2-Hydroxymuconic semialdehyde dehydrogenase catalyzes the conversion of 2-hydroxymuconic semialdehyde (HMS) to an enol form of 4-oxalocrotonate which is a step in the catechol-meta cleavage pathway. A tomC gene encoding 2-HMS dehydrogenase of Burkholderia cepacia G4, a soil bacterium that can grow on toluene, cresol, phenol or tricholoro ethylene, is identified in between catechol 2,3-dioxygenase gene and HMS hydrolase gene, its sequence is analysed and the enzyme is characterised. The 2-HMS dehydrogenase from B. cepacia G4 is able to oxidize many meta cleavage products (MCPs) in the presence of NAD+. The oxidative activity of the enzyme is spectrophotometrically detected with the observation of a gradual time dependent decrease in absorbance at A375 in the presence of 2-HMS and NAD+. All the tested MCPs served as substrates for 2-HMS dehydrogenase and thus it is evident that the enzyme has broader substrate specificity. But the activity of the enzyme is much lower for both 3-methyl HMS and 2-hydroxy 6-oxo 6-phenylhexa 2,4-dienoic acid (HOPDA) when compared with 2-HMS, 4-methyl HMS and 4-chloro HMS. Km and Vmax of 2-HMS dehydrogenase for 2-HMS, 4-Methyl HMS, 4-chloro HMS and for NAD+ are calculated from Lineweaver-Burk plots. The kinetic parameters showed that the enzyme has high catalytic efficiency in terms of Vmax/Km towards 4-methyl HMS followed by 2-HMS but very low for 4-chloro HMS. On the other hand the catalytic efficiency of the enzyme is overall low for NAD+ in the presence of different MCPs. The sequence analysis shows the open reading frame (ORF) corresponding to tomC consists of 1458 base pairs with ATG initiation codon and TGA termination codon. This gene can encode a polypeptide of molecular weight 52 KDa containing 485 amino acid residues. The deduced amino acid sequence of 2-HMS dehydrogenase encoded by tomC gene from B. cepacia G4 exhibited a highest 78% homology with that of corresponding enzyme encoded by aphC gene of Comamonas testosteroni, 64%-78% homology with those of reported HMS dehydrogenases, and 29%-70% homology with those of different kinds of dehydrogenases. From the alignment of amino acid sequence putative cofactor NAD+-binding regions and catalytic residues were identified. The 2-HMS dehydrogenase from B. cepacia showed significant phylogenetic relationship not only with the same enzyme from other bacteria, also with different dehydrogenases from evolutionarily distant organisms.

[PC1-23] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]

Differential regulation of gene expression by RNA polymerase II in response to DNA damage

Heo JeongHwa°, Han JeungWhan, Lee HyangWoo, Cho EunJung

Lab. of Biochem. & Mol. Biol., College of Pharmacy, Sungkyunkwan University

RNA polymerase II (pol II) is known to cycle between hyperphosphorylated and hypophosphorylated forms during transcription cycle. These extensive phosphorylation/dephosphorylation event occurs in the C-terminal domain (CTD) of the largest subunit of pol II which consists of a tandemly repeated heptapeptide motif with consensus of YSPTSPS. Since different phosphorylation pattern of CTD is the hallmark of transcription regulation, we asked whether changes in CTD phosphorylation is involved in global regulation of transcription in response to DNA damage.
Taking the advantage of chromatin immunoprecipitation assay, we have demonstrated that pol II or pol II with nonphosphorylated CTD uniformly associated with a transcribed gene from a promoter to a coding region. While TFIIF (Tfg2) or TFIIH (Kin28) associated predominantly at a promoter region. With several antibodies against selected transcription factors and also antibodies against different CTD phosphorylation epitopes, we describe the response of transcription complex in terms of CTD phosphorylation and its role in gene expression upon DNA damage induced by various sources.

[PC1-24] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]