Phosphoglyceric Acid Mutase

A COMPUTER SIMULATION STUDY*

JOHN MANTLES AND DAVID GARFINKEL

From the Johnson Research Foundation, Department of Biophysics and Physical Biochemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104

SUMMARY

By digital computer simulation, it was shown that a sequential mechanism for the 2,3-diphosphoglyceric acid-dependent (DPGA-dependent) muscle phosphoglyceric acid mutase reaction cannot be disproved by kinetic analysis of the available velocity data, contrary to the suggestion of Grisolia and Cleland (Biochemistry, 7, 1115 (1968)). At the high concentrations of DPGA and 3-phosphoglyceric acid (3PGA) used in the experimental velocity assay, the rates of product formation calculated from the sequential and the ping-pong mechanisms were found to be very similar; possible experiments to distinguish them are suggested. A sequential mechanism model (supported by other types of experimental data) was fitted to the velocity data over the range 0.5 to 6 mM DPGA and 1 to 40 mM 3PGA. Calculated kinetic parameter values are presented. Competitive substrate inhibitions required 2 molecules of 3PGA bound per molecule of apoenzyme, and 1 of DPGA bound per phosphoglyceric acid mutase-DPGA complex. At high 3PGA and DPGA the enzyme protein is modified into a form with an increased turnover number as a function of 3PGA and DPGA concentrations. Binding constants depend on the ligand concentrations, in accord with experiment.

studies extensively, its complex behavior and the consequent difficult experimental problems have limited our understanding of the reaction (3). Sutherland, Posternak, and Cori (4) first demonstrated that 32P-labeled DPGA phosphoryl groups could interchange with PGA3 phosphoryl groups. Pizer and Ball and Cascales and Grisolia (7), using 32P-labeled substrates, found that the DPGA need not dissociate for each PGA turnover, but that the resulting ratio of DPGA- to PGA-bound 32P depended on the ionic strength and the DPGA and PGM concentrations. PGM does not incorporate inorganic 32P into either PGA3 or DPG4 (8). An enzymatically active PGM-DPGA complex which is able to transfer its labeled phosphoryl groups as described above has been isolated; the DPGA is bound irreversibly to PGM in the absence of excess DPGA and PGA, and the phosphoryl ester bonds are altered (6, 8). A PGM-PGA3 complex has also been isolated (6). These findings support the postulated sequential binding of substrates as the DPG-dependent PGM reaction mechanism (6).

<table>
<thead>
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<th>Abbreviations</th>
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<tbody>
<tr>
<td>PGA</td>
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<tr>
<td>PGA3</td>
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<tr>
<td>PGA2</td>
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<tr>
<td>DPGA</td>
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<tr>
<td>PGM</td>
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<tr>
<td>PGMO</td>
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<tr>
<td>PGM-D</td>
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<tr>
<td>PGM-P</td>
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<tr>
<td>PGM-D-3</td>
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<tr>
<td>PGM-D-2</td>
</tr>
<tr>
<td>PGM-13</td>
</tr>
<tr>
<td>PGM-I3-3</td>
</tr>
<tr>
<td>PGM-D-ID</td>
</tr>
<tr>
<td>PGM-P-ID</td>
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<tr>
<td>DPGMO</td>
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The models can be expanded as desired by adding the appropriate alterations, or to include intermediate reaction complexes. To illustrate the possible binding site initial velocities. No attempt was made in these models to simulate both the sequential and the ping-pong reaction mechanisms. Only ordered binding was simulated, since the random and the ping-pong mechanisms both yielded the same calculated turnover number. The simulation also permitted a critical evaluation of other types of data, the design of additional experiments, and a better definition of the behavior of the enzyme and the problems of studying it.

**METHODS**

The methods of King and Altman (15) and Cleland (16) and digital computer simulation were used to study the reaction schemes. An on line simulation technique with the Digital Equipment PDP-6 computer with teletype and oscilloscope display system proved to be the most flexible, comprehensive, and efficient method of studying the models and comparing their behavior with the experimental results. The input consisted of chemical equations (see Reference 17 for a description of the equation form), steady state ligand concentrations, and rate constants. The simulation program (MATRX) transformed these statements into differential equations and solved them at the steady state conditions (derivatives = 0) by a matrix inversion.2 The output was the initial velocity for each set of DPGA and PGA3 concentrations and the percentage distribution of the enzyme among the various complex forms (available as a printout). The calculated initial velocities were displayed on the oscilloscope in a Lineweaver-Burk plot for on line model development.

