regulation and control of 2,3-bisphosphoglycerate (2,3-BPG) metabolism in human erythrocytes. 2,3-BPG is a major modulator of haemoglobin oxygen affinity and hence is vital in blood oxygen transport. A detailed mathematical model of erythrocyte metabolism was presented in the first two papers. The model was refined through an iterative loop of experiment and simulation and it was used to predict outcomes that are consistent with the metabolic behaviour of the erythrocyte under a wide variety of experimental and physiological conditions. For the present paper, the model was examined using computer simulation and Metabolic Control Analysis. The analysis yielded several new insights into the regulation and control of 2,3-BPG metabolism. Specifically it was found that: (1) the feedback inhibition of hexokinase and phosphofructokinase by 2,3-BPG are equally as important as the product inhibition of 2,3-BPG synthase in controlling the normal *in vivo* steady-state concentration of 2,3-BPG; (2) H^+ and oxygen are effective regulators of 2,3-BPG concentration and that increases in 2,3-BPG concentrations are achieved with only small changes in glycolytic rate; (3) these two effectors exert most of their influence through hexokinase and phosphofructokinase; (4) flux through the 2,3-BPG shunt changes in absolute terms in response to different energy demands placed on the cell. This response of the 2,3-BPG shunt contributes an [ATP]-stabilizing effect. A 'cost' of this is that 2,3-BPG concentrations are very sensitive to the energy demand of the cell and; (5) the flux through the 2,3-BPG shunt does not change in response to different non-glycolytic demands for NADH.

Key words: concentration control coefficient, coresponse coefficient, elasticity coefficient, flux control coefficient, Rapoport–Luebering shunt.

INTRODUCTION

The discovery that 2,3-bisphosphoglycerate (2,3-BPG) plays an important role in blood oxygen transport was reported over 30 years ago [1,2]. This discovery stimulated a significant amount of work on 2,3-BPG, particularly during the 1970s, but it is salutary to note that many issues relating to 2,3-BPG metabolism have remained unresolved. One aspect that was yet to be fully understood is how 2,3-BPG concentration is modulated by changes in tissue oxygen demand in the body. This modulation of concentration becomes apparent in various disease states and environmental changes. For example, in conditions where oxygen transport is compromised, such as anaemia, congenital heart disease and high altitude, higher concentrations of 2,3-BPG are evident [3,4].

A basic model for these 2,3-BPG increases during hypoxia was presented by Duhm and Gerlach [5]. An important aspect of their model was that it identified both blood pH and oxygen as important effectors of 2,3-BPG concentration.

An initial response to acute hypoxia is hyperventilation, which causes respiratory alkalosis. If this alkalosis is prevented, the usual accumulation of 2,3-BPG is prevented [5,6]. This result implicates blood pH in the control of 2,3-BPG concentration. The importance of blood pH in controlling 2,3-BPG concentration is also supported by a number of studies. Increases in pH above the usual physiological value result in 2,3-BPG accumulation, while decreases result in depletion [7–10]. This pH-dependence is dramatic; the steady-state concentration of 2,3-BPG in glycolysing erythrocytes decreases from its usual steady-state value of ≈ 7 mM to zero with a decrease of only 0.4 pH unit [8].

Oxygen acts as an external effector of 2,3-BPG concentration, owing to its influence on haemoglobin (Hb) oxygen saturation. Hb oxygen saturation affects 2,3-BPG metabolism in two ways: (1) deoxygenation of erythrocytes leads to an increase in intracellular pH of 0.07–0.14 pH unit due to deoxygenated Hb (deoxy-Hb) having a higher affinity for protons than oxygenated Hb (oxy-Hb) [11,12]; (2) deoxy-Hb binds many phosphorylated glycolytic intermediates with greater affinity than oxy-Hb. Thus a change in oxygen tension can significantly affect the free concentrations of glycolytic intermediates and effectors and thus significantly affect 2,3-BPG metabolism [13–17].

The difficulty in accounting for the changes in 2,3-BPG concentration that occur with changes in pH and oxygen is that they alter the activities of 2,3-BPG synthase (BPGS) and 2,3-BPG phosphatase (BPGP) in several interrelated ways. The activity of BPGS is believed to be limited by the low cellular levels of 1,3-BPG and by the high level of its inhibitory product, 2,3-BPG [18,19], whereas the activity of the phosphatase depends primarily on the concentration of activatory anions, such as P_i,

Abbreviations used: 2,3-BPG, bisphosphoglycerate; deoxy-Hb, deoxygenated haemoglobin; oxy-Hb, oxygenated Hb; Met-Hb, methaemoglobin; MCA, Metabolic Control Analysis; HK, hexokinase; PFK, phosphofructokinase; Eno, enolase; PK, pyruvate kinase; BPGS, 2,3-BPG synthase; BPGP, 2,3-BPG phosphatase.

[1] This is the third of a series of three papers on this topic; the first two papers are [24,25].

[2] To whom correspondence should be addressed (e-mail p.kuchel@biochem.usyd.edu.au).

and the inhibitor 3-phosphoglycerate [20]. Thus the activities of the enzymes which synthesize and degrade 2,3-BPG are highly dependent on the concentrations of some glycolytic intermediates. Since pH and oxygen are known to affect a number of glycolytic enzymes to different extents [21,22], determining the mechanism by which these effectors influence 2,3-BPG concentration is a difficult task. For example, the activities of many glycolytic enzymes are strongly dependent on pH [22]. Also, the association of many glycolytic metabolites and effectors with Hb and Mg^{2+} are highly dependent on pH and oxygenation state [17].

