

Interaction Studies Between CDK and Epigallocatechin Gallate From *Solanum nigrum*

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Abstract

Epigallocatechin gallate is the most abundant catechin, it is present in plants, *Solanum nigrum* is a medicinal plant with many phytochemicals in them. The cell cycle is a normal process although if controlled can lead to cancer, the uncontrolled proliferation of the cells are controlled by the antioxidant compounds present in them. This present study is to done on the fact that antioxidants in plants act as anticancer agents.

Keywords: Epigallocatechin gallate, *Solanum nigrum*, phytochemicals, antioxidants, anticancer agents

1. Introduction

EGCG is the most abundant catechin in plants and is a potent antioxidant that may have therapeutic applications in the treatment of many disorders (e.g. cancer). In a high temperature environment, an epimerization change is more likely to occur; however as exposure to boiling water for 30 straight minutes only leads to a 12.4% reduction in the total amount of EGCG, the amount lost in a brief exposure is insignificant. In fact, even when special conditions were used to create temperatures well-above that of boiling water, the amount lost scaled up only slightly.

1.1 Cell cycle

Cell division is one of the most conspicuous features of life, and thus several elements of the control of cell division are common to both prokaryotes and eukaryotes (Amon, 1998; Leatherwood, 1998). Marcos Malumbres and Mariano Barbacid (2005) reported that recent genetic evidence obtained with gene-targeted mice has shown that Cdk4 and Cdk6 are not needed for entry into the cell cycle after mitogenic stimuli and organogenesis; however, they are essential for the proliferation of some endocrine and hematopoietic cells. These findings had important implications both for our current understanding of the role of CdKs in regulating the mammalian cell cycle and for their potential use as therapeutic targets in cancer.

1.2 Cyclin dependent Kinase (CDK)

CDKs are the catalytic subunits of a large family of heterodimeric serine/threonine protein kinases whose best-characterized members are involved in controlling progression through the cell cycle. According to the latest versions of the human and mouse genomes, there are 11 genes encoding CDKs and 9 other genes encoding CDK-like proteins with conserved primary structure. Because the catalytic activity of these CDKs requires binding of a regulatory subunit, the term CDK is often used for the active heterodimeric complex. The activating partners of the cell-cycle CDKs, are molecules that are synthesized and degraded during each cell cycle and thus have been designated 'Cyclins'. Although this property has been used to define this Kinase family, not all activating partners of CDKs are synthesized and destroyed in a cyclical fashion. Indeed, the physiological role of most CDKs and their activating partners remains unknown.

1.3 Activation of cyclin dependent Kinase

Activation of CDKs also requires phosphorylation of conserved Thr residues within their T-loops, such as residues Thr161 and Thr160 in human CDK2. This activating phosphorylation is catalyzed by CDK-activating kinases (CAKs) (Kaldis, 1999). Upon phosphorylation of Thr160 by CAK, the T-loop moves away and the Cyclin A-CDK2 complex becomes fully active (Jeffrey *et al.*, 1995; Russo *et al.*, 1996).

1.4 G1 Cyclin-dependent kinases

In mammalian cells, there are two classes of CDKs that function at the G1/S phase transition (Sherr, 1994; Reed, 1997; Draetta, 1994). CDK4 and its close relative CDK6 are driven by three D-type Cyclins: D1, D2 and D3. The primary target of these activities is pRb and related proteins (Grana, 1998). Cyclin E accumulates very close to the G1/S phase transition and specifically activates

CDK2. Although Cyclin-E – CDK2 has a secondary role in phosphorylating pRb (Zhang *et al.*, 2000; Harbour *et al.*, 1999), after Cyclin-D – Cdk4/6 the critical target(s) of Cyclin-E – CDK2 in regulation of S phase is not known.

1.5 Regulation of Cyclin - dependent Kinase activity by Cyclins

Cyclin abundance oscillates during the cell cycle as a result of programmed synthesis and degradation, thereby assuring a limited window of CDK activation. Over expression of D-type Cyclins or Cyclin E during early G1 leads to premature S phase entry (Ohtsubo *et al.*, 1993; Quelle *et al.*, 1993; Resnitzky *et al.*, 1994) suggesting that the G1 Cyclins are at least partially rate limiting for S phase entry and confirming that regulation of CDK activity by Cyclin accumulation has biological consequences.

1.6 Cyclin-dependent Kinase function

Even in yeast, using sophisticated genetic analysis, only a few key targets have been identified. This is probably due to the output of CDK function being achieved by the phosphorylation of many proteins and not by the activation of linear signal cascades that are easy to identify both genetically and biochemically. One notable exception is the mammalian retinoblastoma susceptibility protein, pRb. pRb is a component of a transcriptional repression module that targets many genes whose products function during S phase or at the G1/S transition. Phosphorylation of pRb by G1 CDKs near the G1/S boundary relieves this repressive activity and allows transcription of S phase genes (Zhang *et al.*, 2000; Harbour *et al.*, 1999).

