

THE THERMAL DEPENDENCE OF FLAGELLAR ACTIVITY IN *STRIGOMONAS ONCOPELTI*

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INTRODUCTION

Although most of the published work on the motion of cilia and flagella has been centred on the physical aspects of the problem, the fundamental processes underlying the propagation of a wave along an organelle are evidently chemical in character. The relation between the chemical and mechanical properties of such a system is to be found in the domain of chemical kinetics, so that energy considerations must ultimately figure in any molecular explanation of flagellar movement. In the present paper we report some observations, over a range of temperatures, of the flagellar activity of *Strigomonas oncopelti*. From these results we evaluate an activation enthalpy and entropy that are characteristic of the motile system and discuss the results in the light of current data on flagellar activity.

Several stroboscopic and high-speed ciné-photographic studies have revealed that cilia generally execute an oar-like beat followed by a recovery stroke offering little resistance to movement, while many flagella maintain two- and three-dimensional waves along their length. Sleigh (1960), using a stroboscopic technique, has shown that the recovery stroke of a peristomial cilium of *Stentor polymorphus* commences before the effective stroke is complete, and that the entire stroke is due to a single flexional wave which passes along the cilium from base to tip. Lowndes (1941) and Brown (1945) have suggested that waves always pass along the flagellum from base to tip; recent work by Walker & Walker (1963) and Holwill (1964*a, b*) have shown that members of the Trypanosomatidae usually propagate planar waves along their flagella from tip to base. Holwill has reported that these organisms can also propagate waves in the reverse direction.

The effect on ciliary and flagellar beating of altering the environment has been the subject of many reports. An increase in temperature has been found to increase the rate of ciliary beating (Gray, 1930; Lucas, 1932; Sleigh, 1956), and in the case of the peristomial cilia of *Stentor*, Sleigh found the frequency of beat to be related to the absolute temperature by the Arrhenius equation which occurs in the theory of reaction rates.

Few descriptions of flagellar movement have been made with sufficient accuracy to allow the theoretical equations derived by Taylor (1951, 1952), Hancock (1953), Gray & Hancock (1955) and Holwill & Burge (1963) to be applied. These equations relate the propulsive velocity of an organism to the wavelength, amplitude and frequency of the flagellar beat and from equations of a similar nature the energy expended externally by an undulating flagellum can be evaluated (Carlson, 1959; Holwill & Burge, 1963). The energy required to deform the flagellum requires a knowledge of the

Young's modulus for the material comprising it but as yet there have been no reliable measurements of this modulus, so that any value for the power dissipated during elastic deformation of an organelle will have little significance.

The elucidation of the fine structure of cilia and flagella by electron microscopy (e.g. Gibbons & Grimstone, 1960; Gibbons, 1961) has led to speculation regarding the function of the various components. Although the evidence is largely circumstantial, the nine peripheral fibrils are generally associated with the contractile process while the two central fibrils are described as either conducting the stimulus for contraction (Bradfield, 1955) or maintaining rigidity of the system (Satir, 1961). The motility of sperm lacking the central pair (Afzelius, 1962) provides evidence that these fibrils do not constitute the compression elements.

Afzelius (1959), Gibbons & Grimstone (1960) and Satir (1961) discuss sliding filament mechanisms for contraction in cilia and flagella but the system is discarded by Gibbons & Grimstone on the grounds that to sustain a wave on a flagellum would require simultaneous extensions and contractions at different points on the same fibril. Many authors support the view that the fibrils themselves undergo active shortening. Each fibril is assumed to be made up of a series of contractile units. The models described by Bradfield (1955), Gray (1955) and Sleigh (1960) are capable of explaining on a physical basis sinusoidal, helical or oar-like beats. In such a model, waves of contraction pass along each of the nine fibrils which are stimulated in a certain sequence depending on the form of beat.

