

Hybolites: novel therapeutical tools based on stochastic automata

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Abstract

Bacterial disease is a major cause of suffering and the scarcity of new molecules that can act on bacteria is a major problem. New strategies for developing such molecules might be based on recent concepts in microbiology. Hyperstructures are large assemblies of molecules and macromolecules that perform functions such as DNA replication and chemotaxis and the interactions between hyperstructures have been proposed to constitute an intermediate level of organisation in cells. An entirely new therapy for bacterial diseases might therefore be devised based on the manipulation of hyperstructures. One way to do this would be to supply cells with hybrid metabolites or *hybolites* made by a pairwise combination of the thousands of small molecules involved in metabolism. Some of these hybolites would be substrates for two, very different, hyperstructures and might do much more than simply inhibit key enzymes and processes within the hyperstructures: they might provoke the assembly of two hyperstructures in the same space or lead to hyperstructures emitting misleading signals. It is conceivable that hybolites might even convert a pathogenic Mr Hyde into an inoffensive Dr Jekyll. The likely action of candidate hybolites on hyperstructure dynamics and hence phenotype might be explored cheaply in silico using stochastic automata such as HSIM.

1 Introduction

In a Howard Hughes lecture given in 1999, Don Ganem rebukes those eminent colleagues who, in the sixties, made optimistic predictions about how infectious disease was conquered and how funding should be redirected to chronic diseases: "Now we know, of course, that that notion (that infectious disease was no longer a problem) was foolish to begin with, that infectious disease, epidemic infection, is a part of the human condition. I'm going to show you that it's really a part of human evolution that we can never get away from infectious disease as a class. We can triumph over individual infectious diseases, but the concept that we're going to be free of infection as a species is a ridiculous one and one that nobody believes anymore". Infectious disease, usually bacterial, causes over 60% of total deaths in the developing world, infectious disease is the third leading cause of

death in Europe (the elderly and weak are most vulnerable) and, despite existing antibiotic therapies and vaccines, infectious disease remains the leading cause of mortality and morbidity worldwide (for references see [reference]). In particular, the problem of bacterial resistance to antibiotics (often due to plasmids that can transfer resistance from one bacterium to another) is far from solved as illustrated by the recent deaths in Israel due to multi-drug resistant *Klebsiella* (in news.yahoo.com/070308/43/6cz1b.html).

So what are we going to do about infectious disease? Vicente *et al.* point out that “the antibiotics that were easy to discover have already been found, and it is likely that the search for new members of existing classes, and certainly for new classes of antibiotics, will involve a substantial amount of high-quality, expensive and laborious research”. Indeed, the cost of bringing a new drug to market is estimated to be more than 800 million Euros whilst “a paradox of the effectiveness of antibiotics is their weak value as marketable goods: patients stop buying them once their health returns, after relatively short courses of treatment”. Apparently, therapeutic success is not guaranteed even when pathogenic bacteria are sensitive to an antibiotic. One reason is persistence – the fact that a bacterial population is phenotypically heterogeneous such that some bacteria are not growing at the time of the antibiotic treatment and therefore survive. Another reason is the antibiotic itself may lead to a biofilm forming in which the bacteria are more resistant to antibiotics.

Exploitation of concepts developed in the field of integrative biology is one approach to tackling bacterial and other diseases. Here, we consider a new therapy for bacterial diseases based on the manipulation of hyperstructures *alias* large assemblies of different molecules and macromolecules that, in our hypothesis, perform particular functions within cells. We propose that hyperstructures may be manipulated by supplying bacteria with hybrid metabolites or *hybolites* and, to decide which *hybolites* to construct, we propose modelling their putative action on hyperstructure dynamics via the stochastic automaton HSIM.

2 Hyperstructures as targets

The concept of a hyperstructure is that of an extended assembly of molecules and macromolecules with a specific function within bacterial cells. Certain hyperstructures are functioning-dependent structures that only form when the constituents of the structure function – and function *together*. Examples of candidate hyperstructures include the prokaryotic equivalent of the eukaryotic nucleolus for making ribosomes, the giant factory for DNA replication, the array of chemotaxis receptors for interpreting gradients of attractants and repellents, transertion structures produced by the coupled transcription/translation/insertion of nascent proteins into membrane (which include nascent flagella and the expressed *lac* operon for metabolising lactose), a competence hyperstructure responsible for DNA uptake, a phosphoenolpyruvate:sugar phosphotransferase system (PTS) responsible for the sensing and uptake of a large number of extracellular sugars and for feeding their products, cytoplasmic sugar phosphates, directly to glycolytic enzymes that may even be part of the same hyperstructure, the divisome that executes cell division, and possibly structures involved in pili formation and virulence.