Another aspect of 2,3-BPG metabolism that had not been fully explored was whether the rate of metabolite flow through the 2,3-BPG shunt is biologically important. In by-passing phosphoglycerate kinase the shunt allows the metabolism of glucose without net production of ATP. In other words, the extent of flux through the shunt relative to the flux through the main glycolytic pathway will affect the glucose/ATP stoichiometry which could vary from 0:1 to 2:1. Thus it has been suggested that the 2,3-BPG shunt 'uncouples' the supply of NADH from the production of ATP [23]. NADH production is particularly important in erythrocytes because it serves to maintain Hb in its functional Fe(II) state by acting as a cofactor in the reduction of methaemoglobin (Met-Hb) [Fe(III)] by NADH:Met-Hb reductase.

These issues were addressed by performing computer simulations and Metabolic Control Analysis (MCA) of 2,3-BPG metabolism. The basis of the analysis is a detailed mathematical model of erythrocyte metabolism that was presented in the two previous papers [24,25]. This model had been refined though an iterative loop of experiment and simulation and was shown to yield predictions of the time dependence of metabolite concentrations that were consistent with the metabolic behaviour of the erythrocyte under a wide variety of conditions. The roles of the important external effectors, H^+ and oxygen, in controlling the concentration were studied in detail. In addition, the effects of ATP and NADH demand on metabolic flux through the shunt were also investigated.

THEORY

An important message to emerge from the detailed study of the kinetics of metabolic pathways is that the metabolite fluxes and concentrations of a metabolic system are dependent on the properties of all the components of the system [26].

Traditionally, systemic metabolic behaviour has been related to the properties of its component enzymes in only a qualitative manner; this often involved relying on the properties of a few 'rate-limiting' enzymes [27,28]. However, the complex interconnections between different metabolic pathways, the existence of moiety conservation cycles, and the enormous number of possible regulatory interactions on enzymes ensure the limited utility of a qualitative approach. A qualitative approach does not allow precise comparison between predictions of a model and experimental data. Thus it is difficult to distinguish between alternative explanations for the same event. MCA allows a quantifiable and rigorous analysis of the regulatory properties of metabolic pathways [29–31]. Theoretical and experimental aspects of MCA have been treated in detail in a number of recent papers and reviews [27,28,32,33]. The following is a brief outline of the central concepts that were used in the present paper.

The major concern of MCA is the quantification of the role of individual reactions in determining pathway fluxes and metabolite concentrations. Fundamental to the theory is the definition of control coefficients and elasticity coefficients.

Control coefficients

For a metabolic system at steady state with i metabolites, j steady-state metabolic fluxes (J_j) and k reactions, the flux control coefficient is defined as:

$$C^{J_j}_{v_k} = \frac{v_k \partial J_j / \partial p_k}{J_j \partial v_k / \partial p_k} = \frac{\partial \ln J_j / \partial \ln p_k}{\partial \ln v_k / \partial \ln p_k} \quad (1)$$

where p_k is a parameter which affects the velocity of reaction k (v_k) only, and where $\partial v_k / \partial p_k$ is determined at the parameter values and concentrations associated with the reference steady state. Thus the flux control coefficient determines the effect that the parameter p_k will have on the system-flux, J_j, if the effect of p_k on the local enzyme rate, v_k, is known. For many systems, the value of the control coefficient is independent of the choice of parameter p_k. Thus the flux control coefficient is a measure of the extent to which reaction k 'controls' the steady-state flux, J_j. Similarly a concentration control coefficient may be defined in a similar manner by considering changes in steady-state concentrations rather than fluxes.

Another feature of flux control coefficients is that for simple enzymic reactions:

$$\frac{e_k}{v_k}\frac{\partial v_k}{\partial e_k} = 1 \quad (2)$$

and hence eqn. (1) reduces to:

$$C^{J_j}_{v_k} = \frac{e_k \partial J_j}{J_j \partial e_k} = \frac{\partial \ln J_j}{\partial \ln e_k} \quad (3)$$

Thus for many reactions the flux control coefficient indicates the sensitivity of a particular flux, J_j, to a variation in the concentration of enzyme, e_k.

Elasticity coefficients

Elasticity coefficients characterize the response of an individual enzyme in isolation to the perturbation of a parameter, determined at some reference state of substrate, product and effector concentrations. It is defined by:

$$\epsilon^{v_k}_p = \frac{p}{v_k}\frac{\partial v_k}{\partial p} = \frac{\partial \ln v_k}{\partial \ln p} \quad (4)$$

where v_k is the velocity of reaction k and p is a parameter or metabolite which affects v_k.

In the present work, control and elasticity coefficients were determined numerically using the mathematical model of erythrocyte metabolism described in the accompaying papers [24,25]. This involved replacing the partial differentials (∂) in the above equations by finite differences; namely $\partial p = 10^{-2}p$.

RESULTS AND DISCUSSION

Interrelations between glycolysis and the pentose phosphate pathway

Tables 1 and 2 show the control coefficients (see the Theory section) estimated for some important fluxes and metabolite concentrations using the model described in the accompanying papers [24,25]. A key result was that the ATPase and 2,3-BPG phosphatase activities exert major control of the rate of glycolysis, while the oxidative load primarily controls flux through the pentose phosphate pathway. These findings are in agreement with that of Schuster et al. [34,35] who had, to date, published the most comprehensive MCA of a mathematical model of erythrocyte metabolism. Also in agreement with their model [35] was the finding that the 2,3-BPG phosphatase reaction

Table 1 Selected flux and concentration control coefficients for the normal *in vivo* steady state of the human erythrocyte

Control coefficients were calculated as described in the Theory section. For definitions of abbreviations not already given, see [25]. The largest control coefficients for each system-flux or concentration are highlighted in **bold**.