Machin (1958) has suggested that if wave propagation takes place by mechanical means, no separate system to control the movement of a wave is necessary, but Holwill (1964*b*) considers that, although a mechanical system could be responsible for wave propagation, a control system is necessary for the initiation of a wave. Both Gray (1955) and Machin (1958) have shown that the presence of a sustained amplitude along a flagellum implies that chemical energy is available throughout its length. The rate of expenditure of this chemical energy is dependent on, *inter alia*, the ambient temperature and thus from the thermal dependence of flagellar activity it may be possible to infer something about the molecular processes underlying the contraction cycle.

KINETICS

The change in length of a biological fibre has in certain cases been related to a first-order chemical reaction; for instance, the hydrothermal shrinkage of collagen (Weir, 1949) and the elongation of muscle (Burge & Elliott, 1963) have both been treated thermodynamically on this basis. The cyclic changes in length which occur along a beating flagellum are shown below to conform to a similar pattern, so that we can use standard expressions of chemical kinetics in our analysis.

Frequency of flagellar contraction. On the basis of a model that we shall discuss elsewhere (Silvester & Holwill, in preparation) let us assume that local longitudinal contractions in the outer fibrils are responsible for the flagellar beat, and that the shortening at any region is due to a characteristic change in shape of contractile units. Any active change in shape is related to the chemical activation of the unit concerned. (We need not discuss here the relation between contractile units and the type of molecular subunit observed in the flagellar structure by, e.g., André & Thiéry (1963) and Pease (1963).) Consider a flagellar fibril with a length which comprises n con-

tractile units and suppose that within a wavelength of contraction that passes down the fibril there are N such units. When waves are passing continuously down the fibril, the rate, dn/dt , at which units are being contracted is given by the velocity of the wave along the fibril in contractile units per second, multiplied by the number of waves passing along the flagellar length at any time. Thus

$$\frac{dn}{dt} = v \left(\frac{n}{N} \right), \quad (1)$$

where v is the wave velocity in contractile units per second. Since the wave transverses N contractile units in a time $1/f$ if f is the frequency of the wave, then

$$v = Nf \text{ contractile units per second} \quad (2)$$

and
$$dn/dt = fn \text{ sec.}^{-1} \quad (3)$$

The rate at which units undergo the chemical activation which, it is assumed, alters their conformation is thus proportional to the number of units present. This rate will depend on the rates of the several chemical reactions which must occur in each contraction-relaxation cycle, so that the slowest reaction will be the rate-limiting step which governs the frequency of beat. It is of course possible that one of the reactions is itself controlled by some extraneous factor (e.g. the elastic properties of the flagellum) but this does not affect the foregoing analysis.

The relation between rate of activation of contractile units and the number of units concerned (equation (3)) is formally equivalent to the equation which defines the nature of a first-order chemical reaction (Hinshelwood, 1940); the constant f is usually represented by the symbol k and is called the limiting rate constant of the system. (Equation (3) would in a normal chemical process give rise to an exponential relationship between length and time, as in the cases of collagen and muscle (*op. cit.*), but in the present cyclic process the molecules are continually returned to their original state through the provision of an extraneous source of energy, so that the number of 'available' units, n , and hence dn/dt , remains effectively constant.)

The limiting rate constant of the system may possibly, in the case of flagella, refer to a rate-limiting step which is not in the narrow sense chemical, as mentioned above; nevertheless, the thermodynamics of activation still apply, and the variation with temperature of k will give information about the energetics of the system.

Derivation of thermodynamic parameters. The statistical treatment of reaction rates (Glasstone, Laidler & Eyring, 1941) yields the relation

$$k = \frac{\mathbf{k}T}{h} \exp \left(\frac{\Delta S^\ddagger}{R} \right) \exp \left(\frac{\Delta H^\ddagger}{RT} \right) \quad (4)$$

in which \mathbf{k} and h are respectively the Boltzmann and Planck constants, R is the gas constant per mole, T the absolute temperature, and ΔS^\ddagger and ΔH^\ddagger are the molar changes in entropy and enthalpy that accompany the activation process. On equating the frequency of flagellar beat, f , with the rate constant, k , we may write, from the above,

$$\ln \left(\frac{f}{T} \right) = \left[\frac{\Delta S^\ddagger}{R} + \ln \left(\frac{\mathbf{k}}{h} \right) \right] - \frac{\Delta H^\ddagger}{RT} \quad (5)$$

so that an experimentally derived graph of $\ln (f/T)$ vs. $1/T$ should be linear and give values of ΔS^\ddagger and ΔH^\ddagger from the intercept and slope, respectively.

