Myosin cross-bridge kinetics in airway smooth muscle: a comparative study of humans, rats, and rabbits

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Lecarpentier, Y., F.-X. Blanc, S. Salmeron, J.-C. Pouray, D. Chemla, and C. Coirault. Myosin cross-bridge kinetics in airway smooth muscle: a comparative study of humans, rats, and rabbits. Am J Physiol Lung Cell Mol Physiol 282: L83–L90, 2002.—To analyze the kinetics and unitary force of cross bridges (CBs) in airway smooth muscle (ASM), we proposed a new formalism of Huxley’s equations adapted to nonsarcomeric muscles (Huxley AF, Prog Biophys Biophys Chem 7: 255–318, 1957). These equations were applied to ASM from rabbits, rats, and humans (n = 12/group). We tested the hypothesis that species differences in whole ASM mechanics were related to differences in CB mechanics. We calculated the total CB number per square millimeter at peak isometric tension (Ψ ×10⁹), CB unitary force (II), and the rate constants for CB attachment (f₁) and detachment (g₁ and g₂). Total tension, Ψ, and II were significantly higher in rabbits than in humans and rats. Values of II were 8.6 ± 0.1 pN in rabbits, 7.6 ± 0.3 pN in humans, and 7.7 ± 0.2 pN in rats. Values of Ψ were 4.0 ± 0.5 in rabbits, 1.2 ± 0.1 in humans, and 1.9 ± 0.2 in rats; f₁ was lower in humans than in rabbits and rats; g₂ was higher in rabbits than in rats and in humans than in rabbits. In conclusion, ASM mechanical behavior of different species was characterized by specific CB kinetics and CB unitary force.

MATERIALS AND METHODS

Rabbit and rat tracheal smooth muscle preparations. We studied 12 samples from rabbits and 12 samples from rats (1 sample/animal). Care of the animals conformed to the recommendations of the Helsinki Declaration. After anesthesia with intraperitoneal pentobarbital sodium (100 mg/kg), the trachea was immediately removed. A tracheal ring consisting of four (from rabbits) or five (from rats) tracheal segments was carefully dissected. The rings were opened with a dorsal midline section through the cartilage to obtain strips of the posterior membranous portion of the trachea as previously described (2). The body weights of the Sprague-Dawley rats and New Zealand White rabbits were 381 ± 37 g and 3.56 ± 0.05 kg, respectively. The ages of the rats and rabbits were 8 and 13 wk, respectively.

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Human bronchial smooth muscle preparations. Bronchial smooth muscle rings (generations 1–3) were obtained from patients undergoing lobectomy or pneumonectomy to remove lung carcinomas (n = 12; 1 sample/patient). The age of the patients was 61.3 ± 3.8 yr. The body weight was 69.8 ± 5.2 kg. None of the patients had a history of atopy or asthma nor were they chronically treated with bronchodilators. Immediately after surgical resection, macroscopically tumor-free tissue was put into a 500-ml airtight container filled with a Krebs-Henseleit solution (in mM: 118 NaCl, 4.7 KCl, 1.2 MgSO4, 1.1 KH2PO4, 24 NaHCO3, 2.5 CaCl2 and 4.5 glucose) bubbled with a gas mixture of 95% O2-5% CO2 and maintained at 37°C and pH 7.4. While the lower end of the strip was held by a stationary clip at the bottom of the bath, the upper extremity of the strip was held in a spring clip linked to an electromagnetic lever system as previously described (21). Supramaximal electrical field stimulation (30 V/cm, 50-Hz alternating current, 10-ms pulse duration, 12-s train duration) was provided through two platinum electrodes every 5 min. Experiments were conducted after a 1-h equilibration period. The optimal initial length (L0; mm) was defined as the resting muscle length corresponding to the maximum active tension.

Mechanical analysis. The tension-velocity (P-V) relationship (37) was derived from the peak velocity (V; L/s) of 6–10 isotonic afterloaded contractions plotted against the isotonic force level normalized per cross-sectional area (P) and by successive load increments from zero load up to the total isometric tension (P0; Fig. 1). The mean muscle cross-sectional area is the ratio of muscle weight to muscle shortening velocity that is measured by means of the zero-load clamp technique (24). Velocity is expressed as a function of time in the same contractions. L0, initial optimal length; EFS, electrical field stimulation.