**MODELS**

Kinetic models, kept as simple as possible, were developed for both the sequential and the ping-pong reaction mechanisms. Only ordered binding was simulated, since the random and the ordered sequential binding both yielded the same calculated initial velocities. No attempt was made in these models to define the binding sites, to illustrate the possible binding site alterations, or to include intermediate reaction complexes. The models can be expanded as desired by adding the appropriate terms to include additional competitive inhibitors, to trace labeled compounds through the system, or to adapt the system for alterations in the protein functions, or to include intermediate enzyme-substrate complexes.

Model I is based on the turnover of PGA ↔ PGA2 by an activated enzyme complex PGM-DPGA (one reaction site per PGM molecule) (Fig. 1). DPGA binds reversibly with PGMO to form an activated PGM-DPGA complex which then reversibly binds with either PGA3 or PGA2 to form an intermediate PGM-DPGA-PGA complex which can dissociate, after the rate-limiting enzymatic conversion into the other PGM-DPGA-PGA complex, into the new PGA and PGM-DPGA.3 The observed competitive substrate inhibitions are simulated by competitive reversible reaction schemes, where two molecules of PGA3 bind with PGMO and one DPGA binds with PGM-D.

A black box modifier function \( F = \frac{k_{a}k_{2}}{k_{a}k_{3}} \) was used to transform PGMO into a new enzyme form, APGMO, with an altered turnover to reproduce the kinetic potentiation and inhibition observed at the high DPGA and PGA3 concentrations. The function is represented for convenience in the form of July 25, 1969

1. Terminal carboxyl group or charge equivalent
2. Phosphorylated or free hydroxyl group on a carbon
3. Substitutions on \( \beta \) carbon and/or presence of \( \gamma \) carbon not critical
4. Either \( \alpha \) or \( \beta \) phosphorylated for acceptor, and both phosphorylated for donor activity

No metal ion requirements

2,3-DPGA phosphatase activity << mutant activity

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**TABLE II**

<table>
<thead>
<tr>
<th>Substrates</th>
<th>( K_s )</th>
<th>( K_i )</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPGA</td>
<td>( 2 \times 10^{-6} ) M</td>
<td>( 2.5 \times 10^{-5} ) M</td>
</tr>
<tr>
<td>PGA3</td>
<td>( 1.4 \times 10^{-5} ) M</td>
<td>( 6 \times 10^{-5} ) M</td>
</tr>
<tr>
<td>PGA2</td>
<td>No reliable value</td>
<td>No reliable value</td>
</tr>
</tbody>
</table>

Ligand-binding specificity and substrate properties

1. Direct from data, rabbit muscle enzyme (5).
2. Direct calculations, chicken breast enzyme (9).
3. Direct from data, chicken breast enzyme (10).
4. Analogue studies, rabbit muscle enzyme (11, 12).
5. Rabbit muscle enzyme (3).
6. Chicken breast enzyme (8, 13).

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* S. Green and D. Garfinkel, manuscript in preparation.
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expanded, it was necessary to include only the counterpart of the PGM reactions of Model I to study its basic kinetic behavior.

RESULTS

A representation of the initial velocities that were calculated by the computer for Model I and Model II are given in a double reciprocal plot of

\[ \frac{1}{\text{velocity}} \text{ versus } \frac{1}{\text{PGA3}} \]

(Fig. 3). The calculated kinetic parameters are listed in Table III; no error limits were calculated for these values because of the interdependent nature of the reaction equations and the technique of visual curve fitting, but a ±30% variation is a reasonable estimation. Only the relative magnitudes of the rate constants as represented in the various kinetic parameters and not the absolute magnitudes, are meaningful with the technique of data analysis (it does not substitute for at least some direct measurement of rate constants).

Both models were fit to the velocity data at 5 and 10 mM DPGA, 1 to 40 mM PGA3. The kinetic expressions for the initial velocities of the models at these substrate concentrations are given in Equations 1 and 2.

\[ V_1 = \frac{V_{\text{max}}}{1 + \frac{K_{\text{PGA3}}}{\text{PGA3}} \left( 1 + \frac{K_{\text{DPGA}}}{\text{DPGA}} \left( \frac{1}{K_{\text{PGA3}}} \right) \right)} \]

\[ V_{\text{II}} = \frac{V_{\text{max}}}{1 + \frac{K_{\text{PGA3}}}{\text{PGA3}} \left( 1 + \frac{\text{DPGA}}{K_{\text{PGA3}}} \left( 1 + \frac{\text{DPGA}}{K_{\text{PGA3}}} \right) \right)} \]

with DPGA ≪ \( K_{\text{PGA3}} \) and PGA3 ≫ \( K_{\text{PGA3}} \) (PGA3 ≫ \( K_{\text{PGA3}} \)) the initial velocity expressions become the same for both models (Equation 3), as shown in Fig. 3.

\[ V = \frac{V_{\text{max}}}{1 + \frac{\text{PGA3}}{K}} \]

where \( K \) is the product of the constant terms in the limiting case.