It should perhaps be noted here that the 'heat of activation', ΔH^\ddagger , arrived at in this way will be slightly different from the activation energy, E , found by the application of the Arrhenius equation

$$k = A \exp \left(\frac{-E}{RT} \right). \quad (6)$$

The Arrhenius equation, often used in chemical kinetics (e.g. Laidler, 1958), has a different temperature dependence from equation (5) so that for reactions in solution

$$E = \Delta H^\ddagger + RT$$

(Glasstone *et al.* 1941) which introduces a difference of about 0.6 kcal./mole at ordinary temperatures.

MATERIAL AND METHODS

The organism selected for this work was the uniflagellate *Strigomonas oncopelti*; this was cultured by the method previously described (Holwill, 1964*b*). The animals were observed under phase-contrast conditions with a Zeiss W.L. research microscope which could be maintained at a constant temperature. The microscope stood on a metal plate which contained a heating element controlled by a thermostat; the apparatus was enclosed in a Perspex box within which the temperature could be regulated. The temperature at the level of the microscope stage was maintained constant to within $\pm 0.5^\circ$ C. and could be varied in the range from 4 to 45° C. In order to attain thermal equilibrium a culture was placed in the enclosure for about 2 hr. before a sample was taken for examination. Specimens could be manipulated without significantly altering the temperature. The pH of the culture medium lay between 6 and 7.

Films were taken at 450 frames per second using a Stalex high-speed camera as described in a previous paper (Holwill, 1964*b*). The frequency, wavelength and amplitude of the flagellar beat were measured in a frame-by-frame analysis of each film.

RESULTS

The movement of *S. oncopelti* under normal conditions has been described in detail elsewhere (Holwill, 1964*b*), so it will suffice to recall only those features of the motion relevant to the present work. The organism has a cylindrical body measuring about 8.2 by 2.6 μ with a flagellum 17 μ long. During forward motion the organism swims with its flagellum preceding the body. Planar waves pass along the flagellum from tip to base with an increase in wavelength and often in amplitude.

The effects of temperature change on the beat frequency are summarized in Table 1. The frequency increased with rise in temperature up to about 30° C. but the average wavelength and amplitude remained essentially constant at 14.4 and 2.4 μ , respectively. Between 30 and 35° C. the beating became erratic and no significant value for the frequency could be determined. Above about 35° C. localized spasmodic bending of the mid-portion of the flagellum occurred and waves were not propagated. In this case the flagellum remained bent so that the distal and proximal ends made an angle of 120° with each other; during the flagellar movement the body of the organism remained stationary while the distal region of the flagellum oscillated through a range of angles of about 30°. The effects of changing temperature were reversible up to about 41° C., but above this temperature all movement ceased. The variations in frequency

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cannot be attributed to a change in the viscosity of the medium with temperature, since the change is too small to have a significant effect on the wave parameters. Assuming that the medium in which the organism swims has a similar viscosity-temperature relation to that of water, a variation in temperature from 4 to 30° C. would produce a change in viscosity from 1.6 to 0.8 centipoise. For such a change the frequency would be expected from previous measurements (Holwill, 1964*b*) to vary from 16 beats/sec. to 17.8 beats/sec.; this variation is negligible compared to the change actually observed.

