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Biophysical Chemistry 54 (1995) 137–142

Biophysical Chemistry

On the expected relationship between Gibbs energy of ATP hydrolysis and muscle performance

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Received 23 June 1994; accepted 11 November 1994

Abstract

Allowing for creatine kinase buffering of changes in adenine nucleotide concentrations, and the known relationship between muscle performance and rate of ATP hydrolysis by myosin, the variation of exerted force with intracellular Gibbs energy of ATP hydrolysis is calculated for voluntary muscle contraction. The resulting relationship is sigmoidal, most of the operating range coinciding with the quasi-linear range around the inflection point. Finger-flexor muscle magnetic resonance spectroscopy data are shown to be in line with this prediction.

Keywords: NMR; Oxidative phosphorylation; Non-equilibrium dynamics; Force-flow relations

1. Introduction

Thanks to the application of nuclear magnetic resonance spectroscopy to muscle in situ, the variation of the concentration of the adenine nucleotides, and of the Gibbs energy of hydrolysis of ATP with muscle performance has been estimated [1–6]. This reinforces the importance of the question how the energetic status of muscle affects its functioning, and the ATP synthesis rate of its mitochondria.

With respect to the latter question there has already been quite some debate. Following the early observation of a hyperbolic correlation between mitochondrial respiration and ADP concentration [7], some authors consider respiration to be controlled exclusively by that concentration [8,9]. Others (e.g., [10]) have been stressing effects of the concentration of inorganic phosphate. A different school of thought considered the oxidation of cytochrome oxidase as the sole rate-limiting step, its degree of reduction being determined by its equilibration with the extramitochondrial phosphorylation potential [11]. Also the school of phenomenological non-equilibrium

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SSDI 0301-4622(94)00129-4

thermodynamics proposed control by the phosphorylation potential or phosphorylation pressure as such, whilst allowing for reciprocal control by the extramitochondrial redox potential [13–15]. Other authors have rather stressed that in vivo regulation of mitochondrial respiration may be more through the supply of redox equivalents. In addition, it was recognized that the coupling mechanisms are proton motive [16], suggesting that proton movement should be in control [17]. Thus, the understanding of the control of intracellular mitochondrial ATP synthesis seemed lamed by paradoxes.

Two ways out of the paradox situation have been paved. First, Groen et al. [18] have falsified the intuitive notion that control resides in only one 'rate-limiting' step. They showed control of respiration of isolated rat-liver mitochondria to be shared by the adenine nucleotide translocator, the substrate translocator, proton permeability and cytochrome oxidase. Second, mosaic non-equilibrium thermodynamics (MNET) [19–21] showed that control by adenine nucleotide concentrations and by the Gibbs energy of hydrolysis of ATP are really two sides of the same coin. This is because of the covariation of ATP, and ADP (cf. also [22]) and because it proved possible to translate enzyme kinetics into non-equilibrium thermodynamics.

The MNET approach clarified two additional aspects: (i) how and why the variation of mitochondrial respiration with extramitochondrial Gibbs energy of ATP hydrolysis could be relatively simple even though so many different chemical and transport processes are involved (Stoner [e.g., [23]] has achieved analogous results for the kinetic formalism) and (ii) why that variation is virtually linear even though the process is far from equilibrium.

These clarifications in terms of metabolic control analysis and MNET have been shown to be pertinent to respiration rate in isolated rat-liver mitochondria [20,24]. It seems of interest to examine if they also apply to actual work done in vivo, e.g., by muscle. At variance with liver, in muscle the covariation of ATP, ADP and inorganic phosphate, is largely dictated by the creatine kinase equilibrium. Moreover the performance of muscle is determined by the myosin ATPase, rather than hexokinase or kinases involved in biosynthetic pathways. This paper addresses what relationship one should expect between Gibbs energy of ATP hydrolysis, and performance in muscle. We find that this relationship should be sigmoidal with the quasilinear region in the normal operating domain. Magnetic resonance spectroscopy results for finger-flexor muscle are in line with this relationship.