1.7 Phylogenetic Tree

A phylogenetic tree or evolutionary tree is a branching diagram or “tree” showing the inferred evolutionary relationships among various biological species or other entities based upon similarities and differences in their physical and/or genetic characteristics. The taxa are joined together in the tree are implied to have descended from a common ancestor.

1.8 GOR method

The GOR method (Garnier-Osguthorpe-Robson) is an information theory-based method for the prediction of secondary structure in proteins. The GOR method analyzes sequences to predict alpha helix, beta sheet, turn, or random coil secondary structure at each position based on 17-amino-acid sequence windows. The four matrices reflect the probabilities of the central, ninth amino acid being in a helical, sheet, turn, or coil conformation. In subsequent revision to the method, the turn matrix was eliminated due to the high variability of sequences in turn regions (particularly over such a large window). The method was considered as best requiring at least four contiguous residues to score as alpha helices to classify the region as helical, and at least two contiguous residues for a beta sheet.

1.9 Docking

In the molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Two approaches use a matching technique that describes the protein and the ligand as complementary surfaces. The second approach stimulates the actual docking process in which the ligand – protein pair wise interaction energies are calculated. Docking program depends on the two components: the search algorithm and scoring function. Hence docking plays an important role in the rational design of drugs.

2. Materials and methods

Solanum nigrum habit is a dicot weed, an African paediatric plant. It is an annual branched herb of up to 90 cm high dull dark green leaves, juicy, ovate or lanceolate, and toothless to slightly toothed on the margins. The classification of *Solanum nigrum* is given below in table-1

Table. 1 Classification of *Solanum nigrum*

Family	Solanaceae
Genus	Solanum
Species	nigrum
Botanical name	Solanum nigrum
Common name	night shade

2.1 Sequence retrieval

Many bioinformatics processes require a sequence as input. Retrieval of sequence is an important step in any bioinformatics analysis. Each and every sequence has its own unique accession number in any database. Sequence can be retrieved either by querying with keywords or by the accession number.

2.1.1 NCBI Database

The sequences used in this study were retrieved from NCBI database (<http://www.ncbi.nlm.nih.gov/>). The National Center for Biotechnology Information (NCBI) is part of the United States National Library of Medicine (NLM), a branch of the National Institutes of Health. The NCBI is located in Bethesda, Maryland and was founded in 1988 through legislation sponsored by Senator Claude Pepper. The NCBI houses genome sequencing data in Gen bank and an index of biomedical research articles in Pubmed Central and Pubmed, as well as other information relevant to biotechnology. All these databases are available online through the Entrez search engine.

2.2 Phylogenetic Tree

The sequence retrieved from NCBI database were multiple aligned and then the phylogenetic tree was constructed. A phylogenetic tree or evolutionary tree is a branching diagram or “tree” showing the inferred evolutionary relationships among various biological species or other entities based upon similarities and differences in their physical and/or genetic characteristics. The taxa joined together in the tree are implied to have descended from a common ancestor.

2.3 GOR method

The GOR method (Garnier-Osguthorpe-Robson) is an information theory -based method for the prediction of secondary structure in proteins. It was developed in the late 1970s shortly after the simpler Chou-Fasman method. The GOR method analyzes sequences to predict alpha helix, beta sheet, turn, or random coil secondary structure at each position based on 17-amino-acid sequence windows. The original description of the method included four scoring matrices of size 17×20, where the columns correspond to the log-odds score, which reflects the probability of finding a given amino acid at each position in the 17-residue sequence. The four matrices reflect the probabilities of the central, ninth amino acid being in a helical, sheet, turn, or coil conformation. In subsequent revisions to the method, the turn matrix were eliminated due to the high variability of sequences in turn regions (particularly over such a large window). The method was considered as best requiring at least four contiguous residues to score as alpha helices to classify the region as helical, and at least two contiguous residues for a beta sheet.

2.4 Pubchem

Pubchem is a database of chemical molecules and their activities against biological assays. The system is maintained by the National Center for Biotechnology Information (NCBI), a component of the National Library of Medicine, which is part of the United States National Institutes of Health (NIH). Millions of compound structures and descriptive datasets can be freely downloaded via FTP. Pubchem contains substance descriptions and small molecules with fewer than 1000 atoms and 1000 bonds.

2.4 Protein data bank

The protein data bank (PDB) is a repository for the 3D structural data of large biological molecules, such as proteins and nucleic acids. The data, typically obtained by X-ray crystallography or NMR spectroscopy and submitted by biologists and biochemists from around the world, can be accessed at no charge on the internet. The PDB is overseen by an organization called the world wide Protein Data Bank.