Table 1. *The effect of temperature on the flagellar wave parameters** of *Strigomonas oncopelti*

Temperature (°C.)	4	10	14	19	22	28
Frequency (sec. ⁻¹)	3.0	4.3	7.1	11.4	16.8	24.0
Wavelength (μ)	14.6	14.5	14.3	14.4	14.4	14.3
Amplitude (μ)	2.4	2.2	2.5	2.3	2.4	2.4

* The values quoted are the mean of measurements on about ten organisms from each of four preparations.

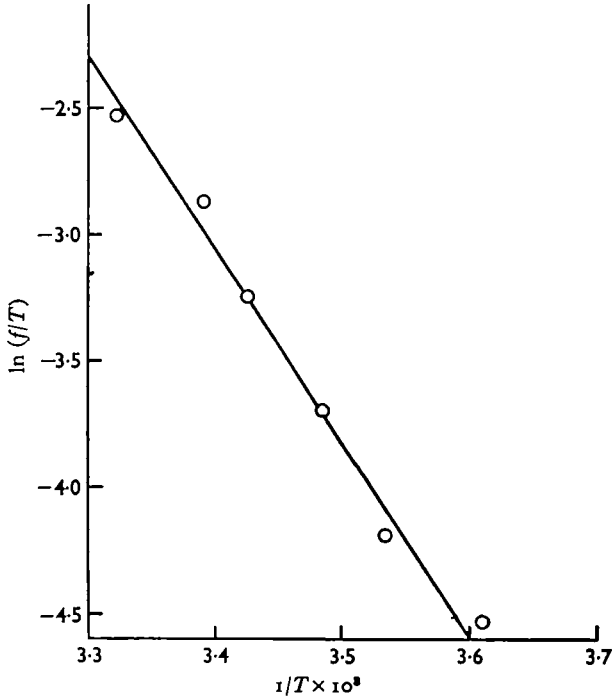


Fig. 1. Graph to show the relation between frequency of flagellar beat (f) and absolute temperature (T) in the uniflagellate, *Strigomonas oncopelti*. This linear presentation allows the calculation of an activation energy, ΔH^\ddagger , and activation entropy, ΔS^\ddagger (see text).

From Table 1 the ratios of beat frequency (f) to the absolute temperature (T) were calculated and a graph of $\ln(f/T)$ against $1/T$ plotted (Fig. 1). The graph is linear within the limits of experimental error showing that the thermodynamic relation between frequency and temperature derived previously (equation 5) holds for the movement of *S. oncopelti*. The slope and intercept of the regression line of $\ln(f/T)$ upon $1/T$ are respectively 7.75° K. and 23.3 leading to a value of 15.4 kcal. mole⁻¹ for ΔH^\ddagger , and of

–0.9 entropy units (e.u.) for ΔS^\ddagger . The standard deviation in ΔH^\ddagger is 0.7 kcal. mole⁻¹ and in ΔS^\ddagger , 2.5 e.u. Since the numerical value of S is less than the estimated standard deviation it is possible that the actual value of ΔS^\ddagger is zero; if this were so, then for the standard deviation quoted, about 36% of the estimated values for ΔS^\ddagger would be expected in any case to give a value at least as low as –0.9 e.u.

DISCUSSION

In the literature on flagellar motility we have been able to find few quantitative observations of thermal effects that would serve as a comparison with the activation values given above. Sleight (1956) has determined the variation with temperature of the frequency of beat of *Stentor* cilia and, using the Arrhenius equation, gives a value for E of 11.3 kcal./mole. We have taken the values from his published graph and replotted them according to our equation (5): we find a value for ΔH^\ddagger of 9.3 kcal. and ΔS^\ddagger is about –20 e.u. Hoffmann-Berling (1955), working with glycerol-extracted models of locust sperm, found a variation of frequency with temperature from which we deduce a ΔH^\ddagger of about 10 kcal. and a ΔS^\ddagger of about –21 e.u. The thermal dependence found by the above authors is thus very similar, but while our value for ΔH^\ddagger , 15.4 kcal., is of the same order as theirs, the activation entropy of almost zero which we find for *S. oncopelti* is somewhat different from their common value of about –20 e.u.