2. Results

2.1. Derivation of expected relationship between muscle power and Gibbs energy of ATP hydrolysis

In contracting muscle, ATP is hydrolyzed by the myosin ATPases, at a rate J_p^a. Exerted power (P) has been shown to be directly proportional to this rate [25]:

$$P = \alpha \cdot J_p^a \tag{1}$$

with α constant and independent of muscle activation. ATP is being synthesized by (i) mitochondrial oxidative phosphorylation plus glycolysis and (ii) glycolysis to lactate. Under conditions of minor to moderate exercise the glycolysis to lactate contribution may be neglected. However, some ATP hydrolysis, J_p^l, occurs which is not coupled to muscle contraction. In our description, we term the latter 'leak'. Writing $-J_p^o$ for ATP synthesis by oxidative phosphorylation, the ATP balance for steady state demands:

$$P = \alpha \cdot \left(-J_p^o - J_p^l\right) \tag{2}$$

At steady state the rate of mitochondrial oxidative phosphorylation may be written as if it is a single enzyme catalyzed reaction [21,23]:

$$-J_p^o = \frac{V_{ADP} \cdot \frac{ADP}{K_{ADP}} \cdot \frac{P_i}{K_p} - V_{ATP} \cdot \frac{ATP}{K_{ATP}}}{\left(1 + \frac{ADP}{K_{ADP}}\right) \cdot \left(1 + \frac{P_i}{K_{P_i}}\right) + \frac{ATP}{K_{ATP}}} \tag{3}$$

ADP, P_i and ATP do not vary independently; their covariation is determined by the presence of sizable concentrations of creatine and a high creatine kinase activity. Assuming the creatine kinase reaction to be near equilibrium

$$\frac{ADP}{ATP} = K_{CK} \cdot \frac{Cr}{CrP} \tag{4}$$

Because the equilibrium constant for creatine kinase (K_{CK}) is smaller than 0.01, the ADP (and AMP) concentrations are less than 10% of the total adenine nucleotide concentration (unless the Cr/CrP ratio exceeds 10). Consequently, ATP can be assumed to be effectively constant. Since already the ATP concentration is much smaller than the total creatine concentration, the terms [ATP] (because of little variation) and [ADP] and [AMP] (because of their small values) may be neglected in the phosphate balance equation [26]:

$$[P_i] + [CrP] + 3 \cdot [ATP] + 2 \cdot [ADP] + [AMP] = \text{constant} \quad (5)$$

leaving as the free variable the amount of phosphate esterified as CrP.

In our calculations we start from the reference state in which half the creatine is phosphorylated. For an arbitrary state, x refers to the concentration of creatine phosphate in excess of that. This leads to the following expressions for $[P_i]$, [CrP] and [Cr]:

$$[P_i] = P_i^{ref} - x \quad (6)$$

$$[Cr] = \frac{(Cr + Cr \sim P)}{2} - x \quad (7)$$

$$[CrP] = \frac{(Cr + Cr \sim P)}{2} + x \quad (8)$$

The myosin ATPase is driven by the Gibbs energy of ATP hydrolysis. We define:

$$\text{driving force} = \Delta G_p - \Delta G_p^{max} = R \cdot T \cdot \ln\left(\frac{ATP \cdot V_{ATP} \cdot K_{ADP} \cdot K_{P_i}}{ADP \cdot P_i \cdot V_{ADP} \cdot K_{ATP}}\right) \quad (9)$$

The apparent equilibrium constant $V_{ATP} \cdot K_{ADP} \cdot K_{P_i}/(V_{ADP} \cdot K_{ATP})$ is determined by the redox Gibbs energy difference as transmitted through the proton electrochemical potential difference ($\Delta\tilde{\mu}_H$); 500 mM^{-1} is a fair number [27]. ΔG_p^{max} is the corresponding theoretical state-4 static head phosphorylation potential.

Eqs. (2–4,6–9) allow calculation, as a function of x, of the rate of mitochondrial ATP synthesis (Eq. (3)), of muscle power output (Eq. (2)) and of the Gibbs energy of ATP hydrolysis (the 'driving force')

