

SEXUAL INDUCTION IN *VOLVOX CARTERI*

A Quantitative Study

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INTRODUCTION

The study of the dependence of reactions on the concentration of various compounds is often of great value in determining reaction mechanisms. In chemical kinetics, it is often found that, according to van't Hoff's rule, the order of dependence of a reaction on the concentration of some compound involved in that reaction is equal to the number of molecules of the compound involved in each reaction event (Pannetier and Sanchay, 1967). This common equivalence has been used in biological as well as nonbiological studies. For example, the finding that the number of bacteriophage plaques is a linear function of bacteriophage concentration was used to conclude that only a single particle is required to produce a plaque (Ellis and Delbrück, 1939). Similarly, the linear relationship between X-ray dose and frequency of mutation supports the view that one X-ray photon can produce a mutation (Schultz, 1936). Chu, Giles, and Passano (1961) showed that the frequency of single-break chromosomal aberrations is a linear function of X-ray dose. However, aberrations requiring two breaks were found to occur as the square of the dose. Thus, each break appears to require only a single X-ray photon and rearrangements requiring two breaks show a second-order dependence on X-ray dose. Gall (1963) was able to infer the strandedness of lampbrush chromosomes from the kinetics of appearance of breaks upon DNase digestion.

This report includes a quantitative analysis of the induction of sexuality in *Volvox carteri f. nagariensis*. Sexuality in this species is induced by a proteinaceous inducer (Starr, 1968 and 1970). The inducer acts to cause the asexual reproductive cells or gonidia to cleave to form

sexual individuals rather than cleaving to form asexual individuals. Thus, the induction involves a shift in development from the asexual pathway to the sexual pathway. The inducer has been partially purified by Starr (1970) and acts at a concentration of about 10^{-15} M. The extremely low concentration of inducer required for induction suggests that perhaps only one or a small number of molecules of inducer are involved in inducing a gonidium to develop sexually.

Results reported below show that at low levels of induction, the frequency of induction goes as the square of the inducer concentration. These results support the view that only two molecules of inducer are required to induce a gonidium to develop sexually. An inducer unit is defined such that one inducer unit per milliliter yields 50% induction. A simple formula is developed allowing one to calculate inducer titer (in units per milliliter) from the fraction induction produced by the inducer.

MATERIALS AND METHODS

The inducer of sexuality was prepared from male strain 69-1b according to the procedure of Starr (1970). The inducer was assayed on the genetically female HK10 strain of *Volvox carteri* as described by Starr (1970). In these experiments, the induced and noninduced gonidia are scored by allowing them to cleave and grow up to mature individuals and scoring those individuals as to whether they are sexual (from induced gonidia) or asexual (from noninduced gonidia). In the assay procedure, the HK10 strain is grown in bubble flasks. Individuals containing young, recently inverted daughters are placed into 18 × 150-mm tubes containing 10 ml of *Volvox* medium with various amounts of inducer added (Starr, 1970). After the gonidia of the daughter colonies have cleaved to form new individuals, the new individuals can be scored.

The results were used to calculate b in the equation $y = ax^b$ where y is either fraction induction or the ratio of induced gonidia to noninduced gonidia, and x is the amount of inducer in arbitrary units. b , which represents the apparent order of dependence, was calculated by using the least squares power curve program (no. 1001-2-ST3) on a Wang 600 programmable calculator.

RESULTS

According to the arguments developed in the introduction, if the induction of a gonidium to develop sexually requires a small number of inducer molecules at low levels of induction one would expect:

$$\text{fraction induction} = k (\text{inducer})^N. \quad (1)$$

Where fraction induction is the fraction of gonidia induced to develop sexually, k is a constant and (inducer) is the concentration of inducer expressed in arbitrary units. N is the order of dependence of induction on inducer concentration and would be expected to be equal to the number of inducer molecules required for the induction of a gonidium. If Eq. 1 holds, it follows that a log-log plot of fraction induction vs. inducer concentration will follow a straight line with a slope of N .

Fig. 1 shows the results of a typical study of induction as a function of inducer concentration. The results from less than 1% induction to about 30% induction closely follow a straight line. In the experiment shown in Fig. 1, the slope is about 1.8. Five separate experiments were performed in which estimates for N were determined by a least squares power curve program on a Wang 600 calculator. The values obtained for N ranged from 1.75 to 2.25. Thus, all values obtained were close to 2. These results support the view that induction of a gonidium to develop sexually involves only two molecules of inducer.

Although Eq. 1 with $N = 2$ is a good representation of the induction obtained where the induction is fairly low ($\leq 30\%$), it cannot be a good representation with high levels of induction. This can be concluded because induction cannot exceed 100%. Consequently, induction cannot increase indefinitely with the square of the inducer concentration. It would be desirable to develop an equation which would closely represent amount of induction both at low and higher levels of induction. One possible approach is suggested by the treatment of Chadwick et al. (1970)

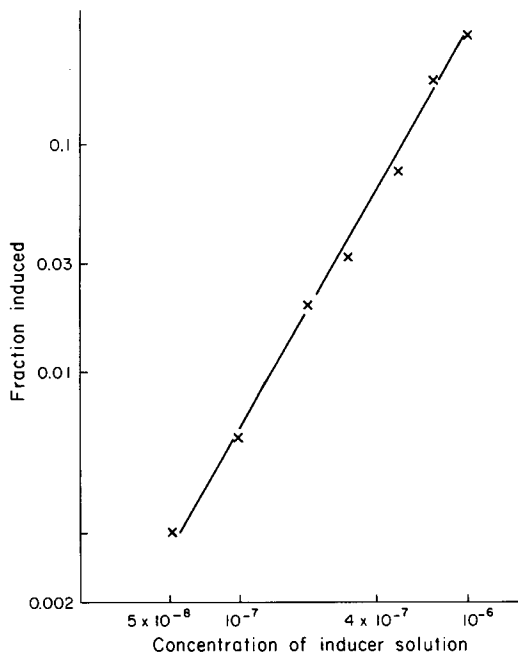


FIGURE 1 Log-log plot of fraction induction vs. inducer concentration. The concentration of inducer is expressed in terms of the concentration of a particular solution of inducer after dilution. The slope of the line drawn is 1.8.