As discussed above, only Model I was developed to fit the experimental data over the full range of DPGA and PGA3 data. Adjustments of the factor \( m \) and \( K_{\text{DPGA}} \) with increasing DPGA at DPGA > \( K_{\text{PGA3}} \) were needed for simulation of the reported data. The initial velocity expression at DPGA ≫ \( K_{\text{PGA3}} \) and without any PGM modification is given in Equation 4 (the same for both Model I and Model II).

\[ V = \frac{V_{\text{max}}}{1 + \frac{K_{\text{PGA3}}}{\text{PGA3}} \left( 1 + \frac{\text{DPGA}}{K_{\text{PGA3}}} \right)} \]

The kinetic expression for the PGM modified system would be similar to Equation 4, and thus the same for both models since DPGA is ≫ \( K_{\text{PGA3}} \); \( V_{\text{max}} \) is increased and the DPGA inhibition term is not included (Equation 5).

\[ V = \frac{V_{\text{max}}}{1 + \frac{K_{\text{PGA3}}}{\text{PGA3}}} \]

model by a two-step reaction sequence. The intermediate complex, DPGMO, and the rate constants \( k_{15} = 10^5 \), \( k_{16} = 10^6 \), \( k_{17} = 4 \times 10^4 \), and \( k_{18} = 10^8 \), therefore, are defined only as a technique to incorporate the modifier function into the model, and are referred to as a dummy complex and dummy rate constants. The APGMO mutase reaction mechanism is similar to that described for PGMO, except that the enzyme activity is increased in the rate-limiting step. The competitive DPGA or PGA3 inhibitions are not significant in the APGM reactions because both DPGA and PGA3 are near binding saturation levels.

![Fig. 1. PGM Model I, sequential mechanism (5 mM → 6 mM DPGA, 1 mM → 40 mM PGA3). ***, 5 mM → 1 mM DPGA; see Table III for values of \( K_1 \) at DPGA > 1 mM. See the text for definition, discussion, and values associated with the "black box."](image-url)
### TABLE III

**Kinetic parameters**

<table>
<thead>
<tr>
<th>pGM Reactions</th>
<th>PGM Model I</th>
<th>PGM Model II</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_d\text{DPGA}$</td>
<td>$k_3 = 4.8 \times 10^{-4} \text{ M}$</td>
<td>$k_2 = 1.5 \times 10^{-4} \text{ M}$</td>
</tr>
<tr>
<td>$K_s\text{PGA}$</td>
<td>$k_{b1}k_{b4} + k_{k4} + k_{a3}$</td>
<td>$4.8 \times 10^{-4} \text{ M}$</td>
</tr>
<tr>
<td>$K_e\text{PGA}$</td>
<td>$\left( \frac{k_{b1}k_{b4}}{k_{a3}} \right)^{\frac{1}{4}} = 1.15 \times 10^{-3} \text{ M}$</td>
<td>$5 \times 10^{-4}$ and $1 \times 10^{-3}$ M</td>
</tr>
<tr>
<td>DPGA $\rightarrow$ 5X $10^{-6}$</td>
<td>$k_{b1}k_{b2} = 5 \times 10^{-4}$</td>
<td>$1.3 \times 10^{-4}$ M</td>
</tr>
<tr>
<td>$K_e\text{PGA}$</td>
<td>$k_{b1}k_{b2}$</td>
<td>$k_{b1}k_{b2} + k_{b4}k_{a3}$</td>
</tr>
<tr>
<td>$K_{eq}$</td>
<td>$PGA_3 = k_3$, $PGA_2 = k_4$, $PGA_2 = k_{a3}$</td>
<td>$PGA_3 = k_3$, $PGA_2 = k_4$, $PGA_2 = k_{a3}$</td>
</tr>
<tr>
<td>Turnover number</td>
<td>54,000 mole PGA3/mole min</td>
<td>60,000 mole PGA3/mole min</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>APGM Reaction</th>
<th>PGM Model I</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPGA $\rightarrow$ 5X $10^{-6}$</td>
<td>$k_{b1}k_{b2} = 4.8 \times 10^{-4} \text{ M}$</td>
</tr>
<tr>
<td>Factor m</td>
<td>$k_{a3}k_{b1} = \frac{4 \times 10^5}{2 \times 10^5}$</td>
</tr>
<tr>
<td>$K_d\text{DPGA}$</td>
<td>$k_{b1}k_{b2} = 4.8 \times 10^{-4} \text{ M}$</td>
</tr>
<tr>
<td>$K_s\text{PGA}$</td>
<td>$k_{b1}k_{b2} = 4.8 \times 10^{-4} \text{ M}$</td>
</tr>
<tr>
<td>$K_e\text{PGA}$</td>
<td>$PGA_3 = k_3$, $PGA_2 = k_4$, $PGA_2 = k_{a3}$</td>
</tr>
<tr>
<td>$K_{eq}$</td>
<td>$PGA_3 = k_3$, $PGA_2 = k_4$, $PGA_2 = k_{a3}$</td>
</tr>
<tr>
<td>Turnover number</td>
<td>203,000 mole PGA3/mole min (≈ 3.75 × 54,000)</td>
</tr>
</tbody>
</table>

