



## KINETIC AND THERMODYNAMIC CONSTRAINTS FOR THE STRUCTURAL DESIGN OF GLYCOLYSIS

REINHART HEINRICH,<sup>§</sup>, FRANCISCO MONTERO,<sup>¶</sup>, EDDA KLIPP,<sup>§</sup>,  
THOMAS G. WADDELL,<sup>‡</sup> and ENRIQUE MELÉNDEZ-HEVIA<sup>†</sup>

<sup>§</sup> Humboldt-Universität zu Berlin, Institut für Biologie, Theoretische Biophysik, D-10115 Berlin, Germany

<sup>¶</sup> Universidad Complutense de Madrid, Departamento de Bioquímica, 28040 Madrid, Spain

<sup>‡</sup> University of Tennessee, Department of Chemistry, Chattanooga, TN, USA.

<sup>†</sup> Universidad de la Laguna, Departamento de Bioquímica, 38206 Tenerife, Canary Islands, Spain

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### 1. INTRODUCTION

Glycolysis is one of the favoured subjects in the field of mathematical modelling of metabolic systems. Different types of simulation models have been developed, for example, a) models based on a very detailed kinetic description of the individual enzymes [1 - 4], b) "Skeleton" models [5] and c) models using for simplification the quasi-steady state approximation [6 - 8]. The aim of the present study is substantially different from that of simulation models. It is tried to explain certain *structural features* of glycolysis which are fixed during the life span of an organism but have been changed in an evolutionary time scale.

Investigations concerning the structural design of metabolic pathways are often based on the hypothesis that, during evolution, systems with certain optimal properties have been selected. Relevant optimization criteria are: maximization of steady state fluxes [9 - 17], minimization of transient times of metabolic pathways [18], minimization of the total osmolarity of intermediates [19, 20], stoichiometric simplicity [21] and maximization of thermodynamic efficiency (cf. [14] for a review). According to the fact that the main biological function of glycolysis is the production of ATP our interest is focused on the implications of the optimization principle of maximal ATP-production rate on the structural design of glycolysis. In particular, we pay attention to the optimal distribution of ATP-producing and ATP-consuming sites with respect to their number and their location along the glycolytic chain.

### 2. MODEL ASSUMPTIONS

Let us consider an unbranched chain of  $n$  reactions with fixed concentrations of the pathway substrate,  $S_0$ , and the end product,  $S_n$ . For simplicity's sake we assume that the rate  $v_i$  of a reaction interconverting two consecutive intermediates  $S_{i-1}$  and  $S_i$  is described by the linear kinetic equation

$$v_i = k_i S_{i-1} - k_{-i} S_i = k_i \left( S_{i-1} - \frac{S_i}{q_i} \right) \quad (2.1)$$

where  $k_i$  and  $k_{-i}$  denote first order rate constants and  $q_i$  is the thermodynamic equilibrium constant of reaction step  $i$ . From the condition  $J = v_i$  ( $J$ : steady state flux) one derives the following equation

$$J = \frac{1}{D} \left( S_0 \prod_{i=1}^n q_i - S_n \right), \quad D = \sum_{i=1}^n \frac{1}{k_i} \prod_{m=i}^n q_m \quad (2.2 \text{ a,b})$$

(cf. [22]). Introducing the overall affinity  $A$  of the pathway

$$A = RT \ln \left( \frac{S_0}{S_n} \prod_{j=1}^n q_j \right) \quad (2.3)$$

( $R$ : universal gas constant,  $T$ : temperature) expression (2.2) may be rewritten as follows

$$J = \frac{S_n}{D} \left( \exp \left( \frac{A}{RT} \right) - 1 \right). \quad (2.4)$$

Whereas in this equation the numerator expresses the thermodynamic properties of the chain the denominator  $D$  depends on the details of the kinetic properties of the reactions as well as on the chain length.