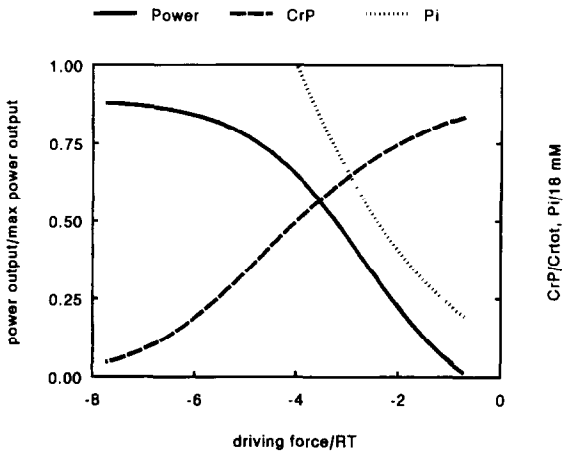

Fig. 1. Predicted variation of muscle output power (calculated as net rate of ATP synthesis; c.f. Eq. (2)) with Gibbs energy of ATP hydrolysis. The dashed and dotted lines give the CrP/total creatin ratio and the inorganic phosphate concentration, respectively. Calculations were based on Eqs. (3–4) and (6–9). $K_{CK} = 1/166$; $V_{ADP} = 1$, $V_{ATP} = 0.08$; $K_{ADP} = 0.0015$ mM [28], $K_{ATP} = 0.15$ mM [28], $K_{Pi} = 2.5$ mM, $J_p^l = 0.05$, $P_i^{ref} = 18$ mM, TCr = Cr + Cr ~ P = 43 mM [29], $\alpha = 1$.

(Appendix I). Consequently, one can calculate how these three functions vary with one another. The solid line in Fig. 1 shows how, according to the above considerations, muscle power is expected to vary with the (concentration dependent part of the) Gibbs energy of ATP hydrolysis, when α of Eq. (1) is constant and steady state is maintained. The estimates for K_{ATP}, K_{ADP}, K_{P_i}, P_i^{ref}, total creatine and ATP concentration used and given in its legend, are based on literature data. The calculated covariation is sigmoidal.

The dashed line in Fig. 1 gives the variation of the CrP to total creatine ratio with the Gibbs energy of ATP hydrolysis. Over the range of 'aerobic' steady states at constant pH = 7.0, this ratio usually varies between 0.9 and 0.3. Accordingly, Fig. 1 suggests that the variation of the delivered muscle power with Gibbs energy of ATP hydrolysis might well be indistinguishable from being linear.

2.2. Comparison of expectations to experimental result

Fig. 2 shows how in an experiment conducted on intact human forearm flexor muscle, power and crea-

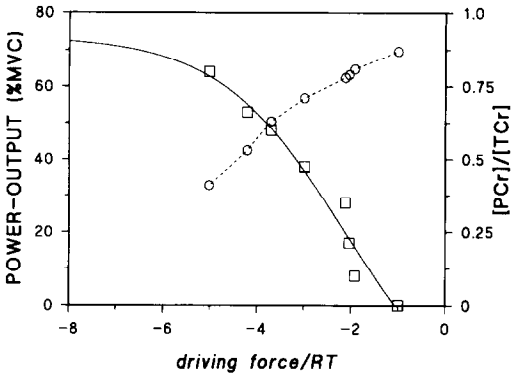

Fig. 2. Variation of power (□, in % MVC) and [PCr]/[TCr] (○) with the Gibbs energy of ATP hydrolysis in intact human finger-flexor muscle, as calculated from ^{31}P MRS measurements of average P_i and PCr levels in muscle cells at various steady states [30,12]. *Driving force / RT* was calculated as $(\Delta G_p - \Delta G_p^{max} - RT)/RT$. The solid line represents the fit of a sigmoidal function (adapted from ref. [21]) to the *force / RT* versus power-output data. Regression equation: power-output = $74 \cdot \{e^{(-0.9-(0.8\cdot[\Delta G/RT]))} - 1\}/\{e^{(-0.9-5(0.8\cdot[\Delta G/RT]))} + 2.4\}$.

tine phosphate varied with the Gibbs energy of ATP hydrolysis. The solid line represents the fit of Eq. (2) to the *force / RT* versus power-output data. Clearly, these experimental results are in line with those predicted by Fig. 1. The creatine phosphate/total creatine ratio varied between 0.87 and 0.41 over the range of power-output levels studied. Intracellular

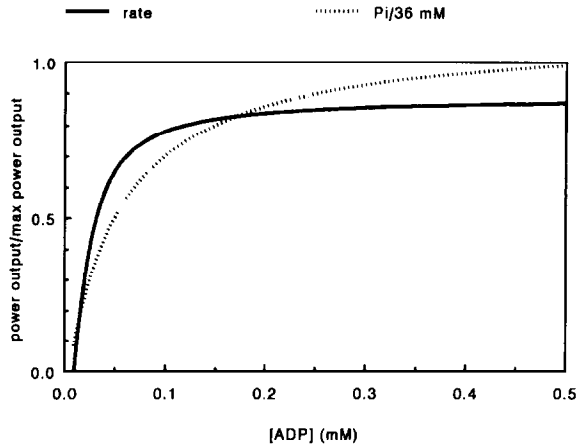

Fig. 3. Predicted variation of muscle power output with ADP concentration as Fig. 1. The dotted line gives the covariation of phosphate concentration with ADP concentration (P_i/36 mM). The full line is not a perfect hyperbola.