Reaction (*i*)	$C^{J_{HK}}_{v_i}$	$C^{J_{PGK}}_{v_i}$	$C^{J_{PK}}_{v_i}$	$C^{J_{BPGS}}_{v_i}$	$C^{J_{G6PDH}}_{v_i}$	$C^{J_{GSSGR}}_{v_i}$	$C^{J_{ATPase}}_{v_i}$	$C^{J_{Ox}}_{v_i}$	$C^{S_{ATP}}_{v_i}$	$C^{S_{2,3\text{-}BPG}}_{v_i}$
HK	0.005	0.028	0.004	**−0.089**	0.000	0.000	0.026	0.000	0.048	**1.142**
GPI	0.000	0.000	0.000	−0.002	0.000	0.000	0.000	0.000	0.000	0.004
PFK	0.002	0.006	0.002	−0.014	0.000	0.000	0.005	0.000	0.010	**0.239**
Ald	−0.001	−0.001	−0.001	0.000	0.000	0.000	−0.001	0.000	−0.001	0.001
TPI	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GAPDH	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGK	0.000	0.000	0.000	−0.001	0.000	0.000	0.000	0.000	0.000	−0.001
PGM	0.002	0.002	0.002	0.002	0.000	0.000	0.001	0.000	0.002	−0.004
Enolase	0.053	0.042	0.054	**0.108**	0.000	0.000	0.042	0.000	0.047	**−0.134**
PK	0.080	0.062	0.081	**0.154**	0.000	0.000	0.062	0.000	0.069	**−0.200**
LDH	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000
LDH(P)	0.001	0.000	0.000	0.000	**0.060**	0.000	0.000	0.000	0.000	−0.001
AK	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
BPGS*	−0.020	−0.055	−0.018	**0.128**	0.000	0.000	−0.056	0.000	−0.064	**0.105**
BPGP*	**0.181**	0.028	**0.186**	**0.834**	−0.001	0.000	0.028	0.000	0.029	**−0.298**
G6PDH	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000
Lactonase	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	−0.001
6PGDH	0.000	0.000	0.000	0.000	−0.001	0.000	0.000	0.000	0.000	0.000
R5PI	0.001	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	−0.001
Ru5PE	0.001	0.000	0.001	0.003	0.000	0.000	0.000	0.000	0.000	−0.001
TK	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000
TA	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000
GSSGR	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000
k_{ATPase}	**0.678**	**0.886**	**0.691**	**−0.133**	−0.003	0.000	**0.887**	0.000	**−0.142**	**−0.836**
k_{ox}	0.016	0.000	0.000	0.000	**0.939**	**1.000**	0.000	**1.000**	−0.001	−0.015
k_{oxNADH}	0.001	0.000	0.001	0.002	0.000	0.000	0.000	0.000	0.000	−0.001

* Since there is no unitary rate constant that can be varied for the synthase reaction, which does not in turn affect the rate of the phosphatase activity, and vice versa, control coefficients were calculated for each unitary step of the BPGS/P mechanism (see [24]); these are presented in Table 2. The values for BPGS and BPGP presented in this Table represent the sum of the control coefficients for the reaction steps involved in the synthase (k_1–k_{13}) and the phosphatase activities (k_4–k_{16}) respectively; the summation theorem (e.g. see [27]) still holds in this case, since the control coefficients for reactions k_4–k_{13} add up to approximately zero for all fluxes and concentrations reported in Table 2.

Table 2 Selected flux and concentration control coefficients for the unitary reactions in the model of BPGS-P

The values were calculated with the metabolic system poised at the normal *in vivo* steady state (see [25]). The largest control coefficients for each system-flux or concentration are highlighted in **bold**.

Rate constant (*i*)	$C^{J_{HK}}_{v_i}$	$C^{J_{PGK}}_{v_i}$	$C^{J_{PK}}_{v_i}$	$C^{J_{BPGS}}_{v_i}$	$C^{J_{G6PDH}}_{v_i}$	$C^{J_{GSSGR}}_{v_i}$	$C^{J_{ATPase}}_{v_i}$	$C^{J_{Ox}}_{v_i}$	$C^{S_{ATP}}_{v_i}$	$C^{S_{2,3\text{-}BPG}}_{v_i}$
k_1	−0.020	**−0.055**	−0.020	0.127	0.000	0.000	**−0.055**	0.000	**−0.063**	0.102
k_2	0.018	0.054	0.019	−0.129	0.000	0.000	0.054	0.000	0.061	−0.097
k_3	−0.018	−0.054	−0.018	0.132	0.000	0.000	−0.054	0.000	−0.062	0.097
k_4	−0.103	−0.041	−0.105	−0.368	0.001	0.000	−0.042	0.000	−0.045	0.204
k_5	0.103	0.041	0.105	0.368	−0.001	0.000	0.041	0.000	0.045	−0.203
k_6	−0.001	0.000	0.001	−0.001	0.000	0.000	0.000	0.000	0.000	0.001
k_7	0.001	0.000	0.001	0.003	0.000	0.000	0.000	0.000	0.000	−0.001
k_8	−0.103	−0.041	−0.105	−0.368	0.001	0.000	−0.041	0.000	−0.045	0.204
k_9	0.103	0.041	0.105	0.368	−0.001	0.000	0.041	0.000	0.045	−0.203
k_{10}	−0.001	0.000	−0.001	−0.004	0.000	0.000	0.000	0.000	0.000	0.002
k_{11}	0.001	0.000	0.001	0.004	0.000	0.000	0.000	0.000	0.000	−0.002
k_{12}	−0.032	−0.053	−0.033	0.051	0.000	0.000	−0.053	0.000	−0.060	0.117
k_{13}	0.032	0.053	0.032	−0.055	0.000	0.000	0.053	0.000	0.060	−0.116
k_{14}	0.133	0.036	0.136	0.548	−0.001	0.000	0.037	0.000	−0.039	−0.241
k_{15}	−0.133	−0.037	−0.136	−0.546	0.001	0.000	−0.037	0.000	0.039	0.241
k_{16}	**0.181**	0.029	**0.185**	**0.834**	−0.001	0.000	0.029	0.000	0.029	**−0.301**

has the largest control coefficient for flux through the 2,3-BPG shunt (column 4 of Table 1). However, in contrast with the findings of those authors, hexokinase (HK), phosphofructokinase (PFK), enolase (Eno), pyruvate kinase (PK), BPGS and the ATPase reactions were all shown to have significant 2,3-BPG shunt flux control coefficients as well. The implications that the

new finding has for the regulation and control of 2,3-BPG metabolism are discussed in greater detail below.