The rate of mechanical energy (\(\dot{E}_{\text{me}}\); W/mm²) per cross-sectional area are expressed as a function of V (17). \(\dot{E}\) is given as

\[
\dot{E} = \Psi_e \frac{h}{2f_1 + g_1} \left[ g_1 + f_1 \frac{V}{\Phi} (1 - e^{-G/V}) \right] \quad (1)
\]

where \(\Psi\) is the CB number per square millimeter (×10⁹) at peak isometric tension; \(f_1\) is the maximum value of the rate constant for CB attachment (s⁻¹) (17); \(g_1\) and \(g_2\) (\(g_2\) appears in Eq. 2) are the peak values of the rate constants for CB detachment (s⁻¹) (17); h is the CB step size (11 nm) (7, 11, 13, 27), defined by the translocation distance of the actin filament per ATP hydrolysis and produced by the swing of the myosin head; e is the free energy required to split one ATP molecule (5.1 × 10⁻⁴ J) (8, 17, 44); l is the distance between two actin sites (36 nm) (32); and \(\Phi = (f_1 + g_1)h/2 = b\) (17).

Calculations of \(f_1\), \(g_1\), and \(g_2\) are given by the following equations (see APPENDIX and Refs. 4, 20, 21)

\[
g_2 = \frac{2V_{\text{max}}}{h} \quad (2)
\]

\[
g_1 = \frac{2wb}{ek\phi} \quad (3)
\]

\[
f_1 = \frac{-g_1 + \sqrt{g_1^2 + 4g_1g_2}}{2} \quad (4)
\]

where \(w\) is the maximum mechanical work of a unitary CB (3.8 × 10⁻¹⁰ J) (17, 44).

The maximum turnover rate of myosin ATPase per site under isometric conditions (\(k_{\text{cat}}\); s⁻¹) is

\[
k_{\text{cat}} = \dot{E}_0/\Psi_e = \frac{h}{2f_1 + g_1} \quad (5)
\]

where \(\dot{E}_0\) is the minimum rate of total energy release (W/mm²), \(\dot{E}_0\) occurs under isometric conditions (V = 0 in Eq. 1), is equal to the product of \(ab\) (15, 17), and is given by

\[
\dot{E}_0 = ab = \Psi_e \frac{h}{2f_1 + g_1} \quad (6)
\]

Thus the \(\Psi\) is

\[
\Psi = \dot{E}_0/(ek_{\text{cat}}) \quad (7)
\]

The \(P_{\text{Hux}}\) is given by (17)

\[
P_{\text{Hux}} = \frac{\Psi \cdot f_1}{I} \cdot \frac{f_1}{f_1 + g_1} \times \left[ 1 - \frac{V}{\Phi} \left(1 - e^{-G/V}\right) \left[ 1 + \frac{f_1 + g_1^2}{2g_2^2} \frac{V}{\Phi} \right] \right] \quad (8)
\]

The maximum \(P_{\text{Hux}}\) (\(P_{\text{Hux,max}}\)) is reached for \(V = 0\) and is assumed to be equal to total \(P_0\), which was experimentally determined.
determined. The mean unitary force per CB in isometric conditions (\(\Pi; \text{pN}\)) equals \(\frac{f_1}{k_{\text{cat}}}\).

\[
\Pi = \frac{w}{l} \frac{f_1}{f_1 + g_1}
\]  

(9)

The mean CB velocity during the stroke size (\(\bar{\vartheta}_c; \mu\text{m/s}\)) is

\[
\bar{\vartheta}_c = \frac{\bar{E}}{\Psi \Pi} \frac{1}{L_0}
\]  

(10)

The time stroke is equal to \(h/\vartheta_c\). The time cycle is equal to \(1/k_{\text{cat}}\). The duty ratio is equal to time stroke/time cycle (36). \(W_M\) equals \(P_{\text{Hux.max}}\). At any given load level, the mechanical efficiency of the muscle is defined as the ratio of \(W_M\) to \(\bar{E}\) and \(E_{\text{eff}}\) is the maximum value of efficiency.

