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Homoserine *O*-Acetyltransferase, Involved in the *Leptospira meyeri* Methionine Biosynthetic Pathway, Is Not Feedback Inhibited

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The *Leptospira meyeri* serovar semaranga *metX* gene was identified by complementation of an *Escherichia coli metA* mutant, i.e., devoid of homoserine *O*-succinyltransferase. However, the MetX protein exhibited a homoserine *O*-acetyltransferase activity in agreement with its similarity to homoserine *O*-acetyltransferases. Reverse transcription-PCR analysis demonstrated that *metX* is the second gene of an operon.

The first step of the biosynthetic pathway leading to methionine is esterification of homoserine (6). However, the precursor differs according to the organism. In members of the family *Enterobacteriaceae*, *O*-succinyl-homoserine is formed by acylation of homoserine in the presence of *O*-succinyl–coenzyme A, catalyzed by homoserine *O*-succinyltransferase, the *metA* gene product (7, 18). In gram-positive bacteria of the genus *Bacillus* and in fungi, esterification of homoserine occurs by an acetylation (3, 9, 13) catalyzed by a homoserine *O*-acetyltransferase.

The genus *Leptospira* consists of two nomenspecies, *Leptospira interrogans* sensu lato (pathogenic) and *Leptospira biflexa* sensu lato (nonpathogenic), which belong to the order *Spirochaetales* (4). Previous studies indicated that *Leptospira* spp. synthesize most of their amino acids by the same biosynthetic pathways as those used by *Escherichia coli* (5).

Our interest in methionine biosynthesis in *Leptospira* arose from the fact that radiotracer studies of biosynthetic pathways did not include any data for the methionine pathway (5). In this paper, we describe isolation and transcriptional organization of the *metX* gene, as well as the enzymatic characterization and regulation of its product, homoserine *O*-acetyltransferase.

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Cloning of a DNA fragment from *Leptospira meyeri* which can complement an *E. coli metA* mutant. We had previously constructed a recombinant cosmid library and cloned the *Leptospira metY* gene, which complemented *E. coli metB* mutants (1). The size of the cloned DNA fragment (25 kbp) in the pb10 recombinant cosmid suggested that other methionine biosynthetic genes might be found on this fragment. In order to test this hypothesis, *E. coli* RC709 (*metF63 pro-22*; R. Clowes) and AB1932 (*metA28 argH1 thi-1 lacY1 lacZ4 galK2 xyl-4* [or *-5*] *tsx-6* F^-; E. A. Adelberg) were used for complementation experiments in supplemented M9 minimal medium (20) at 30°C. Both strains were electroporated with pb10. pb10 weakly complemented *metA* but not *metF* mutants. Further subcloning experiments allowed us to locate the *Leptospira* DNA able to complement *E. coli metA* and *metB* mutants more precisely within a 6.8-kbp fragment. Plasmid pb12 carrying a 6.8-kbp *Pst*I fragment from pb10 in the pBR322 vector (22) still complemented *E. coli metB* and *metA* mutants (Fig. 1). Plasmid pa13, generated by cloning a 3.9-kbp *Xba*I-*Pst*I restriction fragment of pb12 into pSU18 (15), did not complement the *metA* mutant. However, the cloning of a 2.5-kbp *Acc*I-*Hin*dIII DNA fragment into pUC18 (26) allowed for complementation of an *E. coli metA* mutant yielding pxc8 (Fig. 1). Thus, *metX* resided on the 2.5-kbp fragment of pxc8, being expressed under the control of the *lac* promoter.

***Leptospira* MetX is similar to yeast homoserine *O*-acetyltransferase.** Nucleotide sequencing of 1.6 kb of the *Acc*I-*Hin*dIII fragment cloned into pUC18 was performed, starting at the *Acc*I site, by dideoxynucleotide chain-termination reactions on double-stranded templates with T7 DNA polymerase (Pharmacia, Saclay, France), [α-^{33}P]dATP (111 TBq/mmol), and synthetic oligonucleotides (20). Analysis of the sequence revealed an open reading frame spanning 1,137 nucleotides. This open reading frame, called *metX*, encodes a 378-amino-acid protein, with a deduced molecular mass of 41,745 Da. The chosen ATG codon is likely to be the correct start codon since there are only 8 nucleotides between *metX* and the stop codon of the upstream *metY* gene (Fig. 1). No convincing putative ribosome binding site could be found upstream of the ATG.

