

A MECHANOCHEMICAL MODEL OF FLAGELLAR ACTIVITY

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ABSTRACT A theory is presented which quantitatively links the physical properties of a flagellum with parameters which characterize the chemical reactions responsible for deforming the flagellum. Realistic values for the wave parameters are predicted when order-of-magnitude values for the appropriate constants are used. The model may be useful in other fields where mechanochemical coupling occurs.

INTRODUCTION

In recent theoretical analyses of the behavior of an undulating flagellum some success has been achieved by representing the organelle as a vibrating beam (Machin, 1958, 1963; Rikmenspoel, 1965). From this type of analysis it is possible to express the wave parameters of the flagellum in terms of its elasticity and the viscosity of the surrounding medium. Other observations (Brokaw, 1968) suggest that this simple approach is not entirely satisfactory, but its moderate success indicates that the method warrants further examination.

Analytical methods of Machin (1958) have shown that to sustain the observed wave forms energy must be provided along the length of a flagellum. This energy will be stored in the flagellum in chemical form before its use. To bend the flagellum a reaction occurs which allows chemical energy to be converted into mechanical energy. It is possible to relate the beat frequency of a flagellum to the rate constant of a first-order chemical reaction associated with the energy conversion (Holwill and Silvester, 1965). This reaction is one which limits the beat frequency of the flagellum although it is not necessarily the one which provides the energy necessary for beating. A kinetic analysis of the system predicts the variation of beat frequency with temperature that is observed in practice (Holwill and Silvester, 1965, 1967; Holwill, 1969, 1970).

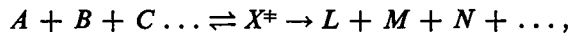
In this paper a consideration of the tension within the flagellum is used to obtain equations which unite the two types of approach described above. The rates of the chemical reactions responsible for flagellar activity depend upon the tensions to which the reactants are subjected, while the same tensions contribute to the forces which bend the flagellum. The relationships obtained on the basis of chemical

kinetics are combined with others based on the theory of bending beams to produce equations which are independent of the tension. In this way the measurable parameters of flagellar wavelength and beat frequency can be related to parameters which characterize the as yet undetermined reactions which bend the flagellum.

REACTION KINETICS

To derive the required expressions it is first necessary to investigate the manner in which the rate of a chemical reaction depends on the tension to which the reactants are subjected.

According to the absolute rate theory (Eyring, 1935) a general reaction,



proceeds through an unstable transition state X^\ddagger (referred to as the activated state) and the rate of the reaction is assumed to depend only on the rate of transition from X^\ddagger to the products $L, M, N \dots$. This rate is determined by the concentration of X^\ddagger which may be calculated if equilibrium is assumed between the reactants and the activated state. Using the absolute rate theory a simple general mathematical treatment of the progress of the reaction is made possible by this equilibrium assumption. A few detailed analyses which do not assume equilibrium have been made (see e.g. Laidler, 1965), but there is no significant discrepancy between these and the method using the equilibrium assumption, provided the activation energy for the process exceeds $5RT$, where R is the gas constant and T the absolute temperature. Holwill and Silvester (1967) have estimated the activation energy for a chemical reaction which they consider limits the flagellar beat frequency. The value they obtain, about 62 kJ mole^{-1} , is greater than $5RT$ (about 12 kJ mole^{-1} at temperatures in the region of 20°C) thus providing some justification for the equilibrium assumption in the present analysis.

From the absolute rate theory, the following relation is obtained for a first-order rate constant k

$$k = P \frac{kT}{h} \exp\left(-\frac{\Delta G^\ddagger}{RT}\right). \quad (1)$$

Here ΔG^\ddagger is the change in the Gibbs free energy in passing from reactants to the activated state, P is a transmission coefficient often taken as unity, and h, k are the Planck and Boltzmann constants respectively.

Suppose that the reactants are subjected to a tension and that the work done by the fiber in respect to its variations in length and tension is very much greater than that due to pressure and volume changes. By analogy with the effects of pressure on reaction rates (e.g. Laidler, 1958) it is found that

$$k_J = k_0 \exp\left(\frac{\Delta l_J^\ddagger}{RT}\right), \quad (2)$$

where k_0 is the rate constant at zero tension, J is the tension, and Δl^\ddagger is a mean change in length associated with activation.

The tension dependence of the rate of a chemical reaction expressed in equation 2 will be used to investigate the behavior of a generalized model of an active flagellum.

A GENERALIZED FLAGELLAR MODEL

For analytical purposes we shall consider a generalized model of a flagellum capable of propagating two-dimensional waves. This model contains an elastic filament which resists the bending induced by forces from two bilaterally symmetric molecular fibers (Fig. 1). The nature of the molecular arrangements need not concern us here, but current ideas favor either a system where localized contraction occurs or one in which filaments slide relative to one another.

