of many of the metabolic actions of insulin (such as stimulation of glucose transport). Similarly, another phosphotyrosine in IRS-1 binds to GRB2 (growth factor receptor binding protein–2), which activates the Ras/Raf/MAP (mitogen-activated protein) kinase pathway. Cohen *et al.* (*1*) now report that leptin antagonizes insulin signaling, consistent with their finding that it decreases insulin-induced tyrosine phosphorylation of IRS-1. As expected, decreased phosphorylation of IRS-1 was associated with decreased binding of GRB-2.

However, other observations are harder to rationalize with current models of insulin action. For example, why was decreased tyrosine phosphorylation of IRS-1 associated with an increase in binding of p85? In addition, leptin was observed to potentiate insulin-induced tyrosine phosphorylation of the insulin receptor. Ordinarily, tyrosine phosphorylation of the receptor results in activation of the receptor tyrosine kinase. Why, then, did leptin inhibit phosphorylation of IRS-1? Finally, Cohen *et al.* showed that leptin antagonizes insulin's ability to decrease mRNA encoding phosphoenolpyruvate carboxykinase (PEPCK), the enzyme catalyzing the rate-limiting step in gluconeogenesis. Previous work showed that insulin's effect on PEPCK requires activation of PI 3-kinase, but not the MAP kinase pathway (*8*). Why did leptin and insulin have antagonistic effects on expression of PEPCK mRNA although both activate PI 3-kinase? Providing answers to these provocative questions has the potential to expand our understanding of the mechanisms of insulin action.

How does leptin act on hepatoma cells? All of the variably spliced isoforms of leptin receptor mRNA encode proteins that bind leptin, but they differ in their cytoplasmic domains (*9*). The longest form (isoform b) is the only one known to activate the Jak-Stat pathway. In fact, mice with a mutant allele of the murine leptin receptor gene [originally named the *diabetes* (*db*) locus] do not express isoform b yet have the same phenotype as mice completely lacking leptin. This observation led to the hypothesis that isoform b mediates all of the biological actions of leptin. However, Cohen *et al.* (*1*) detected only isoform a (a short form that does not activate the Jak-Stat pathway) in human liver and cultured hepatoma cells. [Others have reported the presence of isoform b in murine liver (*10*).] If isoform a mediates the actions of leptin in liver, the molecular mechanism whereby it antagonizes insulin action will be of particular interest.

Because *ob*/*ob* mice develop insulin resistance in the total absence of leptin, it is likely that leptin is only one of a number of factors responsible for the induction of insulin resistance in obesity. Nevertheless, the new results (*1*) will generate renewed interest in the possible role of leptin in NIDDM in humans. It would be both disappointing and ironic if a peptide with the potential to cause people to lose weight exacerbated insulin resistance, thereby predisposing them to NIDDM. We hope that clinical trials will demonstrate that leptin does not have this unfortunate toxicity.

References

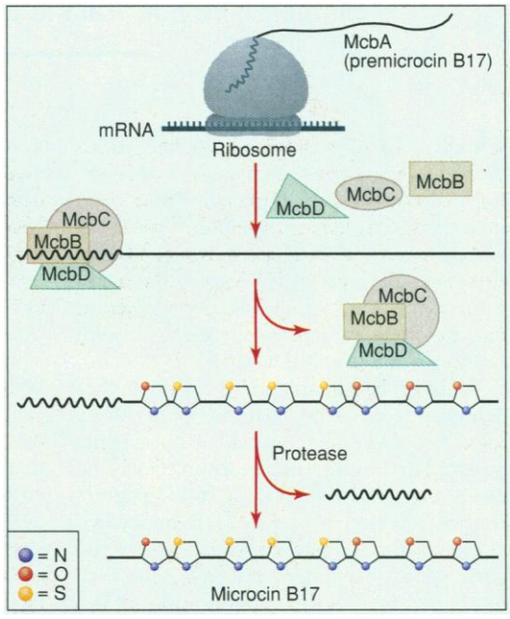
1. B. Cohen, D. Novick, M. Rubinstein, *Science* **274**, 1185 (1996).
2. Y. Zhang *et al.*, *Nature* **372**, 425 (1994).
3. M. A. Pelleymounter *et al.*, *Science* **269**, 540 (1995); J. L. Halaas *et al.*, *ibid.*, p. 543; T. W. Stephens *et al.*, *Nature* **377**, 530 (1995).
4. L. A. Campfield, F. J. Smith, Y. Guisez, R. Devos, P. Burn, *Science* **269**, 546 (1995).
5. R. S. Ahima *et al.*, *Nature* **382**, 250 (1996).
6. J. D. McGarry, *Science* **258**, 766 (1992).
7. G. S. Hotamisligil, N. S. Shargill, B. M. Spiegelman, *ibid.* **259**, 87 (1993); G. S. Hotamisligil *et al.*, *ibid.* **271**, 665 (1996).
8. R. A. Gabbay *et al.*, *J. Biol. Chem.* **271**, 1890 (1996).
9. L. A. Tartaglia *et al.*, *Cell* **83**, 1263 (1995); G. H. Lee *et al.*, *Nature* **379**, 632 (1996); S. C. Chua Jr. *et al.*, *Science* **271**, 994 (1996).
10. N. Ghilardi *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 6231 (1996).