**Values of Huxley's equation constants.** A stroke size of 11 nm has been determined by means of optical tweezers (11, 13) and is supported by the three-dimensional structure of the crystallized myosin head (7, 27). The distance between two actin sites is equal to 36 nm (32). The free energy required to split one ATP molecule per contraction site is \(5.1 \times 10^{-20}\) J and the maximum mechanical work of a single CB is equal to 0.75e, so that it is \(3.8 \times 10^{-20}\) J (17, 44).

**Statistical analysis.** Data are expressed as means ± SE. The parameters of the three species were compared with analysis of variance. Univariate associations between quantitative parameters were assessed with correlation coefficients. Partial correlation coefficients adjusted for species (introduced as two dummy variables) are also reported. Pearson correlation coefficients for each species (rabbit, rat, and human) were used for intraspecies analysis.

**RESULTS**

The mathematical formulation needed to apply Huxley’s equations (17) to smooth muscle and to calculate CB rate constants, \(\Pi\), and \(\Psi\) is described in APPENDIX. This allowed estimation of the CB characteristics of ASM in the three species under study. Total tension, \(\Pi\), and \(\Psi\) were higher in rabbits than in humans and rats but did not differ between humans and rats (Fig. 2). There was a linear relationship between total tension and \(\Psi\) (Fig. 3); the partial correlation coefficient adjusted for species was \(r = 0.999\) (\(P = 0.001\)). The Pearson correlation coefficients indicated a significant correlation between total tension and \(\Psi\) in humans (\(r = 0.917; P = 0.001\)), rats (\(r = 0.983; P = 0.001\)), and rabbits (\(r = 0.996; P = 0.001\)). Conversely, no linear relationship was observed between total tension and \(\Pi\) (Fig. 3). \(V_{\text{max}}\) was higher in rabbits than in rats and in rats than in humans (Fig. 2).

The \(f_1\) was lower in humans than in rabbits and rats but did not differ between rabbits and rats (Fig. 4). The \(g_2\) differed significantly in the three species and was higher in rabbits than in rats and in rats than in humans (Fig. 4). Both the \(k_{\text{cat}}\) and \(g_1\) were higher in rats than in humans and did not differ between rats and rabbits (Fig. 4). However, \(g_1\) did not differ between humans and rabbits and \(k_{\text{cat}}\) was lower in humans than in rabbits (Fig. 4). There was a linear relationship between \(V_{\text{max}}\) and \(k_{\text{cat}}\) (Fig. 5); the partial correlation coefficient adjusted for species was \(r = 0.480\) (\(P = 0.004\)). The Pearson correlation coefficients indicated a significant correlation between \(V_{\text{max}}\) and \(k_{\text{cat}}\) in rats (\(r = 0.614; P = 0.034\)) and rabbits (\(r = 0.745; P = 0.005\)) but not in humans (\(r = 0.004; P = 0.991\)).

Peak efficiency was higher in rabbits than in humans and rats but did not differ between humans and rats (Fig. 6). There was a linear relationship between
Eff\textsubscript{max} and both II and \(k\text{cat}\) (Fig. 7). For the relationship between Eff\textsubscript{max} and II (Fig. 7), the partial correlation coefficient adjusted for species was \(r = -0.982\) (\(P = 0.001\)). The Pearson correlation coefficients indicated a significant correlation between Eff\textsubscript{max} and II in humans (\(r = 0.986; P = 0.001\)), rats (\(r = 0.987; P = 0.001\)), and rabbits (\(r = 0.981; P = 0.001\)). For the relationship between Eff\textsubscript{max} and \(k\text{cat}\) (Fig. 7), the partial correlation coefficient adjusted for species was \(r = -0.684\) (\(P = 0.001\)). The Pearson correlation coefficients indicated a significant correlation between Eff\textsubscript{max} and \(k\text{cat}\) in humans (\(r = -0.892; P = 0.001\)) and rats (\(r = -0.858; P = 0.001\)) but not in rabbits (\(r = -0.858\)).
In our study, we proposed a formalism of Huxley’s equations (17) to ASM from different species. We then used these equations to compare CB number, unitary force, and kinetics in rabbit, rat, and human ASM.