The observed patterns of flagellar bending require chemical energy to be available at all points along the flagellum (e.g. Holwill, 1966). To produce bending each fiber must, effectively, be capable of local contraction and extension. At a given point on an individual fiber two chemical reactions may occur, one to elongate the fiber, the other to shorten it.

From absolute rate theory the velocities V_L and V_S of these reactions may be written

$$V_L = C_L \exp\left(\frac{\Delta l_L^\ddagger + J}{RT}\right), \quad \text{and} \quad (3)$$

$$V_S = C_S \exp\left(\frac{\Delta l_S^\ddagger + J}{RT}\right), \quad (4)$$

where J is the local tension of the reactants while C_L and C_S are temperature-

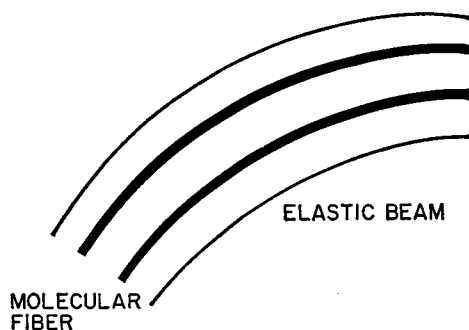


FIGURE 1 A bilaterial arrangement of molecular fibers capable of bending the elastic beam which contains them. With correct phasing of the activation of the two fibers, the elastic beam can execute planar undulations similar to those observed on the flagella of many organisms.

dependent coefficients which are in general functions of the concentration of the reactants. Δl_L^* and Δl_S^* are the changes in length associated with activation of the two processes.

The relative extension (e) of a fiber will be governed by the changes ($\pm \epsilon$) in length which occur during a single shortening or elongating reaction, together with the effects of elasticity in the fiber. We can write

$$\frac{de}{dt} = \alpha \frac{dJ}{dt} + \epsilon \left[C_L \exp\left(\frac{\Delta l_L^* J}{RT}\right) - C_S \exp\left(\frac{\Delta l_S^* J}{RT}\right) \right], \quad (5)$$

where α is an elastic constant. If J and e are sufficiently small that second and higher powers of them can be neglected, equation 5 may be rewritten in the form

$$\frac{de}{dt} = \alpha \frac{dJ}{dt} + pJ - qe + g, \quad (6)$$

where p , q , and g are constants. Equation 6 represents the response of the molecular fiber for small values of J and e . In actual flagella the assumptions of small J and e may well be invalid in certain segments of the contraction-extension cycle, but they will be made here to show that the mechanochemical approach can produce a model capable of generating waves similar to those on flagella.

WAVE PROPAGATION ALONG FLAGELLA

The response described by equation 6 can be incorporated into equations relating to a beam bending in a viscous fluid as derived by Machin (1958).

In his analysis Machin (1958) obtained the following expression for the small displacement y of an elastic filament distorted by active bending moments $M(x, t)$ in a viscous medium:

$$\frac{\partial^2 M}{\partial x^2} + a \frac{\partial^4 y}{\partial x^4} + b \frac{\partial y}{\partial t} = 0. \quad (7)$$

a is a constant describing the elastic properties of the beam while b is a constant proportional to the viscosity of the medium. If the bending moment arises from equal and opposite tensions in the bilaterally arranged fibers (Fig. 1), then

$$M = cJ, \quad (8)$$

where c is a constant, and if the distance between the two fibers is $2d$ (Fig. 1), then the extension of the "outer" fiber per unit length is

$$e = d \frac{\partial^2 y}{\partial x^2}, \quad (9)$$

provided the curvature is small.

From equations 6-9 the following differential equation may be obtained

$$(cd + a\alpha) \frac{\partial^5 y}{\partial x^4 \partial t} + (qcd + ap) \frac{\partial^4 y}{\partial x^4} + b\alpha \frac{\partial^2 y}{\partial t^2} + bp \frac{\partial y}{\partial t} = 0. \quad (10)$$

A solution of equation 10 is

$$y = [\exp(r_1 t) + K \exp(r_2 t)] \{E_1 \exp(-ikx) + E_2 \exp(-kx) + E_3 \exp(ikx) + E_4 \exp(kx)\}, \quad (11)$$

where r_1 and r_2 are given by taking the alternative signs of the root in the equation

$$r = \frac{1}{2b\alpha} \{-[pb + k^4(cd + a\alpha)] \pm \sqrt{p^2 b^2 + 2pbk^4(cd + a\alpha) + k^8(cd + a\alpha)^2 - 4bk^4\alpha(qcd + ap)}\}. \quad (12)$$

Equation 11 will be oscillatory provided r is complex, but sustained oscillations of finite amplitude are only possible if r is purely imaginary. This is the situation which would reasonably correspond to the observed behavior of flagella, and it is therefore of interest to investigate it further.