**Fig. 2.** PGM Model II, ping-pong mechanism (5 and 10 μM DPGA, 1 to 40 mM PGA3).

**DISCUSSION**

A comparison of the results of the simulation and the relationships of the rate laws for Model I and Model II shows that curve fitting cannot be used to differentiate between these two proposed mechanisms. The complex enzymatic activity of the pGM system plotted in Figure 3 does not lend itself to a straightforward kinetic analysis. The characteristic pattern of the Lineweaver-Burk plot for a two-substrate system with either a sequential mechanism (intersecting lines) or a ping-pong mechanism (parallel lines) is obscured in the available velocity data by the competitive DPGA and PGA3 inhibition and by the

**Fig. 3.** Calculated velocities of PGA2 production. --- Model I; --- Model II, 5 μM DPGA; --- Model II, 10 μM DPGA. Experimental data points (9): O, 5 μM; A, 10 μM; □, 100 μM; +, 1 mm; O, 2 mm; A, 4 mm; X, 6 mm DPGA.
apparent PGM modification. The distinct initial velocity expressions for Model I and Model II (Equations 1 and 2) at DPGA ≪ \( K_{d,DPGA} \) can be reduced to the common form given in Equation 3 in the limiting case when PGA3 ≫ \( K_{d,PGA3} \). This similar behavior of the two mechanisms and their agreement with the system is illustrated by the simulated results at 5 and 10 \( \mu M \) DPGA, 1 to 40 \( \mu M \) PGA3 (Fig. 3). The rate laws at DPGA ≫ \( K_{d,DPGA} \), without PGM modification (Equation 4), and at DPGA ≫ \( K_{d,DPGA} \) and PGA3 ≫ \( K_{d,PGA3} \), with PGM modification (Equation 5), are independent of the basic mechanism. PGM activity measured at PGA3 < \( \frac{1}{2} (K_{d,PGA3}) \) and DPGA < \( \frac{1}{2} (K_{d,DPGA}) \) would remove this complication in the kinetic data and permit a unique simulation. Since the presently used coupled enolase assay system is limited at low PGA3, a rapid, more sensitive technique must be developed.

An alternative method of establishing the PGM reaction mechanism is to investigate the reaction intermediates. The simulation technique can be used to estimate the steady state binding constants of the proposed enzyme forms. In an incubation of DPGA and PGM with PGA3 << \( K_{d,PGA3} \), the sequential mechanism would yield the PGM-D form while the ping-pong mechanism would favor the PGM-P form. As discussed above, a PGM-DPGA complex has been obtained from an incubation mixture of PGM0 and DPGA and has been shown to be an active enzyme complex. PGM-P has not been isolated either by the method used to demonstrate the phosphoenzyme of a parallel mechanism for the phosphoglyceromutase system or by the techniques used to demonstrate the other PGM complexes (2, 6, 8, 18, 19). Since these findings, in contrast to the velocity data, can be simulated only by a sequential mechanism, Model I is a better representation of the PGM reaction than Model II. Model I is a very simplified representation of the PGM reaction, however, since no intermediate reaction complexes are included (data on their presence or behavior are very limited). These intermediate complexes are necessary to explain the dissociation of DPGA, the intermolecular phosphoryl transfer, and the binding site properties. The PGM reaction might even include a phosphorylated enzyme-substrate intermediate complex, although not a stable free phosphoenzyme form as postulated in the ping-pong mechanism.