Eq. (2.1) for the individual rates and Eq. (2.2) for the steady state flux may be applied also for chains with bimolecular reactions involving cofactors, if they are considered as external reactants. In this case one has to replace the kinetic constants  $k_i$  and  $k_{-i}$  by apparent first-order rate constants which are obtained as products of the corresponding second-order rate constants  $\kappa_i$  and  $\kappa_{-i}$  and the concentrations of those external reactants participating in the corresponding reaction steps. Since we are interested in glycolysis we consider the case that ATP and ADP may participate in the reaction chain either at ATP-producing sites (*P-sites*) or at ATP-consuming sites (*C-sites*). Both C-sites and P-sites are denoted as *coupling sites*. Reactions which are involved neither in ATP-production nor in ATP-consumption are denoted as *O-sites*. The concentrations of ADP, ATP and of free inorganic phosphate are considered to be constant.

Coupling of the  $i$ -th reaction to ATP consumption or ATP production will change the thermodynamic properties. For the equilibrium constant of the uncoupled reaction,  $q_i$ , we have  $q_i = k_i/k_{-i}$ . The equilibrium constant of the coupled reaction reads  $q'_i = \kappa_i/\kappa_{-i}$ . The equilibrium constants  $q'_i$  and  $q_i$  are related to each other by

$$\text{C-sites: } q'_i = q_i K^{-1}, \quad \text{P-sites: } q'_i = q_i K, \quad (2.5 \text{ a,b})$$

where  $K$  denotes the apparent equilibrium constants for the interconversion of ADP into ATP under physiological conditions ( $K \ll 1$ ). By necessity, changes in the equilibrium constants as given in Eqs. (2.5 a) and (2.5 b) are brought about by changes in the forward and backward rate constants. We use the following relations between the first-order rate constants  $k_{\pm i}$  of the uncoupled reactions and the apparent first-order rate constants  $k'_{\pm i}$  of the coupled reactions

$$\text{C-sites: } k'_i = \frac{k_i}{\alpha_i}, \quad k'_{-i} = k_{-i}\beta_i \quad (2.6)$$

$$\text{P-sites: } k'_i = k_i\gamma_i, \quad k'_{-i} = \frac{k_{-i}}{\delta_i} \quad (2.7)$$

where, for simplicity's sake, we have neglected the effect of the concentrations of ATP and ADP on the apparent first order rate constants.

Taking into account relations (2.5 a,b) the coupling parameters  $\alpha_i, \beta_i$  and  $\gamma_i, \delta_i$  must fulfil the relations  $\alpha_i\beta_i = \gamma_i\delta_i = K$ . The coupling parameters can be expressed as functions of  $q_i$  and  $K$ . For that we use the rate equation proposed for a "perfect" catalyst. It reads under the condition that only diffusional constraints are operative

$$v_i = \frac{k_d E_i (S_{i-1} q_i - S_i)}{1 + q_i} \quad (2.8)$$

(cf. [13]). In this equation  $E_i$  denotes the concentration of the enzyme which catalyzes step  $i$  and  $k_d$  represents the diffusional upper limit for rate constants characterizing the binding of the substrates or products to the enzyme. A comparison of Eqs. (2.1) and (2.8) yields

$$k_i = \frac{k_d E_i q_i}{1 + q_i}, \quad k_{-i} = \frac{k_d E_i}{1 + q_i} \quad (2.9 \text{ a,b})$$

Introducing into these equations the equilibrium constants of coupled reactions from Eq. (2.5) one derives from Eqs. (2.3), (2.6) and (2.7) for the coupling parameters

$$\alpha_i = \frac{K + q_i}{1 + q_i}, \quad \beta_i = \frac{K(1 + q_i)}{K + q_i}, \quad (2.10 \text{ a,b})$$

$$\gamma_i = \frac{K(1 + q_i)}{1 + q_i K}, \quad \delta_i = \frac{1 + q_i K}{1 + q_i} \quad (2.10 \text{ c,d})$$

Since  $K < 1$  one obtains from Eq. (2.10)  $K < \alpha_i, \beta_i, \gamma_i, \delta_i < 1$ .