Application of Huxley’s equations to smooth muscle. In our study, we proposed a formalism of Huxley’s equations (17), adapted to smooth muscle devoid of sarcomeric structure. In his princeps study, Huxley wrote: “It is natural to ask whether the mechanism proposed here for striated muscle could account also for the contraction of smooth muscle. On general grounds, it is to be expected that the mechanism is fundamentally the same in both types, so that it would be unsatisfactory to postulate for one type a mechanism that clearly cannot exist in the other…” The ultrastructure of smooth muscle strongly differs from that of striated muscle. In particular, there is no Z-line structure, even if the attachment of actin filaments to dense bodies is reminiscent of that found at Z lines of striated muscle (10, 18). In Huxley’s equations, “s” represents the sarcomere length. In smooth muscle, the precise ultrastructural substratum for s remains uncertain. In the present study, s is equal to 2 μm. Huxley’s equations have been previously applied to smooth muscle in swine carotid artery (14) and bovine trachea (12), with a fixed value of the parameter of muscle pseudoperiodicity (s = 2.2 μm) in both studies.

Two other ultrastructural parameters appear in Huxley’s equations (17), i.e., the distance between two actin sites and the unitary displacement step or power stroke. The monomer G-actin units are arranged on a nonintegral helix, with subunits repeated at 5.5 nm along two chains that twist around, with crossover points 36 nm apart. The pitch of the polymerized actin helix, i.e., the distance between two actin sites, is 36 nm in all actin isoforms from eukaryotic cells, i.e., in both muscle and nonmuscle actins. In eukaryotic cells, sequences of actin are more highly conserved than almost any other proteins (32). Both smooth and skeletal muscle myosins produce similar unitary displacement (i.e., similar power stroke) of ~10–11 nm when measured with optical tweezers (11, 13, 19). For these reasons, a power stroke value of 11 nm was chosen in our study. Moreover, the power stroke has recently been estimated to be on the same order of magnitude on the basis of crystallography analysis of the myosin motor domain of smooth muscle (7).

The Huxley original theoretical model (17) has been validated by using the experimental data of Hill (15) with the values of the asymptotes -a and -b of the P-V relationship, the product ab = Eo (i.e., the maintenance heat), Go of the P-V relationship = Po/la = gθ(f1 + g1), Φ = b, and w/e = 0.75 (15, 17). In tracheal smooth muscle, Mitchell and Stephens (24) have shown that Vmax values mathematically derived from conventional isotonic afterloaded force-velocity curves [as in Hill’s (15) study] are valid estimates of zero-load velocity because they are not significantly different from values obtained by direct measurement with the zero-load clamp technique (as in our study). The values of the asymptotes -a and -b and consequently of G are not different in the two types of force-velocity curves. The maximum work (w) done by one CB has been determined in quick release experiments and is at least 3.7 × 10^-20 J (44), which is a value very close to that used in our study.

In skeletal myosin filament, there is a bipolar helical arrangement of CBs of opposite polarity, which project from each side of a central bare zone. In smooth myosin filaments, CBs along a rodlike filament with no central bare zone project in opposite directions on opposite sides of the filament. The myosin heads along an entire side have the same polarity along the entire length of the filament (45). The Huxley (17) formalism applied on the ribbonlike myosin filament structure may induce an additional factor of 2 compared with the current calculations.

Unitary force of myosin head and muscle total force. The CB unitary force values in our study (Fig. 2) were on the same order of magnitude as those previously measured by means of the laser trap in both smooth and skeletal muscles (11, 13, 25) and in intact skeletal muscle (4, 20, 21). Total force per cross-sectional area, i.e., the product of CB unitary force and CB number per square millimeter, appears to be slightly lower in ASM than in skeletal muscle. Because the CB myosin unitary force is of the same order of magnitude in smooth and skeletal muscles (13), this difference may be partly explained by a lower myosin concentration in smooth muscle than in skeletal muscle (26). Accordingly, our results show that the total number of active CBs was lower in ASM compared with that previously reported in skeletal muscles (4, 20, 21). In addition, we found that CB number per square millimeter was an important determinant of total tension in ASM as attested to by the linear relationship between total tension and CB number per square millimeter (Fig. 3). No relationship was observed between total tension and CB unitary force.

