STUDIES ON REGULATION OF THE PEROXISOMAL \( \beta \)-OXIDATION AT THE 3-KETOTHIOLOLASE STEP

Dissection of the Rat Liver Thiolase B Gene Promoter

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1. SUMMARY

The peroxisomal 3-oxoacyl-CoA thiolase (thiolase) is the last enzyme involved in the \( \beta \)-oxidation of fatty acids. The enzyme cleaves long chain fatty acyl-CoA to generate acetyl-CoA and shortened acyl-CoA. The enzyme is nuclear encoded, synthesized in the cytoplasm and transported into peroxisomes. The thiolase B gene is inducible by the peroxisome proliferator compounds, like other genes involved in \( \beta \)-oxidation of fatty acids in peroxisomes.

The importance of studying thiolase is that it generates acetyl-CoA which is the precursor for the synthesis of molecules like cholesterol and fatty acids. The structural and functional analysis of thiolase at molecular level may add to the knowledge of fatty acid metabolism and further the obesity phenomenon. It is known that several genes mediate lipid homeostasis in target organs like liver, adipose tissue and are regulated by peroxisome proliferator activated receptors (PPAR\( \alpha \) and PPAR\( \gamma \)). To elucidate the mechanism of induction of rat liver thiolase B gene, an upstream 2.8kb fragment containing promoter element has been subcloned and partially sequenced. The sequence analysis revealed a putative PPRE (Peroxisome Proliferator Response Element) of AGACCT TGAACC sequence at –681 to –668 [Kliever et al. (1992) Nature 358:771–774]. By transient expression of a luciferase reporter gene in HeLa cells, we conclude that the identified
PPRE could be functional in induction of thiolase B gene, but other sequences of genes might be involved.

2. INTRODUCTION

The peroxisomal 3-oxoacyl-CoA thiolase (thiolase) is involved in the final reaction of fatty acids β-oxidation. The enzyme cleaves long chain fatty acyl-CoA to generate acetyl-CoA and chain-shortened acyl-CoA. The importance of studying thiolase is that it generates acetyl-CoA which is the precursor for the synthesis of molecules like cholesterol and fatty acids. It is now established that several genes mediate lipid metabolism in target organs like liver and adipose tissue, and are thus regulated by several Peroxisome Proliferator-Activated Receptors (PPARs).

In rat liver at least 3 genes encode for peroxisomal thiolase of which thiolase B is inducible by peroxisome proliferators.1 To elucidate the mechanism of induction of thiolase B, an upstream 2.8kb fragment containing the promoter element has been sub-cloned and partially sequenced. The sequence analysis revealed a putative PPRE (Peroxisome Proliferator Response Element) AGACCT T TGAACC at –681 to –668.2,3

To analyzes the functional elements in the 2.8 kb fragment, several deletions were made in the 5’ and the 3’ region in a plasmid containing TK promoter and the sequence encoding luciferase. Transfection assays were performed with these various deleted constructs in HeLa cells. Preliminary transfection results seem to suggest that this localized PPRE element is not the only one in controlling thiolase B expression by PPARα.

3. MATERIALS AND METHODS

3.1. Structure of PTBTK luc Plasmid and Deleted Constructs

a. Tool (Fig. 1): a 2.6kb genomic DNA fragment of the 5’-upstream region of PTB gene was inserted between HindIII and BamHI sites in a pBLluc vector. (Fig. 1)
b. Preparation of 5’ or 3’ deleted upstream constructs in pB luc vector (Table I see also Fig. 2)
c. Use of HeLa cell line (human cervical carcinoma) as host cell for transient expression.
d. Techniques of transfection of 2 × 10⁶ cells/dish of HeLa cells in transient expression [6µg of luciferase reporter plasmid and co-transfected with pCMV-βgal(4µg) and +/- pCMV-PPARα or pCMV Not (2µg)].

Luciferase activity has been normalized with β-galactosidase activity.

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**Figure 1.**