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A KINETIC MODEL OF PHOSPHOFRUCTOKINASE FROM *PLASMODIUM BERGHEI*—APPLICATION OF A NOVEL PROCEDURE FOR MODEL DISCRIMINATION

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Abstract—A novel statistical procedure is applied to discriminate between several alternative kinetic models of the glycolytic control enzyme phosphofructokinase from the malarial parasite *Plasmodium berghei*. Among the Monod-models, assuming different reaction mechanisms and various modes of allosteric regulation by ATP, F6P and pH, one model was favoured which was shown to provide an adequate description of the data.

INTRODUCTION

As in man, phosphofructokinase is an important control enzyme in malarial parasites. Therefore, the kinetic characterization of this enzyme is an essential step in gaining a deeper understanding of the energy metabolism of the parasites.

MATERIALS AND METHODS

The kinetic measurements were carried out with stroma-free lysates of parasites isolated from infected mouse red blood cells. PFK-activity was measured at pH 6.5, 6.8 and 7.2, respectively (366 nm, 37°C). The substrate concentrations were varied between 0.03–3.0 mM F6P and 0.04–4.8 mM ATP [1].

Owing to the complex kinetic behaviour of the enzyme, several kinetic models of different complexity were fitted to the $N = 172$ data by a weighted least-squares procedure. Common statistical tests for checking the goodness-of-fit (χ^2-, runs-, signs-, and Wilcoxon-tests), as well as the usual kinetic plots, failed or were insufficient for model discrimination. Thus, two novel tests [2] were used, whose applicability to enzyme-kinetic data has been shown elsewhere by the authors [3].

The mathematical methods are described in Ref. [3]. Repetitional measurements and additional investigations indicated a constant experimental error which was estimated to be $s = 0.035 v_{max}$. The weights used in all the calculations were $w_i = s_i^{-2}$.

RESULTS

The dependence of PFK-activity on ATP and F6P, respectively, is shown in Figs 1a–c. After the rejection of obviously unsuitable rate laws, six models were involved in model discrimination. By application of the T_{kl}-test the differences between models a3, a4 and c4 were found to be not significant at the level of $\alpha = 5\%$ (Table 1), i.e. the three models are equally well-suited to describe the data. By the U_k-test all rate laws except model a4 [equation (1)] could be rejected;

$$V = \frac{v_{max}\,\mathrm{MgATP}\,\mathrm{F6P}}{(\mathrm{MgATP} + K_{ma})(\mathrm{F6P} + K_f)} \cdot \frac{1}{1 + L_0 \left[\dfrac{\left(1 + \dfrac{H^+}{K_{h1}}\right)\left(1 + \dfrac{H^+}{K_{h2}}\left(1 + \dfrac{\mathrm{MgATP}}{K_{ma1}} + \dfrac{\mathrm{ATP}}{K_{a1}}\right)\right)}{1 + \dfrac{H^+}{K_{h1}} + \dfrac{\mathrm{F6P}}{K_{f1}}} \right]^n} \quad (1)$$

Table 1. Discrimination between six models using the T_{kl}-test and the test statistics U_k

	Model l						
Model k	a1	a2	a3	a4	b4	c4	U_k
a1	0	−4.77	7.78	7.94	4.92	5.56	20.1
a2		0	8.44	8.38	5.55	5.94	22.2
a3			0	1.96[a]	−4.25	−0.90[a]	2.22
a4				0	−4.26	−1.42[a]	1.01[a]
b4					0	4.00	8.49
c4						0	3.81

[a]Not significant at this level of significance ($\alpha = 5\%$).