It is also noteworthy that the values of the control coefficients calculated in the present work are significantly different from those reported by Rapoport et al. for early 'skeleton' models of glycolysis [36,37]. For example, Rapoport et al. [37] reported that the enzyme sequence HK → PFK was the most significant control point for glycolytic flux with an overall control coefficient in the range 0.52–1.32, depending on the degree of inhibition of PFK by ATP [37]. In contrast we found a value of 0.007 (taking the flux through HK to be the glycolytic flux) with the control residing principally in the ATPase(s). Our conclusion is similar to that of Schuster et al. [35], who report an overall control coefficient of 0.094 for the HK → PFK enzyme sequence. The discrepancy is primarily due to the simplified rate equation that was used by Rapoport et al. [37] to describe the HK → PFK enzyme sequence, which ignored the feedback inhibition of HK by glucose 6-phosphate and the feedback activation of PFK by AMP, as well as the action of other important effectors [25].

A surprising result to emerge from the MCA was the large control coefficients calculated for the enolase reaction (row 9 of Table 1). This enzyme has received little study in the erythrocyte, and hence the kinetic parameters used in the model were uncertain. However, given the important role of Mg^{2+} in the catalytic mechanism of Eno (see [25]), this enzyme becomes important in controlling flux under conditions of Mg^{2+} deficiency.

Regulation and control of 2,3-BPG concentration

Feedback inhibition by 2,3-BPG

The large values of $C^{S_{2,3\text{-BPG}}}_{v_i}$ for HK and PFK (rows 1 and 3, respectively, in Table 1) indicate that the feedback inhibition of these enzymes by 2,3-BPG are important for controlling 2,3-BPG concentration. The elasticity coefficients for the activities of HK, PFK, and BPGS with respect to [2,3-BPG] were calculated to be −0.13, −0.33, and −0.84 respectively. This indicates that the synthase is the most susceptible of these reactions to changes in [2,3-BPG] around the normal *in vivo* steady state. These elasticities were calculated by taking into account metabolite binding to Hb and Mg^{2+}. The product of the elasticity coefficient for each enzyme and its respective control coefficient:

$$C^{S_{2,3\text{-BPG}}}_{v_i}\epsilon^{v_i}_{S_{2,3\text{-BPG}}} \quad (5)$$

gives the partial internal response coefficient for 2,3-BPG [27]; this is a measure of the control that the 2,3-BPG inhibition of the reaction, whose rate is v_i, has on the steady-state concentration of 2,3-BPG. These were calculated to be −0.15, −0.08 and −0.09 for HK, PFK, and BPGS respectively. Hence, in the normal *in vivo* steady state of the erythrocyte, the feedback inhibition of HK and PFK by 2,3-BPG is as important in controlling the 2,3-BPG concentration as is the product inhibition of BPGS by 2,3-BPG.

Control of 2,3-BPG concentration by H^+ and oxygen

The modulation of 2,3-BPG concentration with respect to the satisfaction of tissue oxygen demand occurs mainly via the external effectors, H^+ and oxygen [3]. The response coefficients:

$$R^{S_{2,3\text{-BPG}}}_{p} = \frac{p}{S_{2,3\text{-BPG}}}\frac{\partial S_{2,3\text{-BPG}}}{\partial p} = \frac{\partial \ln S_{2,3\text{-BPG}}}{\partial \ln p} \quad (6)$$

where p is $[H^+]$, or % oxy-Hb, and $S_{2,3\text{-BPG}}$ is the steady-state concentration of 2,3-BPG, were calculated to be −1.7 and −0.58 respectively. This result indicates that these effectors are strong modulators of 2,3-BPG concentration. The influences of H^+ and oxygen on the steady-state concentrations of 2,3-BPG are also shown in Figures 1 and 2. The value of −1.7 for $R^{S_{2,3\text{-BPG}}}_{[H^+]}$ is in good agreement with the value of −1.8 that can be calculated from the data of Astrup et al. [10]; those authors demonstrated that a rise in intracellular pH of 0.01 unit causes the steady-state 2,3-BPG concentration to increase by ≈ 0.3 mM. The accuracy of the % oxy-Hb response coefficient is considered below, and in this and the entire subsequent analysis, % oxy-Hb

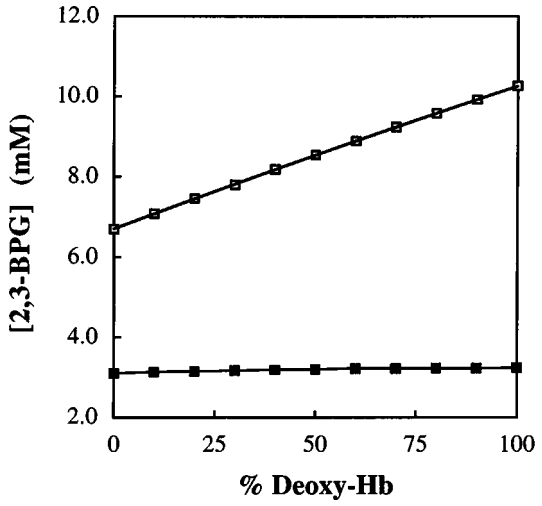

Figure 1 Steady-state concentrations of total 2,3-BPG (□) and free 2,3-BPG (■) as a function of the percentage of deoxy-Hb

The values were calculated using the model described in [24,25].