To put these values in perspective, we may perhaps compare them with the values calculated by Burge & Elliott (1963) from published data on the isometric tension decay of tortoise muscle and the isotonic elongation of the stomach-wall muscle of rabbit. The respective values were $\Delta H^\ddagger = 19$ kcal. and $\Delta S^\ddagger = 23$ e.u., and $\Delta H^\ddagger = 20$ kcal. and $\Delta S^\ddagger = 21$ e.u. Finally, since it is recognized that ATP plays a part in flagellar motion (e.g. Tibbs, 1962) we give the values quoted in Laidler (1958) for the formation and the breakdown of ATP-ATPase complexes: these are respectively $\Delta H^\ddagger = 20.4$ kcal., $\Delta S^\ddagger = 44$ e.u. and $\Delta H^\ddagger = 12.4$ kcal., $\Delta S^\ddagger = -8$ e.u.

Since these activation parameters refer only to a transition state in a chemical reaction, they tell us nothing about the net changes in the reaction and give no guide, for instance, to the work available for flagellar contraction. All the values given are, however, of an order which is consistent with the reactions of ions in solution, and the values of ΔH^\ddagger are smaller than those concerned in the breaking of covalent bonds, which have energies of the order of 50 kcal/mole.

It is difficult to assign a significance to values of ΔS^\ddagger in solution, as they are influenced by so many environmental factors (pH, ionic strength, dielectric constant, charge or polar nature of the reactants, etc.; see Glasstone *et al.* (1941), p. 405 *et seq.*). Reactions between ions with charges of the same sign tend to be slower than 'normal'* and this is reflected in a negative contribution to the entropy of activation; if the electronic charges of the ions concerned are z_a , z_b , the contribution for an aqueous medium is given (*loc. cit.*) by

$$\Delta S^\ddagger \approx -10z_a z_b \text{ e.u.}$$

The value, $\Delta S^\ddagger = -20$ e.u., given by the data of Sleight (1956) and Hoffmann-Berling (1955) is thus consistent with the picture of ions of the same sign reacting in solution.

* 'Normal' reactions are those in which the rate constant agrees with the prediction of a simple collision theory of reaction rates (Glasstone *et al.* 1941, p. 5.)

The fact that both these authors find an increase of frequency on increasing the ionic strength of the medium is indicative of the same situation. Theoretically (Glasstone *et al.* 1941) one would expect to find a relation of the form

$$\log_{10} f = \text{constant} + 1.02 z_a z_b \sqrt{\mu},$$

where f is the rate constant (equated in this context with the frequency of flagellar beat) and μ the ionic strength of the medium. On plotting the observations of Hoffmann-Berling (1955) in this form one does, in fact, obtain a straight line of positive slope, implying that z_a and z_b have the same sign. The activation entropy of almost zero that is observed with *S. oncopelti* does not appear to fit in the picture suggested above and it is an open question whether this value is due to a fortuitous combination of environmental factors or whether it has a special significance for the flagellar motion. Further experiments with varying ionic strength may shed light on this.

Although we have been able to deduce activation parameters by physical observation of flagellar activity, one can only say that they pertain to some chemical reaction which has a limiting effect on the contraction-relaxation cycle and which thus controls the frequency of beat. Any further clarification of the significance of these parameters will depend on the emergence of an acceptable model of flagellar activity and the identification within the cycle of the rate-limiting process.

SUMMARY

The flagellar beat frequency of *Strigomonas oncopelti* was found by cinémicrophotography at temperatures in the range 4–45° C. The thermal dependence is described by an activation enthalpy (ΔH^\ddagger) of 15.4 kcal./mole and an activation entropy (ΔS^\ddagger) of –1 e.u. ΔH^\ddagger is comparable with published data on cilia and glycerol-extracted sperm. Values of ΔS^\ddagger (–20 e.u.) deduced from the literature suggest a rate-limiting reaction between ions of like charge although the present value does not support this idea.

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