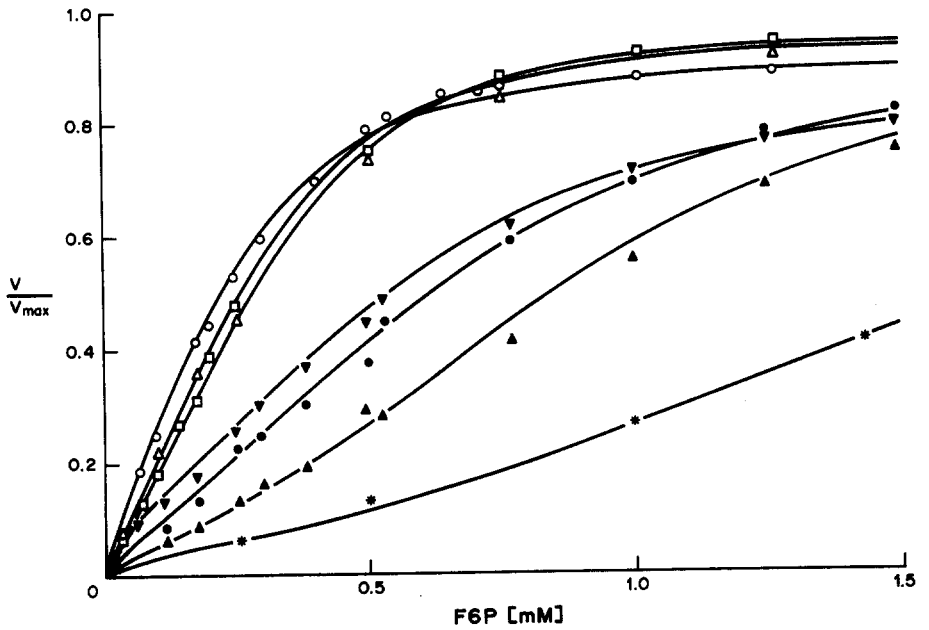

Fig. 1a. F6P-dependence for: [ATP] = 0.4(○), 0.8 (△) and 1.0 mM (□) at pH = 7.2; [ATP] = 0.2 (▼), 0.4 (●) and 0.8 mM (▲) at pH = 6.8; and [ATP] = 0.4 mM (*) at pH = 6.5.

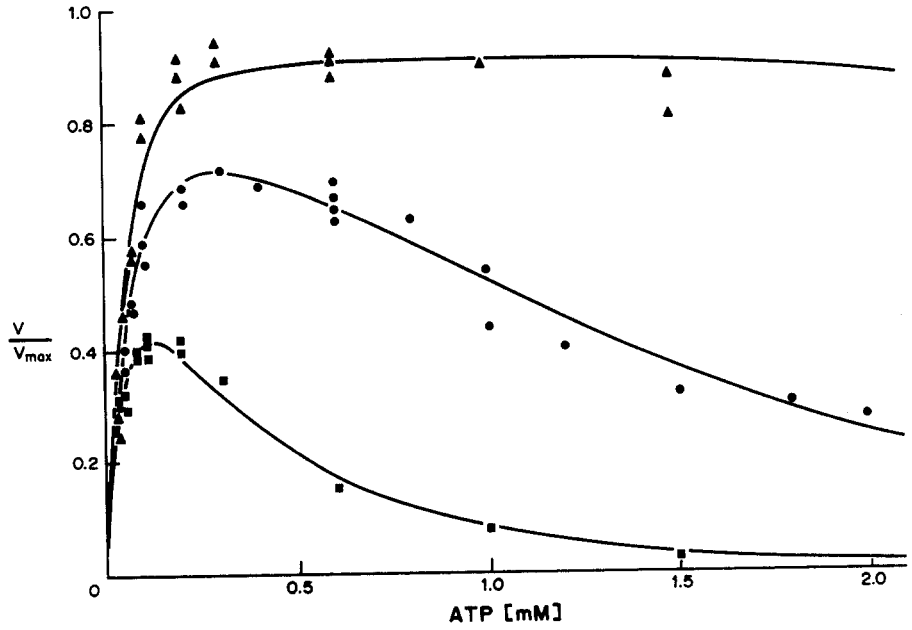

Fig. 1b. ATP-dependence at pH = 7.2 (▲), 6.8 (●) and 6.5 (■), respectively, for [F6P] = 1.0 mM.

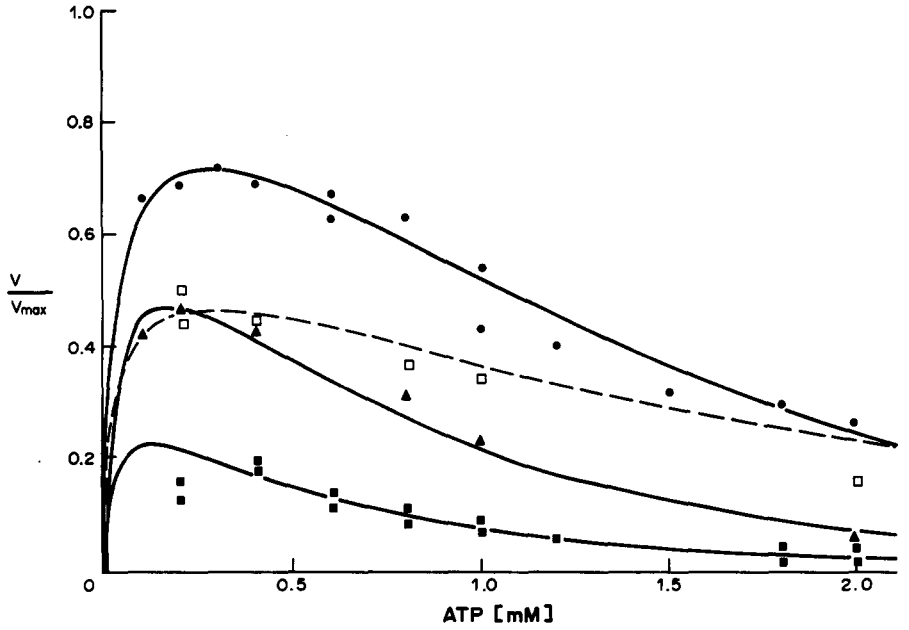

Fig. 1c. ATP-dependence for: [F6P] = 0.2 (■), 0.45 (▲) and 1.0 mM (●) at pH = 6.8; and [F6P] = 0.2 mM (□) at pH = 7.2. The dashed line corresponds to model a4 for pH = 7.2.

