Bistable Bacterial Growth Rate in Response to Antibiotics with Low Membrane Permeability

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(Received 6 July 2006; published 19 December 2006)

We demonstrate that growth rate bistability for bacterial cells growing exponentially at a fixed external antibiotic concentration can emerge when the cell wall permeability for the drug is low and the growth rate sensitivity to the intracellular drug concentration is high. Under such conditions, an initially high growth rate can remain high, due to dilution of the intracellular drug concentration by rapid cell volume increase, while an initially low growth rate can remain low, due to slow cell volume increase and insignificant drug dilution. Our findings have implications for the testing of novel antibiotics on growing bacterial strains.

DOI: 10.1103/PhysRevLett.97.258104 PACS numbers: 87.17.Ee, 87.17.Aa

Bacterial infection is a major cause of human suffering and death. Therefore, design of new antibiotics and development of more efficient ways to deliver already existing antibiotics are mandatory. Antibiotics with low membrane permeability have been regarded as clinically less interesting, although they often are very efficient when they have reached their intracellular targets. The current Letter addresses this class of antibiotics and how its members could become clinically more useful by taking into account some striking relations between bacterial growth and intracellular drug concentration. We develop a general dynamic model for the intracellular concentration of these antibiotics and show that the bacterial growth rate will be bistable in response to the antibiotic if the membrane permeability is sufficiently small and the intracellular response to the antibiotic is sufficiently sensitive.

The total concentration, \( a \), of an antibiotic in a bacterial cell depends on its net flow over the membrane, \( J_{\text{mem}} \), and its dilution by cell volume growth, \( J_{\text{gr}} \). When the drug transport into and out from the cell is passive [3], we model the time evolution of \( a \) by the ordinary differential equation

\[
\frac{da}{dt} = c(a_{\text{ex}} - a_{\text{fr}}) - \frac{\mu a}{J_{\text{mem}}} - \frac{\mu a}{J_{\text{gr}}}
\]

for the in-flow and the out-flow: \( J_{\text{mem}} = c_1(a_{\text{ex}} - c_2a_{\text{fr}}) = c_2(c_1c_2^{-1}a_{\text{ex}} - a_{\text{fr}}) \). Here, \( c_2 \) replaces \( c \), and \( c_1c_2^{-1} \) is a scale factor for the external antibiotic concentration \( a_{\text{ex}} \) in Eq. (1).

Since the growth rate \( \mu \) is a function of the concentration of bound drug, \( a-a_{\text{fr}} \), it follows that also \( \mu \) is a function of the total intracellular drug concentration \( a: \mu = \mu(a) \). We will show that biologically motivated constraints on the functions \( a_{\text{fr}}(a) \) and \( \mu(a) \) in Eq. (1) in conjunction with a sufficiently low permeability \( c \) lead to bistability for a range of external drug concentrations \( a_{\text{ex}} \). Our analysis deals with stationary states during exponential growth and the approach to such stationary states; the treatment can in a straightforward manner be extended to cases in which the growth rate varies with external conditions or in which different bacteria experience different growth conditions, like in biofilms [4].

We will first make the natural assumption that the real valued and continuous function \( a_{\text{fr}} = a_{\text{fr}}(a) \) is smaller than the total intracellular concentration \( a \), i.e., \( a_{\text{fr}} \leq a \), and that both the free and bound intracellular drug concentrations increase with increasing \( a \), so that \( da_{\text{fr}}(a)/da = a'_{\text{fr}} \leq 1 \). We further assume that \( a_{\text{fr}} \) is concave in \( a \) (\( a'_{\text{fr}} \geq 0 \)), since \( a'_{\text{fr}} \) is expected to increase as the target binding sites become saturated. When \( a \rightarrow \infty \), all binding sites are occupied so that \( a'_{\text{fr}} \rightarrow 1 \). Some functions, \( a_{\text{fr}}(a) \), that fulfill the criteria are illustrated in Fig. 1(a) and the corresponding convex membrane flow functions \( J_{\text{mem}}(a) = c(a_{\text{ex}} - a_{\text{fr}}(a)) \) in Fig. 1(b). Here, \( J_{\text{mem}}(0) = ca_{\text{ex}} \), the slope of \( J_{\text{mem}}(a) \) is determined by \( -ca'_{\text{fr}} \), and \( J_{\text{mem}}(a) \) is zero when \( a_{\text{fr}}(a) = a_{\text{ex}} \).