The wavelength ($2\pi/k$) and frequency ($\omega/2\pi$) of the oscillation are given by

$$2b + k^4(cd + a\alpha) = 0 \quad (13)$$

$$\omega^2 = -\frac{p}{\alpha} \left(\frac{ap + qcd}{cd + a\alpha} \right). \quad (14)$$

For real values of k and ω it is clear from equations 13 and 14 that p must be negative and that $(ap + qcd)$ must be greater than zero, since all constants except p and q are necessarily real and positive. In the next section we will show that realistic values for the various parameters can yield orders of magnitude for k and ω which are found in real flagella.

In an oscillatory solution of equation 10 the first and third terms in the braces of equation 11 represent propagating waves in opposite directions. The other two terms represent an exponential increase and decrease in displacement. The solution which applies to a flagellum requires the specification of the boundary and initial conditions appropriate to this case. These boundary conditions are not known for a real flagellum (Machin, 1963), but the form of a solution can be obtained for various ideal situations in which the proximal end of the flagellum is freely hinged, rigidly clamped, or free, while the distal end is free. For any combination of these conditions it can be shown that $|E_1| = |E_3|$ so that the amplitudes of the propagating waves are equal and the system will exhibit standing waves. Such a system cannot produce a propulsive force, and is thus not a reasonable model of a flagellum. It is only when

the transverse and angular impedances at least at one end of the flagellum are neither zero nor infinite that $|E_1|$ and $|E_2|$ are not equal, so that one of the progressive waves will dominate, thereby allowing propulsion to occur. A more detailed discussion concerning the impedances is given by Machin (1963) who shows that a propagating wave form will arise at least in the situation where the impedances at one end of the flagellum are such that energy absorption by a viscous-type mechanism occurs and that a source of energy is also present. Without more knowledge about the function of the various structural elements of a flagellum it is not possible to express the impedances in mathematical terms. It is interesting to note, however, that Rikmenspoel (1966), using a different type of analysis, has shown that the wave velocity along the type of elastic system envisaged here is largely independent of the exact form of the boundary conditions.

ESTIMATION OF WAVELENGTH AND FREQUENCY

We will assume that the boundary and initial conditions of the system are such that progressive waves of finite amplitude are sustained by the model system.

To obtain orders of magnitude for the wavelength ($2\pi/k$) and the beat frequency ($\omega/2\pi$) predicted by equations 13 and 14 it is first necessary to estimate values appropriate to flagella for the various parameters in equations 13 and 14. A preliminary requirement for this is the assumption that bending forces arise in a particular structural component of the flagellum. It is not possible to assign a function with any certainty to a particular flagellar feature, but in the majority of models of flagellar bending so far proposed the outer ring of nine fibers in the $9 + 2$ axonemal complex figures prominently as the origin of the bending force. There are several ways in which the nine fibers could be used to produce planar bending, but it is reasonable to suppose that both c (equation 8) and d (equation 9) will have values of the order of the radius of the nine-fiber ring. From electron microscope studies a typical value for this radius is 0.075μ (e.g. Gibbons, 1967), so that c and d are each of the order of magnitude $10^{-1} \mu$.

The parameter a (equation 7) is the stiffness of the elastic beam and can, in principle, be calculated for a flagellum, if a particular structural feature is assumed to have the elasticity which opposes the bending forces. Since several authors (Holwill, 1966; Rikmenspoel, 1966; Rikmenspoel and Sleight, 1970) obtain values in the region of 10^{-22} N m^2 for the stiffness of certain cilia and flagella, we will assume this value for the present calculation.

In equation 7 the coefficient b involves the viscosity (μ) of the fluid in which the flagellum moves and has the form

$$b = \frac{4\pi\mu}{2 - \ln R}, \quad (15)$$

where R is the Reynolds number for the system. For an aqueous environment and a

Reynolds number of 10^{-3} (e.g. Holwill, 1966), b has an order of magnitude 1 m N sec.

An estimate of the elastic constant α can be made by assuming that the molecular forces which give the fiber its elastic properties are, in common with many materials, about one order of magnitude weaker for small strains than those involved in covalent bonding. A realistic value for the order of magnitude of α is then 10^9 N^{-1} .

Both p and q (equation 6) are difficult to estimate with accuracy, but by expanding the exponentials of equation 5 we see that p has the same order of magnitude as $\epsilon C \Delta f^\ddagger / RT$ in which the subscripts L and S have been omitted. The activation length may be positive or negative (Glasstone et al., 1941) but commonly observed values for $\Delta f^\ddagger / RT$ are of the order 10^{10} N^{-1} . The parameter C will approximate the number of molecular changes involving extension or shortening per unit length of a fiber. Using the data of Silvester and Holwill (1965) the value for C has an order of magnitude of $10^{11} \text{ m}^{-1} \text{ sec}^{-1}$. Taking a value of 100 nm for ϵ we obtain an estimate for p of $\pm 10^{12} \text{ N}^{-1} \text{ s}^{-1}$. In an oscillatory system, as we have already seen, it is necessary that p should take the negative value.

