

PERSPECTIVES

then a question of whether colonization of the GI tract by antibiotic-resistant strains is competitive with the indigenous, less-resistant commensal enterococci. Because of the numerical advantage of indigenous enterococci within the healthy consortium, colonization of the GI tract by a few resistant cells would most efficiently result from a noncompetitive process.

What new traits would an enterococcus require to enable it to colonize the gut in a manner that is noncompetitive? Traits predicted to influence selection of a habitat by a microorganism include new pathways for carbohydrate metabolism, new surface proteins that enable attachment to different host epithelial cell types, production of antimicrobial molecules such as bacteriocins, and development of activities that limit host defenses. These are precisely the new activities that are encoded by the enterococcal pathogenicity island (3, 11). This pathogenicity

island encodes a cytolysin that has antibacterial properties, several surface adhesins, several new carbohydrate utilization pathways, and enzymes such as a bile acid hydrolase, which may permit colonization of areas of the intestine closer to the bile duct. From a successfully colonized GI tract, multiple antibiotic-resistant enterococci are well positioned to cause the urinary tract, bloodstream, and surgical-wound infections frequently observed in hospitalized patients.

Human and bacterial genome studies are beginning to reveal the underlying basis for the highly coevolved relationship between humans and microbes. On the basis of emerging information, testable models can be advanced for how the antibiotic-resistant pathogens of the 21st century are able to infiltrate the GI tract consortium and use it as a staging ground for causing infections that are increasingly difficult to treat. With more than 500 microbial species present in the GI

tract consortium, publication of the genome sequences for a leading commensal strain (2) and a "commensal gone bad" (3) represents only a beginning. The insights gained into the biology of this important "organ" are a clear testament to the fertility of this line of pursuit.

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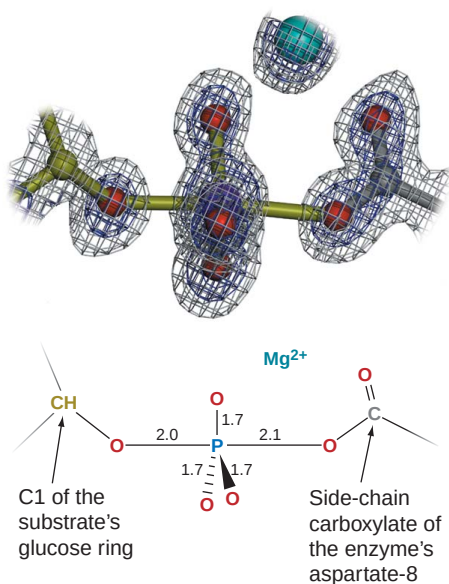
CHEMISTRY

Seeing Is Believing

Jeremy Knowles

Enzymologists, not to speak of physical-organic chemists, have always yearned for a movie of the reactions they study. They'd like to know the precise structures, not just of starting materials and products, but of each of the reaction intermediates and transition states in between. On page 2067 of this issue, Lahiri *et al.* (1) take a step along this path, and illuminate one of the most common reactions in metabolism: the enzyme-catalyzed transfer of a phospho group.

For reactions of small molecules in solution, we don't learn much about the nature of reaction intermediates by looking at ensembles of reactants battered by random solvent collisions and in a multitude of energy states and extents of progress to product. [Much information will doubtless come from studying reactions of single molecules (2), but the methods for such scrutiny are still young.] Enzyme-catalyzed reactions, in contrast, occur in the defined matrix of the active site, and here we can know the precise environment that the substrate experiences. Even though this author once disloyally criticized those who seemed to think that the crystal structure of an enzyme would reveal the whole of its catalytic mechanism ["Making a model of a horse from photographs does not necessarily tell us how fast it can run" (3)], the opportunity for examining the structures



Precise grip. The stretched pentacoordinate oxyphosphorane formed between glucose 1,6-(bis)phosphate (green) and aspartate-8 (gray) of phosphoglucomutase. (Bond lengths are in angstroms. At 1.2 Å resolution there is no connectivity in electron density between the apical ligands and phosphorus, because the resolution is higher than these bond lengths.)

of enzyme-bound intermediates is real. Thus, by soaking native enzyme crystals with substrate or product, we can often obtain the structures of enzyme-substrate or enzyme-product complexes. Further, be-

cause most enzymes are catalytically active in the crystal lattice, we may be able to drive a multistep reaction to an intermediate stage and determine the structure of that intermediate. Much mechanistic information has been gleaned from the crystal structures of acyl-enzymes, phosphoryl-enzymes, and other reasonably stable intermediates. Indeed, we often know more, structurally and mechanistically, about the course of an enzyme-catalyzed reaction than we do about its uncatalyzed counterpart.

In the quest for the details of more transient species, enzymologists have often stopped the reaction part way, by modifying the enzyme (for example, excising a catalytic group) or by modifying the substrate (that is, synthesizing an enzyme inhibitor). But these approaches are never truly satisfying, because one is left looking at the structure of an enzyme complex that cannot react, and trying to deduce—from that—what the actual catalytic situation might be.

It is much more informative to analyze the structure of real substrates bound to active enzyme. On occasion, this approach yields exciting information about the nature of the catalytic act itself. Lahiri *et al.* (1) have determined the crystal structure of the active (that is, phosphorylated) form of the enzyme phosphoglucomutase in the presence of its natural substrates. This enzyme catalyzes the interconversion of glucose 1-phosphate and glucose 6-phosphate. The catalytic cycle involves two phospho-group transfers, one from the phospho-enzyme to glucose 1-phosphate (or glucose 6-phosphate) to give the common intermediate glucose 1,6-(bis)phosphate, and the second from the bisphosphate to the enzyme to regenerate the phosphoenzyme and liberate one of the glucose monophosphates.