Vmax and θo. Vmax and θo were ~10-fold lower in smooth than in skeletal muscle, as previously reported (3, 9, 37). Accordingly, in in vitro motility assays, purified smooth muscle myosin also propels actin filament at one-tenth the velocity of skeletal muscle myosin (42). Our results showed no relationship between...
CB kinetics and \(k_{\text{cat}}\). Our results showed a longer time cycle \(= 1/k_{\text{cat}}\) and time stroke in ASM (Fig. 6) than that previously reported in skeletal muscle (4, 20, 21). In smooth muscle, the duration of two major steps of the CB cycle (i.e., \(1/f_1\) and \(1/g_2\)) was roughly 10 times longer than in skeletal muscle. These results corroborate the fact that the duration of the ATPase cycle is longer for smooth than for skeletal muscle (34). Moreover, the mean attached time is longer in smooth than in skeletal muscle myosins (13). A dephosphorylated "latch-bridge" model has been proposed to explain the mechanics and energetics of smooth muscle. Dephosphorylation may produce a noncycling latch bridge that has a slow detachment rate (6). Huxley's formalism (17) has been adapted to the latch-bridge model (14). This model can be used to quantitatively predict stress maintenance with reduced phosphorylation, CB cycling rates, and ATP consumption. The apparent rate constants \(f(K)\) and \(g(K)\) represent the average behavior of the CB population in smooth muscle and vary linearly as a function of the phosphorylation level. Taking into account the proportionality constants, the rate constant values for CB attachment \((f_1)\) and detachment \((g_1\) and \(g_2\)) found by Hai and Murphy (14) are on the same order of magnitude as those in our study (Fig. 4). It is widely accepted that the slow cycling rate of latch CBs in smooth muscle contributes to the high economy of tension maintenance during prolonged isometric contraction (6, 33). A similar approach has recently been used by Fredberg et al. (12), who suggested that excessive airway narrowing in asthma may be associated with the destabilization of dynamic processes and the resulting collapse back to static equilibrium. Because smooth and skeletal muscle myosins do not markedly differ in their unitary force and power stroke but rather in their kinetics (13), enzymatic and mechanical differences may be partly due to functional differences and/or differences in their primary amino acid sequence.

Differences in CB mechanics and kinetics between the species studied. In our study, \(V_{\text{max}}\) was particularly low in human ASM compared with the values in rabbit and rat ASM (Fig. 2) (3, 23) as well as in canine (37) and mouse (9) tracheae. CB velocity was higher in human ASM than in the two other species, corresponding to a shorter time stroke (Fig. 6) and suggesting that \(V_{\text{max}}\) and \(\dot{\theta}_o\) are modulated by different molecular mechanisms. Differences in \(\dot{\theta}_o\) in rabbit, rat, and human ASM were due to differences in CB rate constants for attachment \((f_1)\) and detachment \((g_1\) and \(g_2\)). All rate constants \((f_1, g_1, g_2)\) and \(k_{\text{cat}}\) were lower in human ASM than in the two other species (Fig. 4). The higher value of total tension observed in rabbit ASM compared with that seen in humans and rats was due to higher \(\dot{\psi}\) and \(\dot{\Omega}\) values (Fig. 2). Interestingly, \(E_{\text{ff}_{\text{max}}}\) and \(E_{\text{ff}1}\) were of the same order of magnitude as the values observed in skeletal muscle (Figs. 2 and 6) (4, 20, 21). This is probably due to the fact that the three rate constants for CB attachment and detachment were proportionally decreased in smooth compared with skeletal muscle (4, 20, 21). As in skeletal muscle, peak efficiency was linearly related to CB unitary force (Fig. 7) (4, 20, 21). In smooth and skeletal muscles, paralogous and orthologous myosin II heavy chain isoforms lead to a considerable range of variations in shortening velocity, CB kinetics, and myosin ATPase activity compared with the relatively small range of variations in efficiency and CB unitary force. Even if significant changes in CB unitary force were observed in our study, total tension appeared to be strongly linked to the total number of CBs (Fig. 3).