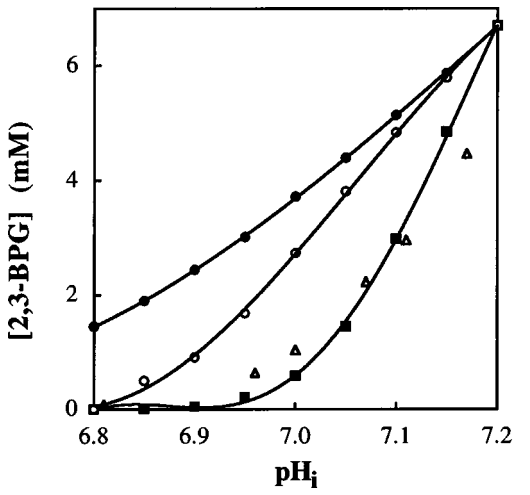

Figure 2 Steady-state concentration of 2,3-BPG as a function of intracellular pH (pH$_i$)

■, Values were calculated using the model described in [24,25], although similar values were calculated if it was assumed that the total concentration of ATP was constant. △, Experimental values of Rapoport et al. [8]. (Note that pH values were converted into intracellular ones using the data of Duhm [11].) ○, Values calculated using the model described in [24,25], but neglecting the pH-dependence of BPGS/P. ●, Values calculated using the model described in [24,25], but neglecting the pH-dependence of BPGS/P, and assuming that the total concentration of ATP was constant.

Table 3 Partial response coefficients for 2,3-BPG concentrations with respect to [H$^+$] and percentage of oxy-Hb

For abbreviations not already defined, see [25]. The largest control coefficients for each system-flux or concentration are highlighted in **bold**.

	$R^{S_{2,3\text{-BPG}}}_{v_i p}$	
Reaction	p = [H$^+$]	p = % oxy-Hb
HK	**−0.53**	**−0.28**
PFK	**−0.71**	**−0.35**
Eno	−0.01	0.01
PK	0.00	0.04
BPGS	−0.23	−0.08
BPGP	−0.21	0.03
ATPase	0.12	0.07

Table 4 Glycolytic flux and 2,3-BPG concentration as a function of Hb oxygen saturation and pH

	Steady state		Quasi-steady state	
Conditions*	HK flux (mmol · litre of erythrocytes^{-1} · h^{-1}	[2,3-BPG] (mM)	HK flux (mmol · litre of erythrocytes^{-1} · h^{-1})	[2,3-BPG] mM
pH 7.20, 100% oxy-Hb	1.41	6.70	1.41	6.70
pH 7.20, 100% deoxy-Hb	1.51	10.3	2.39	6.70
pH 7.27, 100% deoxy-Hb	1.46	11.9	2.71	6.70

* Note that all other external parameters were held at the concentrations shown in Table 3 in [25].

was used instead of oxygen tension. In calculating % oxy-Hb it was assumed that each Hb molecule is either totally oxygenated or totally deoxygenated. The latter assumption is clearly an oversimplification, but it was considered to be prudent, since there is no information available on metabolite–Hb binding constants for partially O_2-saturated Hb species.

The partial response coefficients for those reactions with the largest values of $C^{S_{2,3\text{-BPG}}}_{v_i}$ were also calculated, namely:

$$R^{S_{2,3\text{-BPG}}}_{v_i,p} = C^{S_{2,3\text{-BPG}}}_{v_i} e^{v_i}_p \quad (7)$$

The results are shown in Table 3; the values indicate that H$^+$ and oxygen exert their strongest effects on 2,3-BPG concentration via HK and PFK.

The ratio of the response coefficient of glycolytic flux with respect to [H$^+$] to the response coefficient of 2,3-BPG concentration with respect to [H$^+$] is −0.07. The low value of this co-response coefficient [27,38] indicates that H$^+$ is effective in modulating 2,3-BPG concentrations without substantially changing the glycolytic rate. A similarly low co-response coefficient is found for oxy-Hb (0.11). Therefore, both H$^+$ and oxygen can modulate 2,3-BPG concentrations without large changes in glycolytic flux. This conclusion can also be made from the data in Table 4, where it is seen that complete deoxygenation of Hb causes a $< 7\%$ increase in the steady-state glycolytic rate, while at the same time it causes a 54–78% increase in steady-state [2,3-BPG]. Table 4 also illustrates the value of comparing model predictions with experimental measurements of the effects of deoxygenation. If 2,3-BPG is kept constant at its initial concentration, the glycolytic rate in this 'quasi-steady state' is significantly larger than at the true steady state. In most experimental estimates of the effect of the oxygenation state of Hb on glycolytic rate, the rate of glycolysis is measured 1–3 h after deoxygenation. From the current model it was calculated that ≈ 10.5 h is required to reach $\approx 90\%$ of the 2,3-BPG levels of the new steady state. Thus these experimental measures are estimates of glycolysis during the transition between the quasi-steady state and the true steady state. Hence the fact that the model predicts significantly higher glycolytic rates in the quasi-steady state upon deoxygenation than has been determined experimentally is not surprising; most experiments have found a 25–50% increase in the glycolytic rate upon deoxygenation [22,39,40].

Another aspect of comparing the predictions of the model with experimental results is due to the role of 2,3-BPG in the Donnan equilibrium [11,41,42]. Because 2,3-BPG has a high negative charge and does not permeate the cell membrane, the intracellular pH decreases as it accumulates. The present model only predicted 2,3-BPG concentrations at a given intracellular pH; it did not account for the fact that 2,3-BPG plays a significant role in determining the intracellular pH value. Most reports of experimental studies of the effect of deoxygenation on 2,3-BPG concentrations do not give the intracellular pH values. Furthermore, while deoxygenation has been reported to increase intracellular pH by 0.07−0.14 unit [5,12], these measurements were made before new steady-state concentrations of 2,3-BPG were achieved. However, if it is assumed that the steady-state intracellular pH, upon deoxygenation, is at the lower end of the above range, then it was calculated that deoxygenation would cause a 78% increase in total [2,3-BPG] (Table 4). This calculated result is in good agreement with the finding of Duhm and Gerlach [5] that, in rats exposed to hypoxic gas mixtures [$\approx 7\%$ (v/v) O_2], there was an increase of 76% in total 2,3-BPG concentration after 24 h.