To obtain an order of magnitude for q it is again necessary to assume a fairly specific mechanism for the chemical reactions underlying shortening and elongation of a fiber. Since the molecule adenosine triphosphate (ATP) is known to be intimately involved in flagellar activity it is natural to consider the values of q that would arise if the fibrillar shortening and extension were the result of the breakdown and formation of an ATP-enzyme complex. If the reactions are of first order then q turns out to be the sum of two real or apparent rate constants, and for the mechanism mentioned above has an order of magnitude of 10^{-3} at reasonable values of the ATP concentration. For convenience the estimates we have made are summarized in Table I.

Substituting these values in equations 13 and 14 we find that the predicted wavelength and beat frequency have orders of magnitude of 10μ and 100 sec^{-1} respectively. These values agree well with the wave parameters observed on real flagella.

TABLE I
ORDERS OF MAGNITUDE OF THE
PARAMETERS INVOLVED IN
THE THEORY

Parameter	Order of magnitude
c	10^{-1} m
d	10^{-1} m
a	10^{-13} N m^2
b	1 m N sec
α	10^9 N^{-1}
p	$-10^{12} \text{ N}^{-1} \text{ sec}^{-1}$
q	10^3 sec^{-1}

DISCUSSION

In this paper we have used chemical kinetics and the theory of bending beams to obtain an expression for the wave parameters of an elastic system (which represents a flagellum) in terms of coefficients associated with the chemical reactions which bend and straighten the beam. To obtain these equations several approximations were made, the most important of which are the specification of small amplitudes and the neglect of higher powers than unity of the tension and extension of the molecular fiber in the expansion of the exponential terms in equation 5. It is likely that none of these approximations is valid for a real flagellum so that the equations 13 and 14 derived here are to be regarded as a first step in the development of nonlinear flagellar models.

Equations 13 and 14 allow specific mechanisms for flagellar activity to be examined for feasibility. For example, they immediately show that a purely passive elastic system (which is characterized by zero values of p and q) cannot sustain undamped oscillations, a fact which has been demonstrated in earlier work (e.g., Machin, 1958). Similarly, certain types of viscoelastic systems in which p and q are both positive cannot account for observed flagellar behavior. Any system in which $q = 0$, such as one involving zero-order rate processes, is also unsatisfactory. As noted earlier, however, a system involving first-order reactions can yield values of p and q which permit the formation of sustained undulations.

It is encouraging that the use of reasonable values for the chemical and physical parameters (Table I) in equations 13 and 14 leads to realistic values for the wavelength and beat frequency of the model. The reliability of the values obtained by this method can be judged only with difficulty, mainly because of the problems associated with the estimation of p , q , and α ; however, the estimates of p , q , and α are unlikely to be in error by more than one or two orders of magnitude. In the most unfavorable case this could result in values one order of magnitude greater for the wavelength and frequency, but in view of the methods used for the estimation, this magnitude of discrepancy is not unreasonable.

Equations 13 and 14 do allow certain predictions to be made about the variation of the flagellar wave parameters with altered physical and chemical conditions, such as viscosity, temperature, and concentration of chemical substances. The results of these investigations will be reported elsewhere, but we may note here that good agreement is obtained in most cases between the experimental results and the predictions of our equations. In these experiments, the values for p , q , and α are obtained graphically and are found to have values of the order of magnitude shown in Table I.

One serious discrepancy of the theory presented here is the prediction of equation 14 that the beat frequency should be independent of the viscosity. In practice the frequency decreases significantly when the viscosity is increased by one or two orders of magnitude. This failure is a direct consequence of the approximations made in

describing the model, and can be eliminated by including nonlinear terms in the analysis. Machin (1963) has shown that the effect of including certain types of non-linearity in the analysis of a similar model is to allow only one propagating wave to be present at any time on the flagellum. We are currently investigating the effects of nonlinearities on the behavior of our model and can say at this stage that the equations predict a dependence of the oscillation frequency on viscosity, although we cannot express this dependence quantitatively.

The results we have obtained are largely independent of the adoption of a specific model for the flagellum, but may be used to predict the properties of the various components of such a model. In a local contraction model, for instance, the elastic coupling between adjacent units would be characterised by the parameter α while this same parameter would determine the nature of the elasticity in the cross-linkages of a sliding filament model. Of more fundamental importance is the expression of the chemical parameters in terms of the physical properties of the system. The formulation of this type of mechanochemical coupling is of importance not only in the field of flagellar activity but in any system, e.g. muscle, peritrich stalks, mitotic spindles, where a chemical reaction leads directly to the development of motive force.

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