Structural differences, particularly with respect to the ATP binding pocket, the actin binding site, and the light chains, may represent the molecular basis for differences in mechanical performance between species. Some authors have also discussed the role of flexible loops (29, 36). Increases have been observed in the ADP release rate, actin-activated ATPase activity, and the rate of actin filament sliding in an in vitro motility assay involving an insert of seven amino acids in a flexible loop (the 25- to 50-kDa loop) on the surface of the smooth muscle myosin head near the nucleotide binding pocket (29). The role of regulatory (RLC) and essential light chains has also been discussed (40). Unlike striated muscle myosin, smooth muscle myosin requires the RLC to be phosphorylated to act as a molecular motor. RLC-deficient myosin moves slowly in vitro motility assays and has a low actin-activated ATPase activity (40). However, differences in flexible loops and/or light chains in rabbit, rat, and human ASM have not been precisely identified. It remains to be determined whether such differences contribute to the differences in CB properties observed in our study.

Allometric factors. Allometry helps to explain species differences in function of physiological variables that have a time-related component. Small animals have higher power-to-weight ratios than large animals. In skeletal muscle, it has been shown that isometric tension exhibits no dependence on animal body size, but \(V_{\text{max}}\) in both fast and slow fibers and maximum power output in fast fibers vary with the \(-\frac{1}{6}\) power of body size (31). In the present study, we did not find any relationship between time-related parameters \((V_{\text{max}}\) and \(k_{\text{cat}}\)) on the one hand and body size on the other hand. By calculation of the partial correlation coefficient, we observed a global linear relationship between \(V_{\text{max}}\) and \(k_{\text{cat}}\), which is in agreement with Bárány’s law (1). However, some discrepancies appeared in relation to this law. As expected from allometric properties, \(V_{\text{max}}\) and \(k_{\text{cat}}\) were lower in humans than in the two other species (rats and rabbits). Conversely, \(V_{\text{max}}\) was higher in rabbits than in rats and there was no difference in \(k_{\text{cat}}\) between rats and rabbits. Other deviations
from the theory of allometry and from Bárány’s law (1) have been previously described (28). Importantly, these deviations have been reported in skeletal muscle from humans, rabbits, and rats. Thus the unexplained deviations have been reported in skeletal muscle from the same three species.

Smooth muscles have been classified into two classes, tonic and phasic (35). Kinetics of the contractile machinery and force development have been found to differ in these two types of smooth muscle. However, the same mathematical formalism can be applied to both of them. Mechanical differences observed in our study may be related to the site along the airways. Isometric tension and \( V_{\text{max}} \) decreased from 0 to 6 generations of airways (22). Thus the lower mechanical performance observed in human smooth muscle may be partly explained by the fact that we used bronchial samples (generations 1–3), whereas tracheae were studied in rats and rabbits. Moreover, in a given species, shortening velocity decreases somewhat with age (43). Thus the lower velocity observed in humans compared with the other species may be partly due to age. Age also influences contractile protein content, calcium handling, Na-K-ATPase activity, and receptor responsiveness (43). Finally, shortening velocity in smooth muscle is linearly related to phosphorylation level of 20-kDa myosin light chain (14), but we have no indication of the phosphorylation level of 20-kDa myosin light chain. However, muscle strips were studied under similar tetanic electrical conditions of stimulation and the same experimental conditions (temperature).

In conclusion, we proposed a new formalism of Huxley’s equations (17) specifically adapted to smooth muscle. ASM mechanical behavior of different species was characterized by CB kinetics and CB unitary force. The present study may have potential interest for studying CB kinetics in the ASM pathophysiology (9, 12, 23, 37), particularly airway hyperresponsiveness.

**APPENDIX**

In Huxley’s original manuscript (17), the velocity \( v \) (in \( \mu \text{m/s} \)) with which the actin filament is sliding past the myosin filament is proportional to the rate of isotonic muscle shortening \( V \) according to \( v = (s/2) \times V \) and \( \Phi = (f_1 + g_1)b/h = b \) (where \( V, \Phi, \) and \( b \) are in \( \text{s}^{-1} \) and \( s \) is in \( \mu \text{m} \)). In Huxley’s study (17), \( E \) per volume unit is expressed as a function of \( m \), the number of sites per volume unit. In the present study, \( E \) per cross-sectional area is expressed as a function of \( \Psi \), and \( s \) was equal to 2 \( \mu \text{m} \).