The deduced amino acid sequence was analyzed by BLAST e-mail servers and CLUSTAL software (10). The amino acid sequence deduced from *Leptospira metX* showed strong similarities with homoserine *O*-acetyltransferases (EC 2.3.1.31) from *Haemophilus influenzae* (8), *Saccharomyces cerevisiae* (MET2) (13), and *Ascobolus immersus* (9) and with deacetylcephalosporin C acetyltransferase from *Cephalosporium acremonium* (*Acremonium chrysogenum*) (16). No similarity was detected with *E. coli* MetA (homoserine *O*-succinyltransferase) (7). Figure 2 gives an alignment of MetX, yeast homoserine acetyltransferase (MET2), and a deacetylcephalosporin C acetyltransferase (39 and 37% identity, respectively). The *Leptospira* MetX protein is smaller than the corresponding similar fungal proteins (378 residues compared to 438 for the enzyme from yeast). There are two domains of similarity (residues 31 to 250 and 264 to 319) with one gap (77 residues in length); the N-terminal and C-terminal parts are not well conserved. Similarity with the putative homoserine *O*-acetyltransferase from *H. influenzae* extends throughout the whole proteins, which have roughly the same size (378 versus 358 residues).

Homoserine *O*-acetyltransferase activity of *Leptospira* is not regulated. The knowledge of the weak and unstable activity of homoserine acyltransferases (25) led us to overexpress the whole *metX* gene. Plasmid pETmetX was constructed by cloning the region containing the entire *metX* gene (from the ATG

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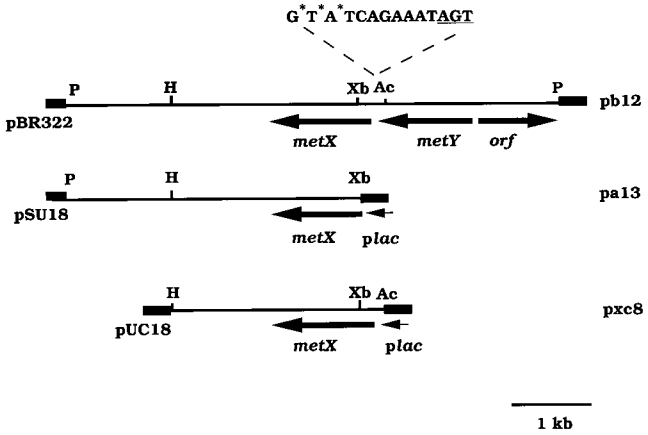

FIG. 1. Subcloning of the *L. meyeri* serovar semaranga *metX* gene. The orientation of the *metX*, *metY*, and *orf* genes within the *Leptospira* insert is indicated by thick arrows. The ATG of the *metX* gene is marked by asterisks, and the stop codon of the *metY* gene is underlined. The *lac* promoter is marked by a thin arrow. Solid boxes represent the vectors. The names of the recombinant plasmids and the relevant restriction sites are indicated: P, *Pst*I; H, *Hin*dIII; Ac, *Acc*I; Xb, *Xba*I.

to the *Hin*dIII site approximately 1.5 kbp downstream from the stop codon [Fig. 1]) in pET20b+ (Novagen, Madison, Wis.), which expresses genes under the control of the T7 promoter. The plasmid pETmetX was transformed into *E. coli* BL21 (DE3) (Novagen), which carries the T7 RNA polymerase gene on λDE3 integrated on the chromosome of BL21. The pellet from 100 ml of cells of *E. coli* BL21(DE3) transformed with pETmetX and induced with IPTG (isopropyl-β-D-thiogalactopyranoside; 0.4 mM) was suspended in 4 ml of ice-cold buffer (50 mM sodium phosphate [pH 7.5] containing 0.5% phenylmethylsulfonyl fluoride, 25% sucrose, 10 mM DL-homoserine, and 2.5 mM dithiothreitol). After sonication, the lysate was centrifuged at 39,000 × *g* for 45 min at 4°C. The supernatant was dialyzed overnight against 2 liters of the above buffer (without sucrose) containing 50% glycerol, and the dialysate was used for enzymatic assay of acyltransferase according to the method in reference 24. The overexpressed MetX protein exhibited a molecular mass of 40 kDa in a sodium dodecyl sulfate-polyacrylamide gel (data not shown) in agreement with that expected.