pH remained within the interval [7.14–7.04] (resting value: 7.08). The fitted estimates for v_s and v_p (expressed in % MVC, relative to the resting state), n and C were (4°); $74 \pm 25\%$ MVC, $31 \pm 64\%$ MVC, 0.8 ± 0.7, and -1.1 ± 0.3, respectively. Clearly, the experimentally accessible range of driving forces and the quasi-linearity of the observed variation allow for a limited accuracy of the determination of these parameters.

Fig. 3 shows how muscle power is expected to vary with ADP concentration: hyperbolically. When considered in detail however, this dependence is not a first order hyperbola: As indicated by the dotted line in Fig. 3 inorganic phosphate varies approximately, but not quite proportionally with ADP at low concentrations of the latter [12].

3. Discussion

This paper has used available information on muscle energetics to estimate how muscle power is expected to vary with the Gibbs energy of ATP hydrolysis, as contraction is being activated at the level of actinomyosin activity.

The predicted quasilinear variation of power with ATP driving force was consistent with an experimental observation. This may serve to demonstrate that such linear variations should cause neither surprise nor assumptions of a near-equilibrium nature. It will be interesting to examine if such linear variation is observed over a broader range of circumstances and also in other muscles [12].

To prevent any conclusion that these results falsify the hypothesis that muscle power output varies hyperbolically with ADP concentration, we have also plotted the latter two variables with respect to one another and found a near-hyperbolic relationship (see also [12]). Clearly our results are consistent both with 'thermodynamic' (by Gibbs energy of ATP hydrolysis) and with 'kinetic' (by ADP concentration) 'control'. Indeed, as we have contended before [24], much of the controversy concerning kinetic versus thermodynamic control is void; in practice ADP, P_i and ATP are covariant [26] and a relationship between flux and ADP can be simply translated

to a relationship between flux and Gibbs energy of ATP hydrolysis (ΔG_p). If the covariation is absent (such as in vitro), both the ADP control and the ΔG_p control are likely to become non-unique, as has been made explicit early on for the latter [20].

An interesting feature of our result is that even though the rate of ATP hydrolysis by myosin was taken as independent of ΔG_p, that rate still varied linearly with it. This effect resulted from the applied steady-state condition, the quasilinear variation of the rate of ATP synthesis with ΔG_p, and the fact that the independent variable was the extent of activation of the actinomyosin, hence (Eq. (1)) of P at constant α. Had the independent variable been different (e.g., inactivation of the mitochondria) the relationships had appeared differently. For instance, had the independent variable been the concentration of mitochondria, the power output would have appeared to be horizontally (i.e., not) related to ΔG_p. When muscle power is varied voluntarily, however, as was done in the experiments of the present paper, muscle energetics may well be characterized by a rather simple, i.e., linear, flow–force relationship. This may help to simplify our analyses of this important process.

Acknowledgements

We thank Profs. K. van Dam, M. Kushmerick, and R. Berger for stimulating discussions and Ms J.E. Wijker for the excellent typing. This study was supported by the Netherlands Organization for Scientific Research (NWO).

Appendix 1

Describing J_p^0, P, and ΔG_p as a function of high-energy phosphate bonds in contracting human skeletal muscle under steady-state conditions at pH = 7.

$$J_p^0(x) = \frac{2.5 \cdot \dfrac{(21.5 - x)}{(21.5 + x)} \cdot (18 - x) - 4.37}{\left(1 + \dfrac{2.5 \cdot (21.5 - x)}{(21.5 + x)}\right) \cdot (1 + (18 - x)) + 54.67}$$

$$P(x) = \alpha \cdot \left(-J_p^0(x) - 0.05\right)$$

$$\Delta G_p(x) = \Delta G_p^0 + RT \ln \frac{8.2}{\left(0.05 \cdot \dfrac{(21.5 - x)}{(21.5 + x)} \cdot (18 - x)\right)}.$$

x represents phosphate esterified as PCr (mM) relative to $[PCr]_{ref}$. The calculation was performed for 73 steady states, $-21 < x < 15$ (software used: MLAB (Civilized Software, Inc., Bethesda, MD, USA)).

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