The ATP-production rate is related to the glycolytic flux  $J$  in the following way

$$J_{\text{ATP}} = (b - a)J = d \cdot J \quad (2.11)$$

where  $a$  and  $b$  denote the number of C-sites and P-sites, respectively. Using in this equation for  $J$  expression (2.2) the equilibrium constants  $q_i$  and the rate constants  $k_i$  must be replaced for coupling sites by  $q_i'$  and  $k_i'$ , respectively. In the following the factor  $d = b - a$  is denoted as excess number of ATP-producing sites.

Concerning the evolutionary optimization of glycolysis we are mainly interested in the implications of the extremum principle

$$J_{\text{ATP}} = \max. \quad (2.12)$$

To identify the optimal structural design, the kinetic properties of chains with different numbers and different locations of coupling sites are compared. No restrictions concerning the number and distribution of coupling sites are made except of

$$a + b \leq n, \quad 2b \leq n. \quad (2.13 \text{ a,b})$$

Relation (2.13 b) follows from the fact that for  $b$  ATP-producing sites the chain must contain the same number  $b$  of sites where substrates are phosphorylated either by ATP or by inorganic phosphate. Taking into account these restrictions different structural designs are obtained by an interchange of the different types of sites within the chain.

The standard free energy change  $\Delta G_{\text{glyc}}^{\circ}$  of the uncoupled interconversion of  $S_0$  into  $S_n$  reads

$$\Delta G_{\text{glyc}}^{\circ} = -RT \ln Q = \text{const.}, \quad Q = \prod_{i=1}^n q_i \quad (2.14 \text{ a,b})$$

( $Q$ : overall equilibrium constant of the chain in the uncoupled state). Using these assumptions alternative pathways are created differing in the sites where ATP is produced and consumed at the conversion of the initial substrate  $S_0$  into the end product  $S_n$ .

Thermodynamically, a chain with  $a$  C-sites and  $b$  P-sites may be characterized by the overall affinity

$$A = RT \cdot \ln \left( \frac{S_0}{S_n} K^{(b-a)} \prod_{j=1}^n q_j \right) \quad (2.15)$$

which is independent of the location of C-, O-, and P-sites along the chain. Since  $K < 1$  the overall affinity decreases as  $b - a$  increases. A positive glycolytic flux is obtained for positive affinity ( $A > 0$ ) which is fulfilled if the excess number of ATP-producing sites does not exceed a certain maximum, that is, for

$$d = b - a < -\frac{\ln(S_0 Q / S_n)}{\ln K} = d_{\text{max}}. \quad (2.16)$$

For  $d > d_{\max}$  the reactions proceed in the reverse way, that is from  $S_n$  to  $S_0$ .

3.. EFFECT OF REPLACEMENTS OF O-SITES BY P- OR C-SITES AND THEIR OPTIMAL LOCATION

The main conclusions concerning the optimal kinetic properties of ATP-producing reaction chains may be derived from the following two theorems.

**THEOREM 1:** (1) The replacement of an O-site by a C-site, that is,  $a \rightarrow a + 1$ , at any reaction increases the glycolytic rate  $J$ . (2) The replacement of an O-site by a P-site, i.e.  $b \rightarrow b + 1$ , decreases  $J$ .

This theorem points to the kinetic effects of a change of the *number of coupling sites*. The kinetic effects of a variation of the *location of coupling sites* at fixed numbers  $a$  and  $b$  are described by

**THEOREM 2:**  $J$  as well as  $J_{\text{ATP}}$  are increased (1) by an exchange of a P-site at reaction  $i$  for an O-site at reaction  $m$  with  $i < m$  and (2) by an exchange of a C-site at reaction  $j$  for an O-site at reaction  $m$  with  $m < j$  provided that the affinity  $A$  and the excess number  $d$  of ATP-producing sites are positive.