Crude cellular extracts of *E. coli* BL21(D23) transformed with pETmetX after induction exhibited a high homoserine *O*-acetyltransferase activity (6.3 μmol/min/mg of protein). However, *O*-succinyl–coenzyme A was not used as a substrate by MetX. It remains to be explained how *Leptospira metX* can complement functionally an *E. coli metA* mutant, albeit weakly. The most likely explanation is that this complementation was possible through the marginal use of the *E. coli* substrate acetyl coenzyme A by the *Leptospira O*-acetyltransferase. Moreover, the *E. coli* enzyme can use both acetyl and succinyl derivatives of homoserine, although *O*-succinylhomoserine is preferred (12). The use of acetyl coenzyme A by *Leptospira* spp. could be related to their unique metabolism. These bacteria use fatty acids as the chief carbon source, which by oxidation give rise to acetyl coenzyme A (11). A high content of acetyl coenzyme A is therefore available within the *Leptospira* cells.

No inhibition of the overexpressed homoserine *O*-acetyltransferase activity was detected with 5 mM methionine and/or with *S*-adenosylmethionine. The lack of feedback inhibition of homoserine *O*-acetyltransferase activity is highly significant since the concentrations of inhibitors in the reaction were at least 10 times higher than the K_m values for the substrates, which were 0.46 ± 0.07 and 0.2 ± 0.06 mM for homoserine and acetyl coenzyme A, respectively. This result for the MetX *Leptospira* enzyme is in contrast to the feedback inhibition by methionine and *S*-adenosylmethionine found for the similar enzymes from *Bacillus subtilis*, *Bacillus polymyxa*, and *Brevibacterium flavum* (3, 21, 23).

***metX* is the second gene of an operon.** As the *metX* and *metY* genes were separated by only 8 nucleotides (Fig. 1), the organization of the two genes into an operon was very plausible. Total cellular RNA was extracted from 500 ml of *Leptospira* cells (10^8/ml) harvested in the exponential growth phase by use of Tri reagent (Sigma Chemical Co., St. Louis, Mo.), and reverse transcription (RT)-PCR was carried out with the Access RT-PCR system (Promega, Lyon, France). A positive control yielded an 821-bp band with primers within *metY* (Fig. 3, lane 3). A band of the expected size (466 bp) was found with

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MetXLm                                                          MPTSEQNEFSHGSVGVVYTQSIRFES  26
MET2Sc                                                  MSHTLKSKTLQELDIEEIKETNPLLKLVQGQRIVQV  36
CEFGAc          MLPSAQVARLKPDPFPPSLSPIPHGAVTFAALAPCHNLPIFSSRQMLRDSLTYSHTSPTMSPQIANRFEASLDAQDIARI  80