Decline of 2,3-BPG concentrations at low pH

During blood storage there is a progressive accumulation of lactic acid due to erythrocyte glycolysis. This results in a decrease in pH and depletion of 2,3-BPG; the mechanism of this pH-dependent depletion has been an issue of debate. It has generally been considered that it is the pH-dependence of glycolysis which results in this decline (e.g. [43]). However, Rapoport et al. [8] postulated that it is the pH-dependence of the BPGS reaction rather than the pH-dependence of glycolysis that is the cause of this decrease.

From Table 3 it is seen that a decline in pH around the normal *in vivo* value leads to 2,3-BPG depletion due to inhibition of both the glycolytic enzymes, HK and PFK, and the BPGS reaction by H$^+$. In other words it is seen that the control of the 2,3-BPG concentration, as a function of pH, is shared primarily amongst HK, PFK, and 2,3-BPG synthase. Figure 2 shows a plot of the steady-state concentration of total 2,3-BPG as a function of pH that is predicted by the model described in [24,25]. If the pH-dependence of the synthase, which was determined in [24], was included in the calculation, then there was a very good match between the experimental data [8] and the simulated values. When this pH-dependence was ignored, it was seen that the pH-dependencies of the glycolytic reactions alone would be responsible for a substantial part of the decrease in 2,3-BPG concentration with pH. These time courses were significantly different when the different assumptions were used for the ATPase, and therefore these differences should be experimentally detectable. Also, during blood storage, the accumulation of P_i

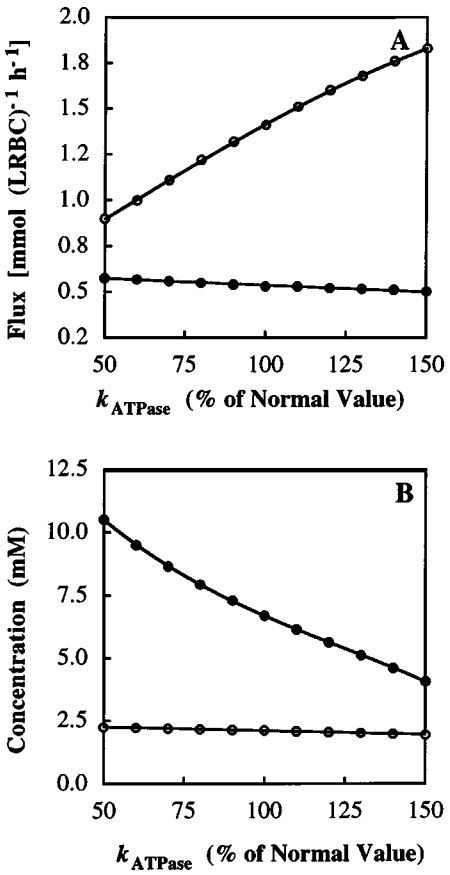

Figure 3 (A) Steady-state glycolytic (○) and 2,3-BPG (●) shunt fluxes and (B) ATP (○) and 2,3-BPG (●) concentrations as a function of the value of k_{ATPase}

that results from the depletion of 2,3-BPG will contribute to the decline of 2,3-BPG, since P_i activates 2,3-BPG phosphatase (BPGP). The response coefficient of 2,3-BPG concentration to extracellular P_i was calculated to be -0.21 under normal *in vivo* conditions.

An interesting hypothesis regarding the decline in 2,3-BPG during blood storage was put forward by Black et al. [44]. They proposed that, under the acidic conditions of blood storage, the reactions of the 2,3-BPG shunt together with phosphoglycerate-kinase form a futile cycle which depletes both ATP and 2,3-BPG. However, the occurrence of this is unlikely, since it requires the rate of the shunt to be higher than the rate of glycolysis; it was calculated in the present work that this would not be the case at pH values down to less than 6.8. However, the calculations were performed using enzyme-kinetic data valid at 37 °C; the different temperature-dependencies of the various enzymes [45] may mean that, at 4 °C, the temperature at which blood is stored, a futile cycle could operate. Black et al. [44] also claimed to have provided evidence for the futile cycle operating in erythrocytes incubated in the absence of glucose. They concluded that the incorporation of radiolabelled P_i into 2,3-BPG was indicative of significant synthase activity under these conditions. This interpretation, however, does not take into account the label exchange that would occur between 3-phosphoglycerate and 2,3-BPG in the phosphatase and phosphoglycerate mutase reactions [24]. From the simulations reported in [24], the rate of this futile cycle in the absence of glucose would be negligible (the rate of the futile cycle was found to be less than 2% of the net rate of ATP synthesis).

Control of 2,3-BPG concentration by total Hb concentration

Another novel result that emerged from the MCA was that the response coefficient for 2,3-BPG concentration to [Hb] is 0.57. This indicates that changes in 2,3-BPG concentration are very responsive to changes in total Hb concentrations around the normal *in vivo* steady-state.

Control of 2,3-BPG concentration by total Mg^{2+}

Another interesting point is that the response coefficient of 2,3-BPG concentration to total [Mg^{2+}] is large (0.84). This finding is in agreement with a study that found that Mg^{2+} supplementation of stored blood causes increases in 2,3-BPG concentration [46]. The largest three partial response coefficients for this magnesium response were: HK, 0.45; PFK, 0.98; and ATPase, -0.28.