NATURAL PRODUCTS

One of Nature's Macromolecular Machines Demystified

JoAnne Stubbe

Enzyme-based macromolecular machines inside bacteria synthesize, with amazing efficiency, a diverse array of medicinally interesting polypeptides and polyketides with unusual ring structures (*1*–*3*). Polypeptides such as cyclosporin are made nonribosomally from pantetheinylated modular protein components and monomeric building blocks such as amino acids. Alternatively polypeptides such as microcins (*4*, *5*) and lantibiotics (*26*) are made by ribosomes from mRNA and then extensively posttranslationally modified by macromolecular machines. On page 1188 of this issue, Li *et al.* report the first purification of the enzymes required to synthesize microcin B17 (see figure). The purification of the essential components of this process lays the groundwork for understanding the chemical basis for these interesting conversions, which may lead to the development of novel compounds with unprecedented properties (*7*).



Making microcin. Biochemical reaction steps in the synthesis of microcin B17 in *Escherichia coli*. The precursor peptide is modified after ribosomal translation to form the microcin product with oxazole and thiazole rings.

Peptide antibiotics with five-member heterocyclic rings are prevalent and varied in structure. They function as antibacterial, antiviral, and antitumor agents and act on diverse targets (*4*, *5*, *8*). The peptide antibiotics made by ribosomes have a number of features in common (see figure). In each case, several gene products are required for their biosynthesis and they are usually part of a single operon. In the case of the lantibiotics and microcins, one gene from the operon codes for the prepeptide. Additional gene products within the operon then convert the natural amino acids within the prepeptide into the novel heterocyclic ring structures. Subsequent to this posttranslational modification, in many cases, the resulting modified peptide is cleaved proteolytically to generate the active natural product (see figure). In addition, the operons usually contain genes that code for proteins that are involved in transport of these peptides out of the host cell, as a defense mechanism when a

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hostile environment is encountered. These operons also contain genes that code for proteins that provide self-protection, that is, self-immunity, from these lethal peptides.

Studies from the Kolter and Walsh laboratories have provided important insight into how the *mcbA* gene product, premicrocin B17, a 69–amino acid polypeptide, is processed into the 43–amino acid active peptide microcin that functions as a putative antibacterial agent by inhibition of bacterial DNA gyrase. Their studies revealed that the 26–amino acid NH_2-terminus, which is proteolytically processed, is required for the gene products McbB, -C, and -D to catalyze the postranslational cyclization of glycine-cysteine and glycine-serine dipeptide modules to five-membered ring heterocycles (oxazolines and thiazolines) and their oxidations. Presumably this tail serves as a scaffolding for the binding of the machine that catalyzes these interconversions. Their preliminary studies also suggest the requirement for both adenosine triphosphate (ATP) and O_2 in these steps. Finally, McbC, when isolated, is yellow and is shown to contain a single flavin mononucleotide cofactor, presumably involved in the ring oxidation.

The stage is now set to answer a number of intriguing questions. Are the heterocyclic rings made processively? What is the smallest peptide recognized by the cyclase genes? Are these cyclases homologous to the cyclases that make similar rings in the bacitracins or the bleomycins? Can understanding the functions of McbB, -C, and -D lead to a module that can make similar heterocyclic rings in other peptides? How do these proteins interact with each other and with the leader sequence? A key to successful genetic engineering will be to develop an understanding of these interactions, so that unwanted side reactions leading to mixtures of products can be avoided.