**PROOF OF THEOREM 1:** Let us consider the replacement of an O-site by a C-site at reaction  $i$  (Part 1 of Theorem 1). According to Eqs. (2.2), (2.5) and (2.6) the flux  $J(O_i)$  with an O-site at reaction  $i$  and the flux  $J(C_i)$  with a C-site at reaction  $i$  read, respectively,

$$J(O_i) = \frac{S_0 Q - S_n}{\sum_{j=1}^{i-1} \frac{Q_{j,n}}{k_j} + \frac{Q_{i,n}}{k_i} + \sum_{j=i+1}^n \frac{Q_{j,n}}{k_j}}, \tag{3.1 a}$$

$$J(C_i) = \frac{S_0 K^{-1} Q - S_n}{K^{-1} \sum_{j=1}^{i-1} \frac{Q_{j,n}}{k_j} + K^{-1} \alpha_i \frac{Q_{i,n}}{k_i} + \sum_{j=i+1}^r \frac{Q_{j,n}}{k_j}}, \tag{3.1 b}$$

with  $Q$  from Eq. (2.14 b) and

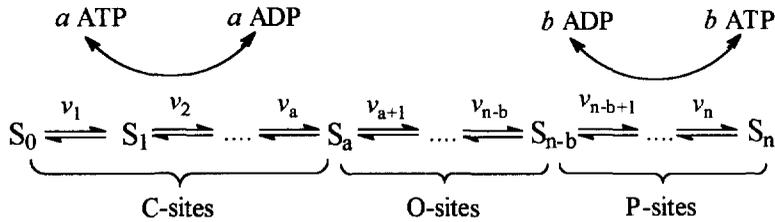
$$Q_{j,n} = \prod_{m=j}^n q_m. \tag{3.1 c}$$

From Eqs. (3.1 a) and (3.1 b) it follows directly that  $J(C_i) > J(O_i)$  if and only if

$$S_0 Q \left( \frac{Q_{i,n}}{k_i} K^{-1} (1 - \alpha_i) + \sum_{j=i+1}^n \frac{Q_{j,n}}{k_j} (K^{-1} - 1) \right) + S_n \sum_{j=1}^{i-1} \frac{Q_{j,n}}{k_j} (K^{-1} - 1) + S_n \frac{Q_{i,n}}{k_i} (K^{-1} \alpha_i - 1) > 0. \tag{3.2}$$

Condition (3.2) holds true under consideration of  $K < 1$  and  $\alpha_i, \beta_i, \gamma_i, \delta_i < 1$  which completes the proof. Part 2 of Theorem 2 can be proved in an analogous way.

It follows from Theorem 2 that  $J_{ATP}$  becomes maximum when all P-sites are located at the lower end of the chain and all C-sites are located at the upper end of the chain (cf. Scheme I)



Scheme I

Therefore, the optimal ATP-production rate reads

$$J_{ATP}(a, b) = \frac{b-a}{D(a, b)} \left( S_0 K^{(b-a)} \prod_{j=1}^n q_j - S_n \right), \quad D(a, b) = D_a + D_o + D_b \tag{3.3 a, b}$$

where

$$D_a = \sum_{j=1}^a \frac{1}{k_j} \alpha_j Q_{j,n} K^{(b-a+j-1)}, \quad D_o = K^b \sum_{j=a+1}^{n-b} \frac{Q_{j,n}}{k_j}, \tag{3.4 a, b}$$

$$D_b = \sum_{j=n-b+1}^n \frac{1}{k_j \gamma_j} Q_{j,n} K^{(n-j+1)}. \tag{30 c}$$

An optimum for the ATP-production rate  $J_{ATP}$  is not only obtained by the proper localization of P- and C-sites at the beginning and the end of the chain, respectively, but also by variation of their numbers  $a$  and  $b$ . For this we consider the special case of equal values for all thermodynamic equilibrium constants ( $q_j = q$ ) and equal values for enzyme concentrations ( $E_j = E$ ).