Regulation and control of flux through the 2,3-BPG shunt

In by-passing phosphoglycerate kinase the 2,3-BPG shunt allows the metabolism of glucose without net production of ATP. Hence it is possible that the shunt will play an important role in 'co-ordinating' the supply of redox equivalents and the ATP requirements of the erythrocyte.

Stabilization of ATP concentration

It has been recognized for some time that the 2,3-BPG shunt assists in maintaining stable ATP concentrations in the face of changing ATP consumption rates [47,48]. This role in altering the stoichiometric relationship between glucose consumption and ATP production has been attributed to the fact that the percentage of the glycolytic flux that passes through the shunt will decrease with an increasing ATP consumption and hence glycolytic rate. In other words, at higher glycolytic rates the ATP/glucose stoichiometry increases. In most models of erythrocyte glycolysis, the kinetics of 2,3-BPGP are modelled such that, at physiological concentrations of 2,3-BPG, 2,3-BPG degradation is an almost zero-order process (e.g. [34,35]). For such a kinetic description, the flux control coefficient for flux through the shunt will be ≈ 1. But by using a more realistic model of BPGS/P [24] it was found that, while the phosphatase activity exerted the most control over flux through the 2,3-BPG shunt, ATPase activity also exerts some control (-0.133; fourth column, third row from bottom in Table 1). However, the change in flux through the 2,3-BPG shunt, as a function of k_{ATPase}, is small; a change in ATPase activity by $+50\%$ leads to only a -7% change in the 2,3-BPG shunt flux in the steady state (Figure 3A). Even so, this change in flux as a function of ATPase activity will enhance the ATP-stabilizing effect of the 2,3-BPG shunt. The 'cost' of this, however, is a large variation in 2,3-BPG concentration as a function of ATPase activity (Figure 3B).

Uncoupling the supply of NADH from the production of ATP

In by-passing phosphoglycerate kinase it has been suggested that the 2,3-BPG shunt may uncouple the supply of NADH from

the production of ATP [23], a process that we have called 'stoichiometry breaking'. However, from Table 1 it is seen that the oxidation of NADH by non-glycolytic processes (k_{oxNADH}) has a negligible control coefficient for both flux through the shunt and 2,3-BPG concentration. A problem with this analysis is that the NAD^+/NADH ratio is essentially fixed by the external parameters, extracellular [lactate] and extracellular [pyruvate]. However, it was found that the response coefficients for flux via the 2,3-BPG shunt and 2,3-BPG concentration to extracellular pyruvate were also very small, namely -0.01 and 0.01 respectively. Thus, even if the activity of k_{oxNADH} changed the external pyruvate-to-lactate ratios, there would be little effect on flux via the 2,3-BPG shunt and 2,3-BPG concentration.

Validity of Control Analysis

In the work presented here, the regulatory and control properties of a mathematical model of erythrocyte metabolism were determined. How well these regulatory and control properties reflect those of the real erythrocyte depends on the quality of the mathematical model. As seen in [25], most of the kinetic data used in model development were determined from isolated enzymes studied *in vitro* and under conditions far removed from the typical intracellular environment. Thus the parameter values determined may not reflect those in the actual intracellular environment [49,50]. However, through an iterative process of experiment and simulation it was possible to refine various key parameters of the model. This led to close conformity between simulations and the 'real' behaviour of the erythrocyte under a wide variety of experimental and physiological conditions [24].

It is acknowledged that it can only be stated that the current model is consistent with a wide variety of metabolic behaviour. The parameter values used in the model are not unique; there would be a large array of parameter values that could be entered into the model to yield similar behaviour. The extent to which these alternative parameter values would alter the values of the control coefficients is sometimes difficult to determine. Also, the model has been based on the best available experimental information, and its predictions may not be in accordance with future experimental findings. As a general description of erythrocyte metabolism the current model will no doubt contain deficiencies. However, by constructing this model, many of the complicated interrelations that are known to occur in erythrocyte metabolism, on the basis of current knowledge, have been elucidated. At worst, the model forms a useful summary, in as consistent a way as possible, of the huge corpus of *in vitro* and *in vivo* kinetic data that have been accumulated over the last ≈ 50 years of study of erythrocyte metabolism. At best it provides new insights that can be summarized in the Conclusions section.

CONCLUSIONS

Several important general conclusions could be drawn from the simulations that reproduced well the experimental data.

(1) MCA of the model presented in [24,25] verified the findings of Schuster et al. [34,35], who found that the energetic and oxidative loads almost independently control the fluxes through glycolysis and the pentose phosphate pathway respectively.

(2) Feedback inhibition of HK and PFK by 2,3-BPG are equally important as the product inhibition of 2,3-BPG synthase in controlling the normal *in vivo* steady-state concentration of 2,3-BPG.

(3) H^+ and oxygen are effective regulators of 2,3-BPG concentration and increases in 2,3-BPG concentrations are achieved with only small changes in glycolytic rate. These two effectors were also found to exert most of their influence through HK and PFK.

(4) Flux through the 2,3-BPG shunt was shown to change in absolute terms in response to different energetic loads placed on the cell via the ATPase. This contributed to the ATP-stabilizing effects of the 2,3-BPG shunt.

(6) However, the 'cost' of this is that 2,3-BPG concentrations are also very sensitive to the energy demands of the cell.

(7) Finally, the flux through the 2,3-BPG shunt was found not to change in response to different non-glycolytic demands for NADH.

This work was supported by the Australian National Health and Medical Research Council, and P.J.M. received an Australian Commonwealth Postgraduate Research Award.