**Calculation of \( g_2 \) (dissociation rate constant at the end of the power stroke).** Because \( \Phi = (f_1 + g_1)b/h = b \) (where \( \Phi, \) and \( b \) are in \( \text{s}^{-1} \) and \( s \) is in \( \mu \text{m} \)). In Huxley’s study (17), \( E \) per volume unit is expressed as a function of \( m \), the number of sites per volume unit. In the present study, \( E \) per cross-sectional area is expressed as a function of \( \Psi \), and \( s \) was equal to 2 \( \mu \text{m} \).

Calculation of \( g_2 \) (dissociation rate constant at the onset of the power stroke). From Eqs. 6 (with \( E_0 = ab \)) and 8 under isometric conditions (with \( V = 0 \) and \( P_{\text{Hux}} = Ga \)), we deduced that

\[
f_1g_1 = \frac{2lab}{\Psi e h} = \frac{vG_a2l}{2\Psi w g_1} \quad \text{(A1)}
\]

Thus \( g_1 = 2wb/ehG \) (Eq. 3).

**Calculation of \( f_1 \).** \( E \) was linearized as a function of \( P_{\text{Hux}} \).

\[
A = \frac{V}{\Phi} (1 - e^{\psi v}) \quad \text{(A2)}
\]

and

\[
B = \frac{f_1}{2l(f_1 + g_1)} \quad \text{(A3)}
\]

By inserting \( A \) and \( B \) in Eqs. 1 and 8, Eq. 1 becomes

\[
\frac{E}{e h^2} = g_1 + f_1 A \quad \text{(A4)}
\]

and Eq. 8 becomes

\[
\frac{P_{\text{Hux}}}{2\psi B} = 1 - A \left(1 + \frac{1}{2} \frac{d^2V}{\Phi} \right) \quad \text{(A5)}
\]

where \( d = 1/G \).

From Eq. A4, we deduced

\[
A = \frac{E}{mehBf_1} - \frac{g_1}{f_1} \quad \text{(A6)}
\]

and from Eq. A5, we deduced

\[
A = \frac{(2\psi B - P_{\text{Hux}}/2\Phi)}{(2\psi B/2\Phi + d^2V)} \quad \text{(A7)}
\]

From Eqs. A4 and A5, we deduced

\[
\dot{E} = \frac{2\psi (eh^2f_1)}{2\Phi + Vd^2} + g_1 (eh^2B) - \frac{eh^2f_1}{Vd^2} P_{\text{Hux}} \quad \text{(A8)}
\]

From Eq. A8 and near isometric conditions, where \( V \) can be neglected (\( V \approx 0 \)), \( E \) can be linearized as a function of \( P_{\text{Hux}} \) as follows

\[
\dot{E} = \frac{\Psi e h f_1}{2l} - \frac{eh^2 f_1}{2w P_{\text{Hux}}} \quad \text{(A9)}
\]

The slope of this relationship between \( \dot{E} \) and \( P_{\text{Hux}} \) has been shown to be equal to \( b; \) then, \( b = eh^2/2w \), so that

\[
f_1 = \frac{2wb}{eh} \quad \text{(A10)}
\]

From Eqs. 3 and A10, we deduced

\[
\frac{2w}{eh} = \frac{g_1 G}{b} = \frac{f_1}{b} \quad \text{(A11)}
\]

Thus \( f_1 = g_1 G \).

Because \( G = g_2/f_1 + g_1 \) (17) and \( f_1 = g_2 G \), by solving the quadratic equation \( f_1^2 + g_1 f_1 - g_2 g_1 = 0 \), we obtained \( f_1 \) as a function of \( g_1 \) and \( g_2 \)

\[
f_1 = \frac{-g_1 + \sqrt{g_1^2 + 4g_2 g_1}}{2} \quad \text{(A12)}
\]

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