Figs. 1A,B show the glycolytic rate  $J$  and the ATP-production rate  $J_{ATP}$ , respectively, as functions of the number of coupling sites for a chain with 10 reactions for special values of the thermodynamic parameters  $Q$  and  $K$ . The curves are calculated on the basis of Eqs. (3.3) and (3.4) by taking into account Eq. (2.11) and the condition  $b \geq a$ . The end points at high  $b$  values are determined by the limited total number of sites (i.e.

$a + b \leq n$ ). Broken lines connect points which are physically unrealistic since there condition (2.13 b) is violated.

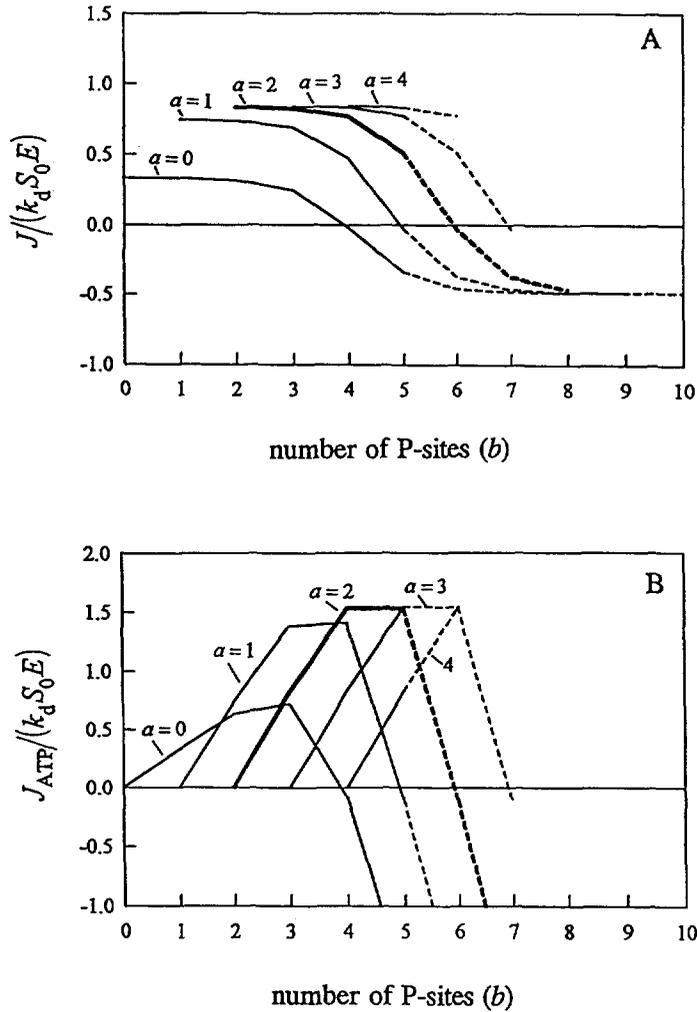


Fig. 1. Glycolytic rate and the ATP production rate for a pathway of 10 reactions.  $J$  (A) and  $J_{ATP}$  (B) are represented as functions of the number  $b$  of P-sites located at the lower end of the chain for various values of the number  $a$  of C-sites at the upper end of the chain according to Eqs. (2.2), (2.11) and (3.4). Parameter values:  $Q = 1024$  ( $q_i = q = 2$ ),  $K = 0.17$ ,  $E_i = E$ ,  $S_0 = S_n$ . The thermodynamic limit is  $d_{max} = 3.94$ . The thick lines connect points for  $a = 2$  which corresponds to the situation of glycolysis.