The exponential growth rate \( \mu = \mu(a) \) is assumed to be a continuous, finite, positive function [Fig. 1(c)] that decreases monotonically with increasing \( a \), such that the dilution flow \( J_{\text{gr}} = a_{\mu} \leq a_{\text{fr}}(a) \). We will, finally, assume that \( d(J_{\text{gr}})/da < 0 \) in some interval of \( a \), implying that here \( J_{\text{gr}} \) decreases more rapidly than \( 1/a \) with increasing \( a \). If, to give an example, the growth rate can be modeled by a Hill function, then \( J_{\text{gr}} = a_{\mu} = a_{K_{\text{H}}}/(1 + (a/K_{\text{H}})^m) \), and the requirement is that \( m > 1 \), meaning that in this special case \( a_{\mu} = 0 \) in the...
of either steady state point. Under the conditions stated above, \( J_{gr} \) will always have an inflection point \( J''_{gr} = 0 \) to the right of its maximum, since \( J''_{gr} = 0 \) also in the limit \( a \to \infty \). Furthermore, by adjusting the external drug concentration \( a_{ex} \), it will always be possible to create an intersection of the \( J_{gr} \) and \( J_{mem} \) curves at the inflection point of \( J_{gr} \), which corresponds to an unstable steady state. By lowering the permeability parameter \( c \), it will, finally, always be possible to create additional intersections of the \( J_{gr} \) and \( J_{mem} \) curves to the left of the maximum and to the right of the inflection point of \( J_{gr} \), both of which fulfill the criteria of a stable steady state [Fig. 1(f)]. Accordingly, the emergence of bistability depends critically on the membrane permeability \( c \).

To further illustrate the importance of the permeability parameter \( c \), we will derive an expression for the highest value of \( c \) that is compatible with bistability for a simple and yet realistic model system, in which the antibiotic inhibits ribosome function and thereby reduces the growth rate \( \mu \). It can be noted that there is a plethora of clinically useful antibiotics with the ribosome as their target [6,7]. In this model, there is a fixed total concentration, \( r \), of ribosomes in the cell, each of which has a single binding site for the drug with the equilibrium dissociation constant \( K_d \), irrespective of the state of the ribosome. We assume rapid intracellular equilibration of drug-bound and drug-free ribosomes with concentrations \( (a - a_{fr}) \) and \( r - (a - a_{fr}) \), respectively. The functional relation \( a_{fr}(a) \), introduced in Eq. (1), between free and total intracellular concentration of the drug is then given by

\[
a_{fr}[r-(a-a_{fr})]=K_d(a-a_{fr})=a_{fr}(a) = \frac{1}{2}\left(a-K_d-r+\sqrt{4aK_d+(K_d+r-a)^2}\right).
\]

On the assumption that the total rate of protein synthesis and the growth rate \( \mu \) are proportional to the concentration of drug-free ribosomes, the functional dependence \( \mu = \mu(a) \) introduced in Eq. (1) is given by [8,9]

\[
\mu(a) = \frac{v_m[r-[a-a_{fr}(a)]]}{\rho_0},
\]

For \( E. \ coli \) cells growing in a rich medium, the average rate of translation \( v_m = 20 \) s\(^{-1} \), \( r = 10 \) \( \mu \)M and the concentration of amino acids in peptide chains \( \rho_0 = 2 \) M [10]. As long as \( K_d < r \), \( J_{gr} = a\mu(a) \) fulfills the above stated criteria for potential bistability. The \( J_{mem} \) and \( J_{gr} \) curves that result from this model with \( c = 0.0001 \) s\(^{-1} \), \( a_{ex} = 2 \) M, and \( K_d = 10 \) nM demonstrate the existence of bistable growth rate [Fig. 2(a)].

How the region of bistability depends on the \( c \) and \( a_{ex} \) parameters is illustrated in Fig. 2(b). Here, the total intracellular drug concentration \( a \) is plotted as a function of the external drug concentration \( a_{ex} \) for three different values of
The critical value \( c^* \) is only a single steady state when \( c > c^* \), while there is only one stable state when \( c < c^* \). The critical value \( c^* \) is given by the expression

\[
c^* = \frac{v_m}{27 \rho_0} \frac{(r - K_d)^3}{r K_d} = \frac{v_m}{27 \rho_0} \frac{r^2}{K_d} \quad \text{when} \quad K_d \ll r. \tag{4}
\]

The critical \( c^* \) value in Eq. (4) was derived by analyzing the solutions to the equation \( J_{\text{mem}}(a) - J_g(a) = 0 \) for varying \( c \)-values. For small \( c \)-values, there are two real and positive roots for \( a \), which indicates bistability for some \( a_{\text{ex}} \)-values. For large \( c \)-values, in contrast, there are only complex valued roots for \( a \), suggesting lack of bistability for all \( a_{\text{ex}} \)-values. The \( c \)-value at which the solutions switch from two real, positive to complex valued \( a \)-roots is \( c^* \). For the parameter values in Fig. 2, \( c^* \approx 0.037 \text{ s}^{-1} \).