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From all the physical-organic work on phosphate esters, we presumed that enzyme-catalyzed phospho transfers would follow one of three paths: “in-line associative” (where the acceptor attacks phosphorus from the side opposite to the leaving group, not unlike an S_N2 reaction in carbon chemistry), “dissociative” (where monomeric metaphosphate is transiently formed, analogously to an S_N1 reaction), or “adjacent associative” (where the acceptor attacks phosphorus from the same side as the leaving group and—after a pseudorotation of the phosphorus ligands—the leaving group departs).

In the 1970s and 1980s, the use of substrates having chiral [^{16}O , ^{17}O , ^{18}O]-phospho groups suggested that all single, enzyme-catalyzed phospho-group transfers proceed with stereochemical inversion at phosphorus (4). That conclusion limited the pathways to those having “in-line” geometry, but it left unanswered the question of whether the mechanism is fully dissociative via metaphosphate (with apical P-O distances of ≥ 3.3 Å and bond orders of zero), S_N2 -like (with apical P-O distances of 1.91 Å and bond orders of a half), or fully associative via an oxyphosphorane (with apical P-O distances of 1.73 Å and bond orders of 1) (5).

With exquisite clarity, the high-resolution crystal structures of Lahiri *et al.* (1) now provide the answer. The coordination

states of the two phosphorus atoms in the intermediate that is formed from the phospho-enzyme and either glucose 1-phosphate or glucose 6-phosphate are quite different. One, at the sugar’s 6-position, has the normal, four-coordinate tetrahedral arrangement of a phosphate monoester. But the other is a stretched pentacoordinate trigonal bipyramidal oxyphosphorane, with the substrate’s C1 oxygen and the carboxylate of the enzyme’s aspartate-8 as its apical ligands (see the figure). The electron density at phosphorus is not ellipsoidal, which argues against the structure being a time average of those of a phosphorylated aspartate and a 1-phosphorylated sugar. The network of hydrogen bonds (and a bound magnesium cation) shows how precisely the enzyme grips this species, to sequester and preferentially stabilize an otherwise unstable entity.

So what is this species? Apical bond lengths of 2.0 to 2.1 Å correspond to P-O bond orders of a quarter to a third, and the structure is thus close to what we’d expect for the transition state of a partly associative in-line displacement (5). Could this actually be the transition state, seductively consistent with Pauling’s view (6) that enzymes are designed explicitly to bind (and thus to stabilize) the transition states of the reactions they catalyze? But transition states are at free energy maxima and could never be ob-

served directly. In this case, we must conclude that the temperature coefficients of the various enzyme-bound species are such that what is a transition state at physiological temperatures has become the most stable intermediate at the very low temperature of the crystallographic work. Or perhaps the uncatalyzed reaction involves a transient intermediate oxyphosphorane and the enzyme has evolved to stabilize that intermediate, lowering in the process the free energies of the two transition states that flank it. Indeed, we must hope that the authors will explore what happens to their structure as the temperature is raised.

But such questions are less important than the fact that the simple, attractive, and anticipated mechanism for enzyme-catalyzed phospho-group transfer has now been so gratifyingly confirmed.

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MATHEMATICS AND BIOLOGY

A Bright Future for Biologists and Mathematicians?

Alan Hastings and Margaret A. Palmer

Why, despite vaccination efforts, is Boulder, Colorado, weathering an outbreak of whooping cough (pertussis)—a potentially fatal illness in young children—this winter? The answer to this biological question comes from the classic mathematical analysis of Kermack and McKendrick, whose threshold theorem calculates the minimum level of vaccination required to prevent an outbreak of an infectious disease (1). This example of how mathematics can help biology was just one of many discussed at a recent series of Quantitative Environmental and Integrative Biology workshops (2) and at a recent NIH-NSF workshop that examined forging

stronger links between mathematicians and biologists (3). A goal of the workshops was to seek answers to the questions: Which biological problems will yield to mathematical analyses, and how should biology and mathematics be integrated to achieve this?

Kermack and McKendrick developed the threshold theorem to determine the conditions under which infectious disease epidemics occur. This theorem has proved crucial for calculating the level of vaccination (less than complete coverage) required to eradicate diseases like polio and smallpox, and for preventing outbreaks of diseases such as pertussis. This theorem relates the occurrence of an epidemic to the number of susceptible individuals, the duration of the infectious period, and the infectivity of the disease. The threshold theorem was initially developed to answer two fundamental biological questions: Why do infectious disease epidemics occur, and

why do they typically die out before all susceptible individuals contract the disease? These questions were answered by using the threshold theorem to develop the SIR (susceptible, infective, removed) model (1), which consists of three differential equations. The SIR model assumes that over the time scale of an epidemic, births and deaths in the host population can be ignored. The model includes the rate of removal (through death or recovery) of infected persons from the group passing on the infection, instead of specifying the more correct but harder to analyze assumption that there is a fixed time period during which an individual can infect others. The threshold theorem was originally illustrated using methods that relied on the graphic display of the number of infective and susceptible individuals during an infectious disease outbreak. The graphic representation of the threshold theorem reveals that the density of susceptible individuals must exceed a certain critical value for an epidemic to occur. This theorem has unquestionable relevance, given heightened concerns about the deliberate introduction of new infectious bioterrorist agents.

Workshop participants agreed that progress in understanding biological problems will depend on mathematical ad-

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