Figs 1a–c. Dependence of PFK-activity on F6P and ATP, respectively, and the fit of model a4 to the data.

This favoured model assumes a rapid-equilibrium random mechanism and allosteric inhibition by ATP and activation by F6P. While by additional investigations, K_{ma}, K_f, L_0 and n were shown to be pH-independent, the dependence of the allosteric activation and inhibition on pH were considered by assuming two virtual protonation steps; ATP binds only to its protonated site and F6P binds only to its unprotonated site (Fig. 2; parameters given in Table 2).

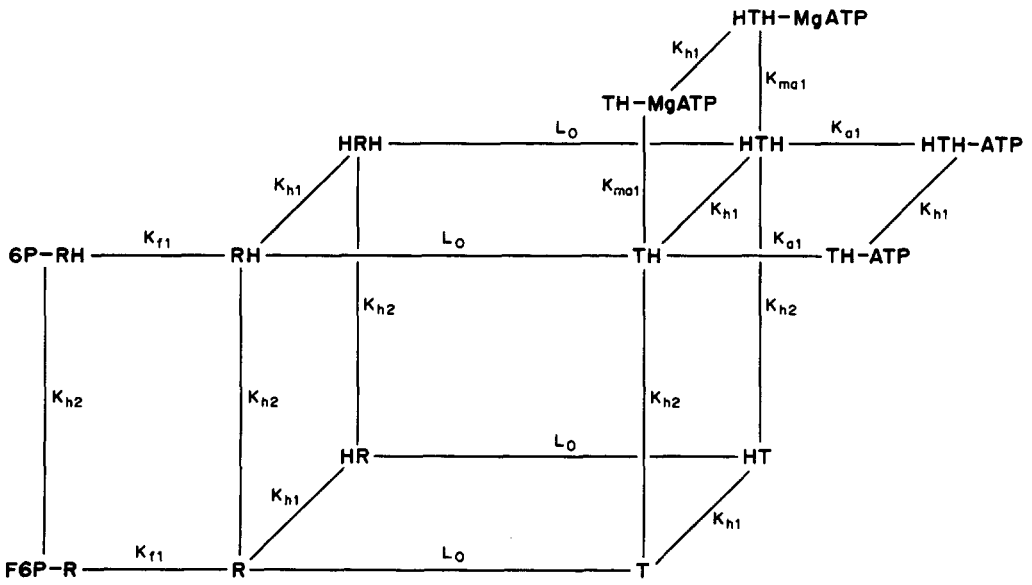

Fig. 2. Proposed kinetic model for the allosteric regulation of malerial PFK (model a4).

Table 2. Estimated parameter values of model a4

K_{ma}	0.031 mM
K_f	0.033 mM
K_{a1}	0.023 mM
K_{ma1}	0.090 mM
K_{f1}	0.064 mM
K_{h1}	0.019 μM[a]
K_{h2}	3.16 μM[a]
L_0	3.55
n	3.79

[a]Corresponds to $pK_1 = 7.72$ and $pK_2 = 5.50$.

DISCUSSION

Although the proposed model of malarial PFK gives no proof for the "real" mechanism of the enzyme it provides an adequate description of the kinetic properties in the relevant ranges of substrate concentrations.

By application of the proposed statistical procedures it was possible to test the adequacy of the model and to get answers regarding the significance of the differences in the goodness-of-fit of the different models.

REFERENCES

1. D. Buckwitz, G. Jacobasch, C. Gerth, H.-G. Holzhütter and R. Thamm. A kinetic model of phosphofructokinase from *Plasmodium berghei*. Influence of ATP and fructose-6-phosphate. *Molec. Biochem. Parasit.* **27**, 225–232 (1988).
2. S. Zwanzig, The choice of approximative models in nonlinear regression. *Math. Opsforsch. Statist. Ser. Statist.* **11**, 23–47 (1980).
3. D. Buckwitz and H.-G. Holzhütter, A new method to discriminate between enzyme-kinetic models. *Computers Math. Applic.* **20**(4–6), 117–126 (1990).