# THE ACTION OF IREHDIAMINE A ON ESCHERICHIA COLI: INHIBITION OF THE MEMBRANE RESPONSE AT LOW TEMPERATURES

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Lowering the incubation temperature reduces the rate of loss of potassium from cells of Escherichia coli produced by a given concentration of the steroidal diamine, Irehdiamine A. This effect can be explained as partly a reduction in the binding of the steroid, and partly a reduction in cooperative effects in the cell membrane which are involved in potassium loss.

The steroidal diamine, Irehdiamine A (IDA), is one of a number of amines which act on cell membranes to produce leakage of ions and inhibit transport of ions and small molecules (1,2) and other energy dependent processes. Its action on membranes of intact E. coli cells has been characterized as a two stage process (2). The initial interaction of the steroid with the cells occurs in a 3-4 minute period (at 30°-35°C) during which there is an accelerated rate of loss of potassium, but no loss of cell viability. After this time cell death begins, the rate of potassium loss falls off, and the binding characteristics of the cells for the steroid change. During the initial period of rapid potassium loss the rate of potassium exit varies sigmoidally with increasing IDA concentration, indicating a cooperative interaction between IDA molecules in producing potassium loss. Binding of the steroid shows cooperativity over the same concentration range, but this appears to depend on events occurring later than the initial loss of potassium (2)

The present report concerns the effect of lowered incubation temperatures on the initial interaction between IDA and the cells. Lowered temperatures reduce the initial rate of potassium loss, the amount of steroid bound, and the extent of cooperation between steroid molecules.

## MATERIALS AND METHODS

The bacterial strain used was W1895 (HfrC, met⁻). Potassium loss was measured with the radioisotope $^{42}K$ using procedures which have been described in detail elsewhere (1,3). Briefly, cells were grown in dilute tryptone broth (1,2) in the presence of $^{42}K$ at 37°C and centrifuged and resuspended in the same medium at the appropriate temperature. Irehdiamine A was added within a few minutes after the temperature shift. Intracellular potassium was determined by filtering samples taken before and at intervals after the addition of IDA through millipore filters, and measuring the $^{42}K$ retained on the filters in a gas-flow counter. Binding of IDA was measured using $^{3}H$-dihydro IDA by centrifugation, and by millipore filtration (2).

## RESULTS

Lowering the temperature of incubation from 37°C to 1°C reduces progressively the initial rate of potassium loss produced by a given IDA concentration (Fig. 1). The $Q_{10}$ for this effect is approximately 2 (Table 1) suggest-

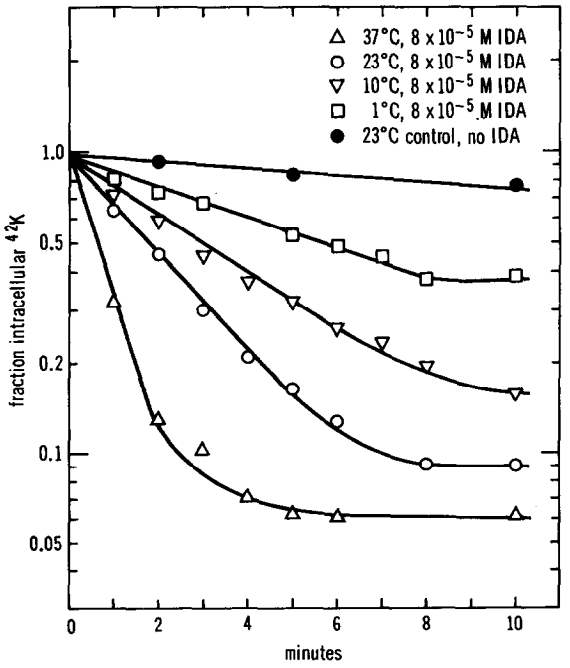


Figure 1. Temperature dependence of IDA-induced potassium loss.