on the binding behavior of the λ -repressor. Using an approach analogous with that of these authors, it was decided to see if the following equation might hold:

$$\frac{\text{induced gonidia}}{\text{noninduced gonidia}} = k' (\text{inducer})^N \quad (2)$$

Where k' is a constant. Eq. 2 will reduce to Eq. 1 under conditions where the fraction induction $\ll 1$. This follows because at low levels of induction:

$$\begin{aligned} \text{fraction induction} &= \frac{\text{induced gonidia}}{\text{total gonidia}} \\ &\approx \frac{\text{induced gonidia}}{\text{noninduced gonidia}} \end{aligned} \quad (3)$$

If Eq. 2 holds, it follows that a log-log plot of the ratio of induced to noninduced vs. concentration of induction should give a straight line with a slope of N . The data shown in Fig. 2 closely follows a straight line with a slope of 2. Least square estimates for N in five separate experiments range from 1.90 to 2.26. It follows that N in Eq. 2 is very close to or equal to the integer 2,

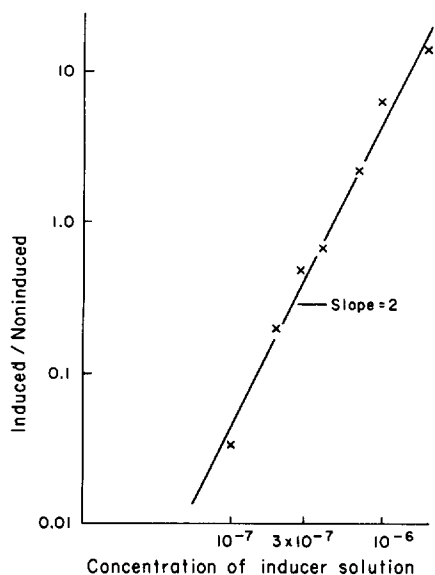


FIGURE 2 Log-log plot of induced/noninduced vs. inducer concentration. The concentration of inducer is expressed in terms of the concentration of a particular solution of inducer after dilution. The solution of inducer used in this experiment was different from that used in Fig. 1, thus explaining the differences in inducer activity.

or:

$$\frac{\text{induced gonidia}}{\text{noninduced gonidia}} = k' (\text{inducer})^2 \quad (4)$$

In *Volvox carteri*, under optimal growth conditions, excess inducer yields 95–100% induction. Under such conditions, Eq. 4 gives a good fit to quantitative induction studies from less than 1% induction to as high as 90% induction. This fit allows one to use Eq. 4 to estimate titer of inducer. If one defines a unit of inducer as the concentration per milliliter yielding 50% induction, then it follows that $k' = 1$ when inducer concentration is expressed as inducer units per milliliter. Where (inducer) is expressed in inducer units per milliliter:

$$(\text{inducer}) = \sqrt{\frac{\text{induced gonidia}}{\text{noninduced gonidia}}} \quad (5)$$

Eq. 5 then allows one to calculate inducer titer from the amount of induction obtained.

DISCUSSION

At low levels of induction, the fraction induction of sexuality in *Volvox carteri* f. *nagariensis* is pro-

portional to the square of the inducer concentration. Such a second-order dependence on inducer concentration supports the view that each induction event involves only two molecules of inducer. Thus, the change in developmental pathway involved in the induction of a gonidium would appear to be determined by a molecular event involving only two molecules of inducer. One possible interpretation of these results is that induction requires the action of two inducer molecules at two different sites. Alternatively, a dimer of the form of the inducer predominant at low concentrations might be required at a single site. Such low concentrations were used in the experiments reported above. The action of such a dimer would be expected to show a second-order dependence on inducer concentration in much the same way that λ -repressor (dimer) binding to DNA shows a second-order dependence on repressor concentration (Chadwick et al., 1970).

The second-order dependence of induction in *V. carteri* does not necessarily reflect that found in other species. Data are available in other species of *Volvox* although no other quantitative studies analogous to those reported here have been performed (Darden, 1966; Kochert, 1968; McCracken and Starr, 1970). Of special interest are the studies on *Volvox rousselletii* (McCracken and Starr, 1970) in which induction appears to show a very high-order dependence on inducer concentration. In the same paper it was reported that the *V. rousselletii* inducer came off in two major peaks from gel filtration chromatography, one with an apparent molecular weight of less than 12,000 and the other with an apparent molecular weight of over 70,000. It is tempting to suggest that the larger molecule is an aggregate of the smaller molecule and the oligomeric aggregate is required for each induction event. Such a suggestion would help explain the high order of dependence of induction in *V. rousselletii*.

A unit of inducer concentration is defined above such that one inducer unit per milliliter is the concentration required to give 50% induction. Using Eq. 5, one can calculate inducer titer from the amount of induction obtained. This should prove of great value in the purification and characterization of the inducer.

SUMMARY

Studies of the fraction of sexual induction as a function of inducer concentration in *Volvox car-*

teri f. nagariensis reveal that induction shows a second-order dependence on inducer concentration. The second-order dependence supports the view that only two molecules of inducer are required to induce a gonidium (asexual reproductive cell) to develop sexually. A unit of inducer concentration is defined. An equation is derived allowing one to calculate inducer titer from the amount of induction obtained.

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