The bistability leads to hysteresis in the growth rate, as demonstrated for \( c = 0.0001 \text{ s}^{-1} \) in Fig. 3(a). The two solid curved lines illustrate the steady state growth rates as functions of the external drug concentration, for bacterial populations initially started out with small (upper curved line) or large (lower curved line) intracellular drug concentration. It is seen how these different initial conditions lead to two different growth rates in the bistable region of \( a_{\text{ex}} \) (0.6 \( \mu \text{M} \)-3 \( \mu \text{M} \)). The dashed curved line illustrates the unstable steady state solution. The arrows show the direction in which the growth rate changes if the system is initialized in different regions of the \((a_{\text{ex}}, \mu)\)-space.

It may take very long times to establish steady state growth in new media with different external drug concentrations, in particular, for cells initially containing a large intracellular drug concentration [Fig. 3(b), dashed lines]. For bacteria initially growing very slowly in a medium with 10 \( \mu \text{M} \) drug concentration, it takes about 50 hours to attain the fast steady state growth to the left of the bistable \( a_{\text{ex}} \) region. For bacteria initially growing rapidly in a medium with 0.1 \( \mu \text{M} \) drug concentration, it also takes quite a long time to attain the slow growth to the right of the bistable region (solid lines). These long times to establish steady state rates of growth for bacteria with different initial intracellular drug concentrations give rise to an experimental problem. That is, to experimentally identify growth bistability and hysteresis, it is essential to allow the growth rates to come near their steady state values as the cell populations are shifted from environments with large or small to an intermediate current drug concentration as in Fig. 3(b). Since, however, the steady state relaxation times are so long, pleiotropic drug effects related to mutations or other effects secondary to their primary inhibition of the target obscure the analysis of the long time (>8 h) growth properties in a fixed external antibiotic concentration (L. Kosenkranius and T. Tenson, unpublished observations).

In conclusion, we have shown how the presence of antibiotics that diffuse slowly over the bacterial cell wall may lead to bistability in intracellular drug concentration and bacterial growth rate. We have also shown hysteresis effects, in that bacteria subjected to increasing external drug concentrations will display larger growth rates in the bistable region than bacteria subjected to decreasing external drug concentrations. We have outlined the general criteria for when drug-dependent growth bistability can arise. We have, in particular, shown that there must be an interval of the intracellular drug concentration \( a \) in which the growth rate is reduced more strongly than 1/\( a \). We have also emphasized the importance of low cell wall permeability for bistability to emerge. In addition, we used a
simple model system, where the drug binds to ribosomes and inhibits protein synthesis, to derive the critical membrane permeability value below, which bistability exists for a range of external drug concentrations.

In the present deterministic analysis, it is assumed that all cells in the population respond identically to the antibiotic drug. In systems with a mixture of slow and fast growers, the latter will rapidly outnumber the former. Variable growth rates are expected in the clinically relevant situation of bacterial biofilm formation. However, relating this interesting situation to the present theory will require a more detailed experimental analysis since the external drug concentration may vary in different regions of the biofilm due to its complex diffusion properties.

Nongenetic biochemical memory effects that are mediated by other means than the rate of growth have previously been described for gene-regulatory and signaling circuits with multiple steady states [11–20]. Particularly interesting for the present work is the persistence phenomenon [11,20], where a fraction of a bacterial population escapes antibiotic treatment by transiently residing in a nongrowing state. This mechanism is thus the opposite of the phenomenon described in this Letter.

The starting condition for the growth of a bacterial culture may have profound effects on the growth rate, seen also in the transient phase before steady state has been established (Fig. 3). The Minimal Inhibitory Concentration (MIC) value is commonly used to characterize how susceptible a bacterial strain is to an antibiotic [21]. The present finding that responses of bacterial populations to antibiotics may be growth history dependent could explain why, in some cases, MIC-estimates are hypersensitive to experimental conditions [22]. When low-permeability drugs are tested, our theory reveals that conditions under which a bacterial growth culture has not reached a high growth rate when the drug is added may result in a lower MIC value compared to the MIC value obtained for the very same culture when exposed under maximal growth rate conditions. Normally, MIC values are obtained for cultures growing in rich media with high growth rates, while the growth rates during clinical infections often are very low, suggesting that MIC values should be estimated for bacteria growing in poor, rather than in rich, media. This is particularly relevant for the testing of new antibacterial compounds, which may be discarded as ineffective from rich medium data although they may turn out to be clinically useful.

We thank Diarmaid Hughes, Johan Paulsson, Martin Lovmar, Hans Bremer, and Arvi Jöers for fruitful discussions. This work was supported by the Swedish Research Council (M.E), The Medical Research Council (M.E), Knut och Alice Wallenbergs Stiftelse (J.E.), and the Wellcome Trust International Grant (No. 070210/z/03/z) (T.T).

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