REFERENCES

1 Benesch, R. and Benesch, R. E. (1967) Biochem. Biophys. Res. Commun. **26**, 162–167
2 Chanutin, A. and Curnish, R. R. (1967) Arch. Biochem. Biophys. **121**, 96–103
3 Meldon, J. H. (1985) Adv. Exp. Med. Biol. **191**, 63–73
4 Harken, A. H. (1977) Surg. Gynecol. Obstet. **144**, 935–955
5 Duhm, J. and Gerlach, E. (1971) Pflügers Arch. **326**, 254–269
6 Lenfant, C., Torrance, J. D. and Reynafarje, C. (1971) J. Appl. Physiol. **30**, 625–631
7 Rapoport, S. M. and Guest, G. M. (1939) J. Biol. Chem. **129**, 781–790
8 Rapoport, I., Berger, H., Elsner, R. and Rapoport, S. M. (1977) Eur. J. Biochem. **73**, 421–427
9 Asakura, T., Sato, Y. and Minikami, S. (1966) Clin. Chem. Acta **14**, 840–845
10 Astrup, P., Rørth, M. and Thorshauge, C. (1970) Scand. J. Clin. Lab. Invest. **26**, 47–52
11 Duhm, J. (1971) Pflügers Arch. **326**, 341–356
12 Labotka, R. J. (1984) Biochemistry **23**, 5549–5555
13 Gerber, G., Berger, H., Jänig, G.-R. and Rapoport, S. M. (1973) Eur. J. Biochem. **38**, 563–571
14 Gupta, R. K., Benovic, J. L. and Rose, Z. B. (1978) J. Biol. Chem. **253**, 6172–6176
15 Hamasaki, N. and Rose, Z. B. (1974) J. Biol. Chem **249**, 7896–7901
16 Bunn, H. F. and Forget, B. G. (1986) Hemoglobin: Molecular, Genetic and Clinical Aspects, Saunders, Philadelphia
17 Mulquiney, P. J. and Kuchel, P. W. (1997) Eur. J. Biochem. **245**, 71–83
18 Rose, Z. B. (1968) J. Biol. Chem. **243**, 4810–4820
19 Rose, Z. B. (1973) Arch. Biochem. Biophys. **158**, 903–910
20 Rose, Z. B. and Liebowitz, J. (1970) J. Biol. Chem. **245**, 3232–3241
21 Jacobasch, G., Minakami, S. and Rapoport, S. M. (1974) in Cellular and Molecular Biology of Erythrocytes (Yoshikawa, H. and Rapoport, S. M., eds.), pp. 55–92, University Park Press, Baltimore
22 Grimes, A. J. (1980) Human Red Cell Metabolism, Blackwell Scientific Publications, Oxford
23 Schröter, W. and Winter, P. (1966) Blut **14**, 15–29
24 Mulquiney, P. J., Bubb, W. A. and Kuchel, P. W. (1999) Biochem. J. **342**, 565–578
25 Mulquiney, P. J. and Kuchel, P. W. (1999) Biochem. J. **342**, 579–594
26 Waley, S. G. (1964) Biochem. J. **91**, 514–517
27 Heinrich, R. and Schuster, S. (1996) The Regulation of Cellular Systems, Chapman and Hall, New York
28 Fell, D. (1997) Understanding the Control of Metabolism, Portland Press, London and Miami
29 Kacser, H. and Burns, J. A. (1973) Symp. Soc. Exp. Biol. **27**, 65–104
30 Heinrich, R. and Rapoport, T. A. (1974) Eur. J. Biochem. **42**, 97–105
31 Heinrich, R. and Rapoport, T. A. (1974) Eur. J. Biochem. **42**, 89–95
32 Fell, D. A. (1992) Biochem. J. **286**, 313–330
33 Cornish-Bowden, A. (1995) Adv. Mol. Cell Biol. **11**, 21–64
34 Schuster, R., Holzhütter, H.-G. and Jacobasch, G. (1988) BioSystems **22**, 19–36
35 Schuster, R. and Holzhütter, H.-G. (1995) Eur. J. Biochem. **229**, 403–418
36 Rapoport, T. A., Heinrich, R., Jacobasch, G. and Rapoport, S. (1974) Eur. J. Biochem. **42**, 107–120
37 Rapoport, T. A., Heinrich, R. and Rapoport, S. M. (1976) Biochem J. **154**, 449–469
38 Hofmeyr, J.-H. S., Cornish-Bowden, A. and Rohwer, J. M. (1993) Eur. J. Biochem. **212**, 833–837
39 Rapoport, I., Berger, H., Rapoport, S., Elsnor, R. and Gerber, G. (1976) Biochim. Biophys. Acta **428**, 193–204
40 Hamasaki, N., Asakura, T. and Minakami, S. (1970) J. Biochem. **68**, 157–161
41 Raftos, J. E., Chapman, B. E. and Kuchel, P. W. (1986) Haematologica **19**, 251–268

42 Raftos, J. E., Bulliman, B. T. and Kuchel, P. W. (1990) J. Gen. Physiol. **95**, 1183–1204
43 Momsen, G. and Vestergaard-Bogind, B. (1978) Arch. Biochem. Biophys. **10**, 67–84
44 Black, J. A., Acott, K. M. and Bufton, L. (1985) Biochim. Biophys. Acta **810**, 246–251
45 Beutler, E. (1984) Red Cell Metabolism: A Manual of Biochemical Methods, 3rd edn., Grune and Stratton, New York
46 Darley, J. H. (1979) Med. Lab. Sci. **36**, 121–140
47 Heinrich, R. and Rapoport, T. A. (1975) BioSystems **7**, 130–136
48 Ataullakhanov, A. I., Ataullakhanov, F. I., Vitvitskii, V. M., Zhabotinskii, A. M. and Pichigin, A. V. (1985) Biokhimiya **50**, 1005–1011
49 Clegg, J. S. (1984) Am. J. Physiol. **246**, R133–R151
50 Srere, P., Jones, M. E. and Mathews, C. (1989) Structural and Organizational Aspects of Metabolic Regulation, Liss, New York

Received 15 March 1999/17 May 1999; accepted 22 June 1999