The glycolytic rate  $J$  decreases for all possible values of  $a$  monotonically with the number  $b$  of P-sites. This property follows directly from Theorem 1, Part 2. For low values of  $a$  ( $a \leq 3$ ) the flux  $J$  may become negative at high numbers of P-sites ( $b - a > d_{\max}$ ), (cf. Eq. (2.16)). For small values of  $b$  with  $b > a$ , the flux  $J$  is rather insensitive to variations of  $b$ . This is in accordance with the result that for  $q_i > 1$  flux control in unbranched chains is mainly exerted by the first enzymic steps, that is, a change of the kinetic properties of reactions at the end of the chain (resulting from the incorporation of P-sites) has little effect on the steady-state flux (cf. [23, 24]). The ATP-production rate  $J_{\text{ATP}}$  shown in Fig. 1 B displays a maximum at variations of the number  $b$  of P-sites. This is explained by the fact that the two factors in Eq. (2.11),  $b - a$  and  $J$ , change in opposite directions at variations of  $b$ . In particular, the increase of  $J_{\text{ATP}}$  results from the insensitivity of  $J$  to variations of  $b$  for low  $b$  values. At higher values of  $b$  the decrease of  $J$  overcompensates the increase of  $b - a$ . The flux  $J$  shown in Fig. 1 A increases with the number  $a$  of ATP-consuming sites at the upper end of the chain, which results from Theorem 1, Part 1. This effect is most pronounced at the transition from  $a = 0$  to  $a = 1$  which makes the first reaction more irreversible due to  $K < 1$ . Since steps behind quasi-irreversible reactions in unbranched chains exert minor flux control, further replacements of O-sites by C-sites at subsequent reactions yield less effect. The maximum for the ATP-production rate is higher for  $a = 1$  (located at  $b = 4$  at the chosen parameter values) than for  $a = 0$  ( $b = 3$ ).

The result that  $J_{\text{ATP}}$  is optimized if the chain contains C-sites at the upper end of the chain and P-sites at the lower end of the chain is qualitatively in accordance with the stoichiometric structure of glycolysis. There, the ATP-consuming reactions catalyzed by hexokinase and phosphofructokinase are located within the first part while the ATP-producing reactions catalyzed by the phosphoglycerate kinase and pyruvate kinase belong to the last part of glycolysis.

*Realistic parameter values:* Using free energy changes the maximal excess number  $d_{\max}$  of ATP-producing sites (Eq. (2.16)) may be expressed as

$$d_{\max} = -\frac{RT \cdot \ln(S_0/S_n)}{\Delta G_{\text{ATP}}} + \frac{\Delta G_{\text{glyc}}^\circ}{\Delta G_{\text{ATP}}} \quad (3.5)$$

where  $\Delta G_{\text{ATP}}$  denotes the free energy change of ATP-hydrolysis under physiological conditions. Since  $\Delta G_{\text{glyc}}^\circ \cong -197 \text{ kJ M}^{-1}$  and  $\Delta G_{\text{ATP}} \cong -50 \text{ kJ M}^{-1}$  (cf. [25]) one obtains for  $S_0 \cong S_n$  the value  $d_{\max} \cong 3.94$ . This number has been used for calculating the curves shown in Fig. 1. However, the overall equilibrium constant  $Q$  and the equilibrium constant  $K$  resulting from the realistic thermodynamic parameters are much higher and much lower, respectively, than those used in Fig. 1.

To introduce into Eqs. (3.3) and (3.4) realistic equilibrium constants  $q_i$  and the corresponding rate constants  $k_i$  and coupling parameters  $\alpha_i$  and  $\gamma_i$  resulting from Eqs. (2.10 a) and (2.10 c) we have to take into account that the existence of  $b$  ATP-producing steps necessitates that the chain contains the same number of phosphorylation steps. If there are  $a$  ATP-consuming reactions the remaining  $b - a$