A satisfactory representation of the inhibition by PGA3 in Model I required a square term in the mass law expression, or two PGA3 molecules binding per PGM0. The isolation of an active PGM-DPGA and a PGM-PGA3 complex, the demonstration of similar structural requirements for binding and phosphate transfer, and the simulation of the PGA3 inhibition by a two-molecule dead end complex and the mutase activity by a PGA3-DPGA complex, indicate that the reactive surface has two binding sites which have similar binding properties but different binding affinities for the first and second PGA3 molecules. The investigation of this hypothesis by equilibrium dialysis, therefore, could provide additional information on the reaction mechanism. The demonstration of two binding constants, \( K_{d,PGA3} \) and \( K_{d,PGA3} \), would further support the sequential scheme and the comparison between \( K_{d,PGA3} \) and the \( K_{d,PGA3} \) values would help define the behavior of the two sites. The finding of only one \( K_{d,PGA3} \) would be equivocal.

The competitive inhibition by PGA3 was simulated by DPGA binding to the active PGM form (PGM-D in Model I and PGM-P in Model II) as discussed above. The increase in \( K_{d,DPGA} \) with increasing DPGA was necessary to correct for the influence that DPGA has on the PGM-D binding affinity. Since \( \mu ^{32} P \) tracer experiments have shown that the PGM affinity for DPGA is altered with increasing DPGA, high ionic strengths (0.5 M maximal affect), and Hg2+ (6, 9, 7, 20), the PGM affinity for other ligands may also be altered with changes in both DPGA and PGA3, but only the change in \( K_{d,PGA3} \) is apparent. The binding constants \( K_{d,DPGA} \), \( K_{d,PGA3} \), \( K_{d,PGA3} \), and \( K_{d,PGA3} \) defined in Model I need to be determined by equilibrium dialysis as a function of the ligand concentration. The activity of the 2,3-diphosphatase elucidated by Zwaig and Miletstein (8) must be considered in the design and analysis of these experiments.

The observed increase of velocity at high PGA3 and DPGA cannot be produced with a basic Michaelis-Menten model or by an isoenzyme model, but can be produced by a potentiation of PGM, which is shown to be DPGA- and PGA3-dependent. The apparent inhibition at the higher DPGA and PGA3 was accounted for by allowing the black box modifier function to assume a local maximum to correspond with the maximum velocity. This change in kinetic activity at substrate saturation levels suggests that the reactive surface of the enzyme is altered (The PGM protein structure and enzymatic activity have been shown to be reversibly altered by Hg2+ ions and, as discussed above, the binding affinities are also influenced by DPGA and by the ionic strength (13)). The mechanism for the PGM structural alteration by DPGA and PGA3 could be either a specific modifier activity or a nonspecific binding effect. The determination of the specificity by substrate analogue studies and the investigation of the PGM protein structural changes are necessary for a better definition of this behavior. It is noteworthy that such an alteration of the enzyme surface was not detected by experimental methods alone, despite much recent interest in allosteric control mechanisms.

The kinetic parameters for the two models (Table III) show some variation because of the different mechanisms, but are generally similar. The results reported by Gribolska and Celnier (Table II) were calculated from a single rate law expression of the ping-pong scheme and thus represent a weighted summation of both the PGM and the APGM reactions (5). Since the only significant difference in the values determined for the corresponding kinetic parameters of the PGM and APGM reactions was between the turnover numbers, the binding constants determined by fitting the PGA3 data with a single rate expression are high. The turnover number and the \( K_{d,DPGA} \) calculated for the PGM reaction are close to the experimental values determined at similar conditions (Table II).

The technique of simulating the experimental data by a digital computer, therefore, proved to be an informative method of studying this complex enzymatic reaction. The use of models to represent the mechanistic schemes showed that the reaction mechanism cannot be identified by the kinetic data. Other aspects of the reaction were also evaluated, resulting in the formulation of new interpretations and in the design of additional experiments to further define the PGM reaction.

Acknowledgment—We are indebted to Dr. L. I. Pizer for helpful discussions and for supervising related laboratory work.

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Phosphoglyceric Acid Mutase: A COMPUTER SIMULATION STUDY
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