Table 1

Comparative Temperature Dependence of IDA-Induced Potassium Loss and IDA Binding

| Temperature (°C) | Rate of Loss[a] (-k) | mM bound/$10^{12}$ cells[b] Estimated by: Centrifugation | Filtration |
|---|---|---|---|
| 37 | .99 | $1.3 \times 10^{-2}$ | $6.3 \times 10^{-3}$ |
| 23 | .38 | $7.9 \times 10^{-3}$ | $3.1 \times 10^{-3}$ |
| 10 | .23 | $6.5 \times 10^{-3}$ | $< 2 \times 10^{-3}$ |
| 1 | .12 | $5 \times 10^{-3}$ | $< 2 \times 10^{-3}$ |

(a) measured at $8 \times 10^{-5}$ M
(b) measured at $5 \times 10^{-5}$ M

Initial rate of potassium loss calculated from the data of Fig. 1: $(^{42}K_{(t)}/^{42}K_{(o)} = e^{-kt})$. Binding was measured 15 min after adding $5 \times 10^{-5}$ M IDA containing $5 \times 10^{-6}$ M $^{3}$H-dihydro-IDA to cells prepared as described in Fig. 1. At 37°C the amount of IDA bound after 15 min may be reduced slightly compared to that bound initially (2).

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ing that it might be accounted for solely by a decrease in the kinetic energy of the IDA molecules in solution (4) and a consequent reduction in their interaction with the cells. Although the binding of IDA is reduced over this same temperature range (Table 1) it is not reduced as greatly as the rate of potassium loss, indicating that bound steroid is not as effective in inducing potassium loss at lower temperature. This conclusion is supported by the results described below.

The relative initial rates of potassium loss produced by various concentrations of IDA at 37°, 23°, 10° and 1°C are shown in Fig. 2. The shape of the curves appears sigmoid as has been reported elsewhere (2), and indicates cooperation between IDA molecules in producing potassium loss. Several characteristics of these curves which are altered by decreasing temperatures

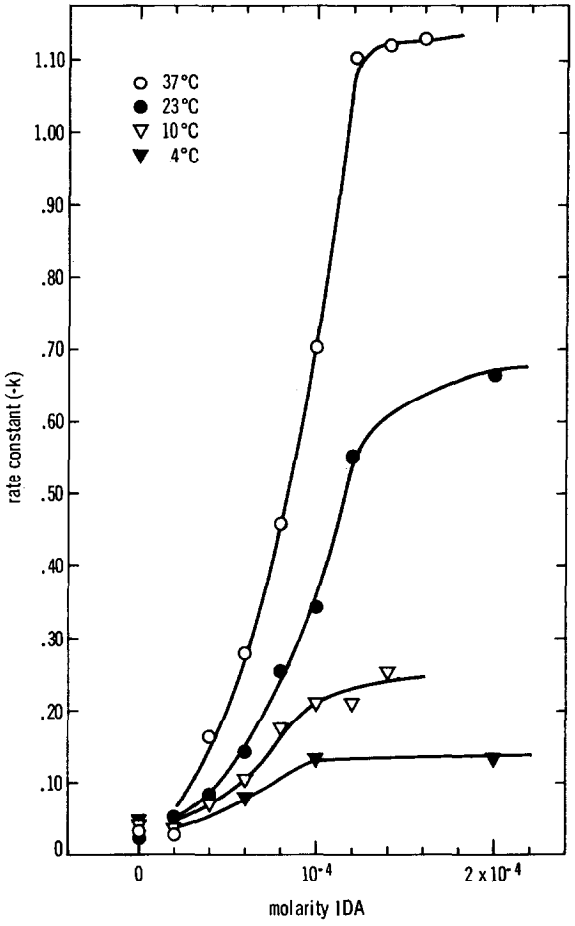


Figure 2. Concentration dependence of IDA-induced potassium loss. Kinetics of loss was measured as described in the legend to Figure 1. Initial rate of loss of potassium was calculated as for Table 1.