phosphorylations have to be carried out by inorganic phosphate as the substrate. In the following these reactions are called  $O_P$  - sites. As a consequence, the chain contains  $n - 2b$  O-sites with no phosphorylation or dephosphorylation. In order to fix the total number of reactions  $n$  at variations of  $b$  and  $a$  the two consecutive reactions  $S_{j-1} + P_i \rightarrow (S_{j-1} - P)$  and  $(S_{j-1} - P) \rightarrow (S_j - P)$  are lumped into one reaction  $S_{j-1} + P_i \xrightarrow{v_j} (S_j - P)$ . Analogously, the C-sites  $S_{j-1} + ATP \xrightarrow{v_j} (S_j - P) + ADP$  result from a lumping of the reactions  $S_{j-1} + ATP \rightarrow (S_{j-1} - P) + ADP$  and  $(S_{j-1} - P) \rightarrow (S_j - P)$  and the P-sites  $(S_{j-1} - P) + ADP \xrightarrow{v_j} S_j + ATP$  from lumping  $(S_{j-1} - P) \rightarrow (S_j - P)$  and  $(S_j - P) + ADP \rightarrow S_j + ATP$ . Using, furthermore, the simplifying assumptions that the standard free energy change of the reactions  $(S_{j-1} - P) \rightarrow (S_j - P)$  equals that of  $S_{j-1} \rightarrow S_j$  and that it is independent of  $j$ , the overall reactions are characterized by the following free energy changes:

$$\text{O-sites: } \Delta G_O = \frac{1}{n} \Delta G_{\text{glyc}} \quad , \quad \text{O}_P\text{-sites: } \Delta G_{O_P} = \Delta G_O - \Delta G_{\text{hydr}} \quad , \quad (3.6 \text{ a,b})$$

$$\text{C-sites: } \Delta G_C = \Delta G_{ATP} + \Delta G_O - \Delta G_{\text{hydr}} \quad , \quad (3.6 \text{ c})$$

$$\text{P-sites: } \Delta G_P = \Delta G_O + \Delta G_{\text{hydr}} - \Delta G_{ATP} \quad , \quad (3.6 \text{ d})$$

where  $\Delta G_{\text{hydr}}$  denotes the free energy change of the hydrolysis of a phosphoryled compound  $(S_j - P)$ . We use the value  $\Delta G_{\text{hydr}} = -13 \text{ kJ/mol}$  which corresponds approximately to the free energy change of the splitting of glucose 6-phosphate into glucose and inorganic phosphate under physiological conditions [26]. For all steps the corresponding equilibrium constants are calculated according to the formula  $q = \exp(-\Delta G/RT)$  with the  $\Delta G$ -values taken from Eqs. (3.6 a) to (3.6 d).

Fig. 2 shows the ATP-production rate for realistic thermodynamic parameters which yield according to Eqs. (2.16) and (3.5) with  $S_0 \cong S_n$  the same  $d_{\text{max}}$ -value as used in Fig. 1. The thick solid line represents the case  $a = 2$ , that is, the situation observed in glycolysis. It is seen that the difference between the curves for  $a = 0$  and  $a \neq 0$  is much more pronounced than for lower  $Q$ -values (cf. inset to Fig. 2). For  $a = 0$  and  $a = 1$  the property is retained that  $J_{ATP}$  exhibits a maximum at variation of the number  $b$  of P-sites. For  $a > 1$  there is also a maximum but the descending part of the curve at high  $b$ -values is missing. This is explained by the restriction  $2b \leq n$  (Eq. (2.13 b)) which for  $n = 10$  does not permit that at increasing  $d$ -values the factor  $K^{(b-a)}$  becomes low enough to produce a significant decrease of the overall affinity  $A$  and of the numerator in Eq. (3.3) for  $J_{ATP}$ .

A striking feature of the curves shown in Fig. 2 is that for  $a \neq 0$  the curves on the left side of the maximum are almost straight lines. This property is due to the fact that for realistic thermodynamic parameters the first reaction, which is a C-site for  $a \neq 0$ , becomes nearly irreversible. This implies full control of the glycolytic flux by reaction 1. Since  $S_0 = \text{const.}$  one obtains  $J \cong \text{const.}$  which means that below the thermodynamic limit  $J_{ATP}$  increases nearly proportionally with  $d = b - a$ .