MetXLm          --LTLEGGETITPLEIAYETYGTLNEKKDNAILVCHALSGDAHAAGFHEGDKRPGWWDYYIGPGKSFDTNRYFIISSNVI  104
MET2Sc          PELVLESGVVINNFPIAYKTWGTLNEAGDNVLVICHALTGSADVAD---------WWGPLLGNDLAFDPSRFFIICLNSM  107
CEFGAc          SLFTLESGVILRDVPVAYKSWGRMNVSRDNCVIVCHTLTSSAHVTS---------WWPTLFGQGRAFDTSRYFIICLNYL  151
                  . **.*   .    .** . * .*   ** ...**.*.. *. .          **   .*  . .** .*.***  * .
MetXLm          GGCKGSSGPLTINGKNG--KPFQSTFPFVSIGDMVNAQEKLISHFGIHKLFAVAGGSMGGMQALQWSVAY-PDRLKNCIV  181
MET2Sc          GSPYGSFSPLTINEETGVR--YGPEFPLCTVRDDVRAHRIVLDSLGVKSIACVIGGSMGGMLSLEWAAMYGKEYVKNMVA  185
CEFGAc          GSPFGSAGPCSPDPDAEGQRPYGAKFPRTTIRDDVRIHRQVLDRLGVRQIAAVVGASMGGMHTLEWA-FFGPEYVRKIVP  230
                *.  ** .* . .       . . **  .. * *  .  ..  .*.  .  * *.***** .*.*.  .  . ... .
MetXLm          MASSSEHSAQQIAFNEVGRQAILSDPNWNQGLYTQENRPSKGLALARMMGHITYLSDEMMREKFGRKPPKGNIQSTDFAV  261
MET2Sc          LATSARHSAWCISWSEAQRQSIYSDPNYLDGYYPVEEQPVAGLSAARMSALLTYRTRNSFENKFSRRSPSIAQQQKAQRE  265
CEFGAc          IATSCRQSGWCAAWFETQRQCIYDDPKYLDGEYDVDDQPVRGLETARKIANLTYKSKPAMDERF-HMAPGVQAGRN----  305
                .*.*  .*.    .  *  ** *  **.  .* *  ...*  **  **  . .** .      ..* . .*
MetXLm          ----------------------------------------------------------------------------GSY  264
MET2Sc          ETRKPSTVSEHSLQIHNDGYKTKASTAIAGISGQKGQSVVSTASSSDSLNSSTSMTSVSSVTGEVKDIKPAQTYFSAQSY  345
CEFGAc          ---------------------------------------------ISSQDAKKEINGTDSGNSHRAGQPIEAVSSY  336
                                                              . . .                          *
MetXLm          LIYQGESFVDRFDANSYIYVTKALDHFSLGTGK--ELTKVLAKVRCRFLVVAYTSDWLYPPYQSEEIVKSLEVNAVPVSF  342
MET2Sc          LRYQGTKFINRFDANCYIAITRKLDTHDLARDRVDDITEVLSTIQQPSLIIGIQSDGLFT-YSEQEF---LAEHIPKSQL  421
CEFGAc          LRYQAQKFAASFDANCYIAMTLKFDTHDISRGRAGSIPEALAMITQPALIICARSDGLYS-FDEHVE---MGRSIPNSRL  412
                * **.  *   **** ** .*  .*   . . .   .  *. .   *..  ** *.  . .   .       .
MetXLm          VELNNPAGHDSFLLPSEQQDSILRDFLSSTDEGVFL                                        378
MET2Sc          EKIESPEATMPSYWSLS                                                            438
CEFGAc          CVVDTNEGHDFFVMEADKVNDAVRGFLDQSLM                                             444
                  ..   .               .  .      .
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FIG. 2. Alignment of *L. meyeri* serovar semaranga *metX* deduced amino acid sequence (MetXLm) with homoserine *O*-acetyltransferase of *S. cerevisiae* (MET2Sc; accession no. P08465) and acetyl coenzyme A-deacetylcephalosporin C acetyltransferase of *Cephalosporium acremonium* (*Acremonium chrysogenum*) (CEFGAc; accession no. P39058). Identical residues are indicated by asterisks, and similar residues are indicated by dots. A hyphen indicates a gap. The alignment was performed by CLUSTAL V.

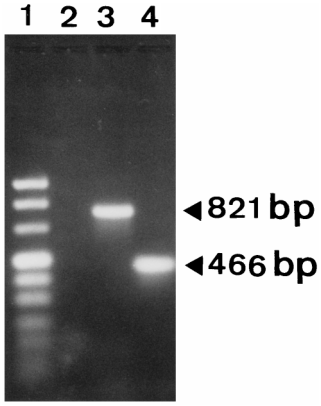

FIG. 3. Transcriptional organization of the *metX* gene assessed by RT-PCR. Electrophoresis of the RT-PCR products was performed on a 1.5% agarose gel. The amplified products span the intergenic (or intragenic) regions as follows: lane 2, *orf-metY*; lane 3, *metY-metY*; lane 4, *metY-metX*. Lane 1, molecular size markers VIII (Boehringer, Mannheim, Germany).

a primer within *metY* and the other within *metX* (Fig. 3, lane 4). No amplification (Fig. 3, lane 2) was observed between *metY* and the upstream *orf* in agreement with their divergent orientation (Fig. 1).

The organization of *Leptospira metX* as the second gene of an operon differs from the situation found in *E. coli*, in which *metA* is transcribed as a single transcriptional unit (17). The absence of a specific promoter for *Leptospira metX* and the absence of regulation at the enzymatic level are in contrast to the multiple regulation of expression of the *E. coli metA* gene by MetJ, MetR, and σ32 and feedback inhibition of *O*-succinyltransferase by methionine plus *S*-adenosylmethionine (2, 14, 19). Though feedback inhibition does not take place for MetX, it was found for the second enzyme (MetY) of the *Leptospira* methionine pathway (1). The most probable interpretation is that the flux of metabolites is differently channeled for *Leptospira*.

Nucleotide sequence accession number. The sequence data reported have been assigned EMBL accession no. Y10744.

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