3 Hybolites

Hyperstructures and the interactions between them have been proposed to constitute an intermediate level of organisation in cells. Importantly for therapy, this intermediate level, in our hypothesis, determines the phenotype. An entirely new therapy for bacterial diseases might therefore be devised based on the manipulation of hyperstructures. One way to do this would be to supply cells with hybrid metabolites or *hybolites* made by a systematic, high-throughput, pair-wise combination of the thousands of small molecules involved in metabolism or cell structure. Some of these hybolites would be substrates for two, very different, hyperstructures and might do much more than simply inhibit key enzymes and processes within the hyperstructures: they might induce the assembly of a functioning-dependent hyperstructure in conditions when normally no such structure should form, or they might provoke the assembly of two hyperstructures in the same space, or they might lead to hyperstructures emitting misleading signals resulting in the bacterium adopting (or trying to adopt) patterns of growth and rates of growth inappropriate for the environment. The hyperstructure hypothesis has corollaries in which hyperstructure dynamics regulate the cell cycle events of chromosome initiation, chromosome partitioning and cell division. Hybolites that affect these dynamics might well result in alterations to the rate of progress through the cell cycle or even to the order of cell cycle events with correspondingly serious consequences for the viability or virulence. Finally, it is conceivable that hybolites might convert a pathogenic Mr Hyde into an inoffensive Dr Jekyll and hence avoid the problem of a killing that selects resistant mutants that survive or creates empty niches to be filled with other bacteria.

4 Feasibility

There are several reports of hybrid molecules that might be used as hybolites. Different phospholipids, with saturated and unsaturated chains, have been linked to a cortisol derivative, novobiocin has been coupled through the 3' or 2'' hydroxyl group and a linker to dioleoylphosphatidic acid, and functionalised lipids have been made with, as head groups, the DNA bases thymidine or adenosine. In addition to a chemical synthesis of hybolites, it may prove possible to develop a biological system to produce them. For example, catalytic antibodies have been obtained for a wide variety of reactions including ester hydrolysis and transesterification, amide hydrolysis, glycosidic bond hydrolysis, and decarboxylation.

5 Targets

In this section we give a few examples of the types of hybolite that might be constructed to manipulate particular hyperstructures.

1. *Sensing* Bacteria use quorum sensing molecules such as homoserine lactone to calculate the density of the population and to either continue growing or stop growing. Hybolites of such molecules linked to other molecules might result in bacteria either continuing to try to grow at high densities or stopping growing at low densities. Bacteria also sense attractants so as to swim up nutritional gradients or repellents so as to swim down gradients. This sensing involves a chemotaxis hyperstructure and hybolites to alter it might be constructed by fusing attractants and repellents.

2. *Transport and metabolism.* Sugars, bases, amino acids and many other small molecules can be imported by bacteria and used in metabolism. Fusing, for example, a sugar such as glucose to an amino acid, might interfere with either the membrane-bound enzyme IIBC^{glc} or the cytoplasmic enzyme IIA^{glc} which are responsible for importing and phosphorylating glucose.
3. *DNA synthesis.* Ongoing replication requires feeding the hyperstructure with the four deoxyribonucleotides (dNTPs) at the rate of about 3000 nucleotides per second. Replication might then be readily perturbed by hybolites that involve fusions between nucleotides and other molecules. The involvement of the membrane in replication has a long history and it is tempting to speculate that the replication hyperstructure may also contain cardiolipin (for references see [reference]). Hence, it might be worth making hybolites from phospholipids such as cardiolipin combined with DNA precursors.
4. *Protein synthesis.* A ribosomal or 'nucleolar' hyperstructure forms in *Escherichia coli* at high growth rates when the synthesis of ribosomes consumes most of the bacterium's resources but is not apparent at lower growth rates. Hybolites might modulate growth rate if they were to interfere with the assembly of this hyperstructure. Such hybolites might be made from the amino acids that constitute ribosomal proteins along with bases such as uracil that are form part of ribosomal RNA.
5. *Cell division.* The synthesis of phospholipids and of peptidoglycan leading to the invagination of the membrane and the cell wall must be coordinated between themselves and with ongoing chromosome replication and segregation. This coordination may be provided by a division hyperstructure. Hence promising hybolites might be made from combinations of phospholipids and precursors of peptidoglycan and DNA

6 Discussion

Over the last decade, a wealth of experimental data on the existence of large intracellular structures or hyperstructures has led to a new but still speculative view of bacteria in which the dynamics of these hyperstructures determines the phenotype. This view leads to the idea that manipulating hyperstructures should result in changes in phenotype and hence to the idea that molecules might be made to cause such changes. Indeed, such molecules might actually be made to prevent bacterial virulence. Here, we have explored briefly the notion of hybrid molecules or hybolites which comprise two molecules that participate in different hyperstructures.

A high throughput generation and testing of hybolites on bacterial pathogens might constitute an attractive strategy for the pharmaceutical industry. There are, however, tens of millions of potential hybolites that might be made and screened. Unfortunately, it is not clear which of them might be effective in an eventual therapy. A complementary rather than alternative strategy would be to develop *in silico* approaches based on stochastic automata such as HSIM. HSIM is being used at present to model the dynamics of hyperstructures such as those involved in glucose transport and metabolism. Addition of virtual hybolites with different characteristics to HSIM might be used to help select those that are worth testing *in vivo*.

Hybolites have been proposed above as a possible panacea to bacterial diseases. Of course, it could be argued that there are eukaryotic equivalents to bacterial hyperstructures (see for example [reference]). If so, and if hybolites really do alter bacterial hyperstructures to affect phenotypes, they may have similar actions on eukaryotic cells. In which case, hybolites might be of value in the treatment of certain chronic diseases.

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References