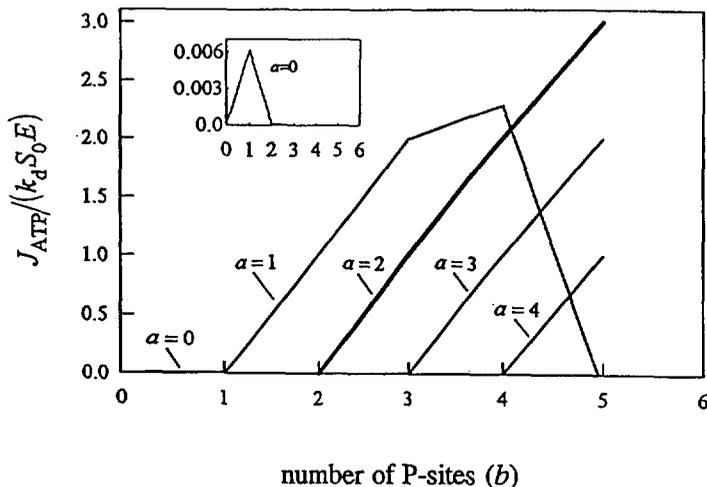


Fig. 2. ATP-production rate for realistic values of thermodynamic parameters for  $n=10$ . The equilibrium constants are  $q_O = 2089.23$ ,  $q_{O_P} = 0.0125$ ,  $q_C = 3.326 \times 10^6$ ,  $q_P = 1.312$  which correspond to the realistic  $\Delta G$ -values given in the text for the various types of sites. Other parameter values:  $E_i = E$ ,  $S_0 = S_n$ ,  $d_{\max} = 3.94$ . The thick lines connects points for  $\alpha = 2$ .

From the curves shown in Fig. 2 it is seen that the  $J_{ATP}$ -optimum for  $\alpha = 2$  is not very much higher than for  $\alpha = 1$ . One could conclude, therefore, that for thermodynamic and kinetic reasons a high ATP-production would be guaranteed by only one ATP-consuming site at the first step of the chain. However, the existence of two ATP-consuming sites at the beginning of glycolysis is also favoured on the basis of the chemical fact that  $\alpha = 2$  allows for a "symmetric pathway" in the degradation of the triose phosphates in the lower part of glycolysis.

#### 4. DISCUSSION

In the present paper it is analyzed whether the design of contemporary glycolysis may be understood on the basis of optimization principles. Since the main metabolic function of glycolysis consists of ATP-production particular attention is paid to the kinetic effects of a change in the location and the number of ATP-producing and ATP-consuming steps. The main result of our investigation is that the optimization of kinetic properties favours pathways where the steps at the upper end of the chain are exergonic or coupled to exergonic processes (as ATP-hydrolysis) and steps at the lower end of the chain are endergonic or coupled to endergonic processes (as ATP-production). This result is in accordance with glycolysis but also with other metabolic systems.

In the present paper many chemical constraints which may favour special structural designs have been neglected. Furthermore, the concentrations of the adenine nucleotides ATP and ADP are considered to be fixed, that is, Eq. (2.2) for the glycolytic flux is derived from the steady-state assumption for the carbohydrates but not for the adenine nucleotides. In a more detailed consideration it would be possible to

treat also the concentrations of ADP and ATP as system variables. Since glycolysis is characterized by a net production of ATP this would necessitate an incorporation of non-glycolytic ATP-consuming processes. This has already been done in several simulation models of glycolysis [2 – 5, 7, 8]. Variable concentrations of the adenine nucleotides would result in nonlinearities in the steady state equations, due to the consideration of bimolecular reactions, as well as in a feedback from the lower end to the upper end of the chain.

Our theoretical approach to explain the structural design of glycolysis in terms of thermodynamic and kinetic parameters is based on the hypothesis that optimization of the net flux through this pathway, in particular, the ATP-production rate, was of utmost importance during evolution. We are well aware that this may be not sufficient for explaining all details of the design of glycolysis. Obviously, other systemic properties should be taken into account in future optimization studies, as stability of steady states, transition times and regulation by internal effectors.

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