A second intriguing area is the basis for the microcins' antibacterial activity. Preliminary evidence suggests that they function as DNA gyrase inhibitors, chemically diverse in comparison with other classes of gyrase inhibitors. What is the mechanism of this inhibition? Do they bind to DNA or protein through intercalation or fit within the minor groove of DNA? Do they form a complex with metals that play a role in the inhibition? The ability to make microcins and microcin analogs should allow the mechanism of cytotoxicity to be understood at the molecular level.

A third area is the mechanism of self-protection provided by the gene product McbG. How does the host organism prevent its DNA gyrase from being inactivated? Does this protein sequester the microcin? Does it modify the microcin so that it can no longer carry out its normal biological function?

The prospect of understanding the biosynthesis and the mechanism of action of microcins most certainly will lead to engineered microcins of semisynthetic origin and define new targets of therapeutic interest. The methods reported by Li *et al.* provide an excellent prototype for studying the biosynthesis of other peptides with modified amino acids (*7*).

References

1. H. Kleinkauf and H. von Dohren, *Antonie Leeuwenhoek* **67**, 229 (1995).
2. T. Stachelhaus and M. A. Marahiel, *FEMS Microbiol. Lett.* **125**, 3 (1995).
3. C. R. Hutchinson and I. Fujii, *Annu. Rev. Microbiol.* **49**, 201 (1995).
4. R. Kolter and F. Moreno, *ibid.* **46**, 141 (1992).
5. G. Jung, *Angew. Chem. Int. Ed. Engl.* **30**, 1051 (1991).
6. H. G. Sahl, R. W. S. Jack, G. Bierbaum, *Eur. J. Biochem.* **230**, 827 (1995).
7. Y.-M. Li, J. C. Milne, L. L. Madison, R. Kolter, C. T. Walsh, *Science* **274**, 1188 (1996).
8. A. Bayer, S. Freund, G. Jung, *Eur. J. Biochem.* **234**, 414 (1995).

UPDATE: ATMOSPHERIC CHEMISTRY

The Elusive Hydroxyl Revisited

William Brune

The elusive hydroxyl radical is a little less elusive these days. This important atmospheric oxidant has traditionally been hard to measure because it reacts rapidly and thus has a small abundance, much less than 1 part per trillion. Over the last 2 decades, instruments have evolved and the resulting hydroxyl measurements are getting better and better. A good way to gauge this improvement is to intercompare instruments, as was done recently in a German cornfield (*1*). These results add important data to the story provided by earlier measurements (*2*), which were discussed in a previous Perspective (*3*).

Scientists conducted the informal intercomparison as part of study called POPCORN, which was held in northeast Germany during August 1994 (*1*). Dorn and colleagues measured OH by absorption (DOAS) over a 3-km path, shrunk into a 39-meter path by passing the laser beam many times between mirrors. This technique is absolutely calibrated because it measures OH absorption directly. It is effectively a local measurement because the mirror separation is short compared to the scale of atmospheric OH variations. Hofzumahaus and colleagues used a second instrument that draws the air sample into a detection cell through a small orifice and detects OH with laser-induced fluorescence (LIF). This technique is a single-point measurement but requires calibration with a quantified OH source. Other instruments measured the meteorology and other chemical species to provide necessary information for the scientific study and to aid in the interpretation of the OH results.

The two OH measurement techniques agree remarkably well. This agreement improves when data from the north-northwest, possibly contaminated by an electric motor on the DOAS instrument, are excluded. The regression of the remaining coincident measurements has a linear relation with a slope of 1.01 ± 0.04 and an insignificantly small intercept. This informal intercomparison of OH measurement techniques is not the first successful one; others have given similarly good results (*2*). However, it is the first successful one for these two techniques and thus gives scientists confidence in them.

Scientists now apparently can measure atmospheric OH. Indeed, various instruments, including five LIF instruments, have recently been part of scientific studies from the ground, ships, and aircraft. It is about time for a formal, blind intercomparison. In the meantime, when the results of recent scientific studies are analyzed and reported, we will discover how much more we need to learn about hydroxyl and atmospheric oxidation.

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References

1. T. Brauers *et al.*, *Geophys. Res. Lett.* **23**, 2545 (1996).
2. G. H. Mount and F. L. Eisele, *Science* **256**, 1187 (1992).
3. W. H. Brune, *ibid.*, p. 1154.