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should be noted: 1) The lowest concentration that produces significantly accelerated potassium loss increases, 2) the maximum rate of loss decreases and, 3) the increase in rate per increment in steroid concentration is reduced. The raising of the 'threshold' IDA concentration, and the decrease in maximum rate are consistant with a reduced binding, and also may reflect a reduced

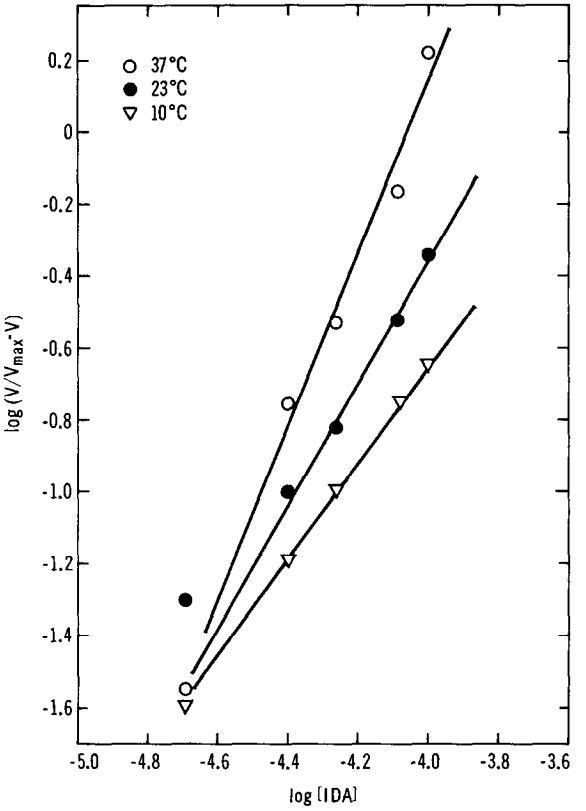


Figure 3. Hill plot of some of the data in Fig. 2.

effectiveness of bound steroid in producing potassium loss.* The third characteristic is indicative of a reduction in cooperation between steroid molecules and is shown more clearly in Fig. 3. Here the data of Fig. 2 for 37°, 23°, and 10°C, and from 2 x $10^{-5}$ to $10^{-4}$ molar, are presented in a Hill plot. The slopes of the curves obtained at 37°, 23° and 10°C are greater than one; however the slopes at 23° and 10°C become progressively less than that at 37°C. This indicates a decrease in the cooperative interaction between steroid mole-

*This interpretation is complicated by the fact that we have experienced difficulty reproducing a given effect at a given concentration. An extreme example of this problem is illustrated by the rates seen at 8 x $10^{-5}$ M in the experiments shown in Figs. 1 and 2. Possible explanations for this have been discussed elsewhere (2). However, it is clear that the qualitative result of a progressive reduction in the rate of loss as the temperature is lowered is reproducible. Interpretations based on comparative data obtained within an experiment such as shown in Fig. 2 are not subject to this complication.

cules, the degree of cooperativity being progressively reduced as the temperature is lowered.

## DISCUSSION

We have observed that the rate of potassium loss at a given IDA concentration is reduced as the temperature is lowered over the range from 37°C to 1°C. This effect at a given concentration might be accounted for by the lowering of the kinetic energy of the molecules in solution such that fewer of them interact with the cells so as to initiate potassium loss. However, measurements of steroid binding at the lower temperatures suggest that the binding is not reduced in proportion to the reduction in the rate of loss, and that events following IDA binding leading to potassium loss may also be affected at low temperatures. Strong support for this interpretation comes from the reduction in cooperativity between steroid molecules observed as the temperature is lowered. Since IDA can act on membrane vesicles as well as intact cells (2), and since potassium loss probably reflects a membrane alteration, it is likely that this reduction is the consequence of an alteration in the cell membrane. Such an alteration could result from a change in the physical state of membrane lipids such as has been reported for Mycoplasm lawdlii (5).

The cooperative nature of the action of the steroid molecules in inducing potassium loss can be explained formally on the basis of a model such as that proposed by Changeux et al. (6), which envisions cooperative interactions between lipo-protein subunits within the membrane. However, there is no reason at present to favor this over some other mechanism (for example, a change in only the lipid phase of the membrane) which might underly the observed cooperativity. What seems clear from these data is that the initial interaction of the steroid with the cells at the higher temperatures must involve a process by which the effect of attack by the steroid molecules on the membrane is to increase the likelihood, or effectiveness, of further attack by additional molecules, and that lowered temperatures inhibit this process.

A delay in the initiation of potassium loss following colicin K treatment has been observed over this same temperature range, suggesting that, whatever the nature of the process underlying the observed cooperativity, it also may play a role in an intermediate step in colicin action (3).

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