3. Results and Discussion

Figure 1 shows the phylogenetic tree for CDK sequence for plant and animal kingdom. Among those 41 organisms were selected and its sequence were retrieved from NCBI database. To construct a phylogenetic tree the sequence was first multiple aligned using FASTA tool. Then the phylogenetic tree was constructed using clustalw software. The result shows *Chlamydomonas reinhardtii* and *Volvox carteri f. nagariensis* are the ancestral organisms for this protein

The phylogenetic tree was constructed for both plant and animal kingdom of CDK family. The evolutionary rates of the plant kingdom and animal kingdom for CDK family members were expected to be different. Therefore phylogenetic analysis was performed by the NJ method (Felsenstein, 1981; Saitou and Nei, 1987). These methods are less likely to mislead than parsimony or compatibility method when the evolutionary rates differ among taxa (Felsenstein, 1978; Saitou and Imanishi, 1989). When taxa have dissimilar rates of substitutions, incorporating the differential rate of character changes at given sites by fitting the amino acid data set to gamma rate distributions facilitates a more accurate phylogeny (Yang, 1996).

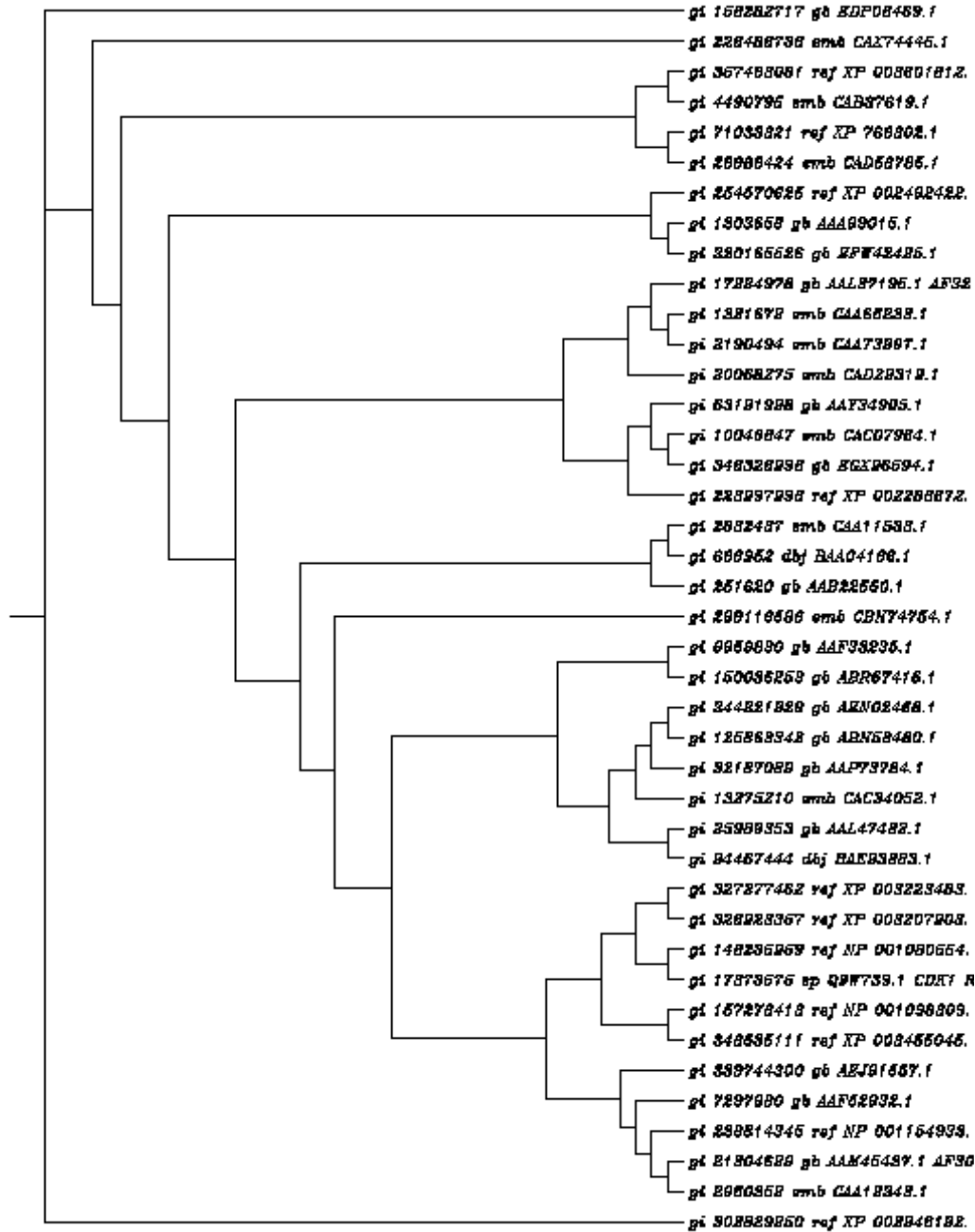


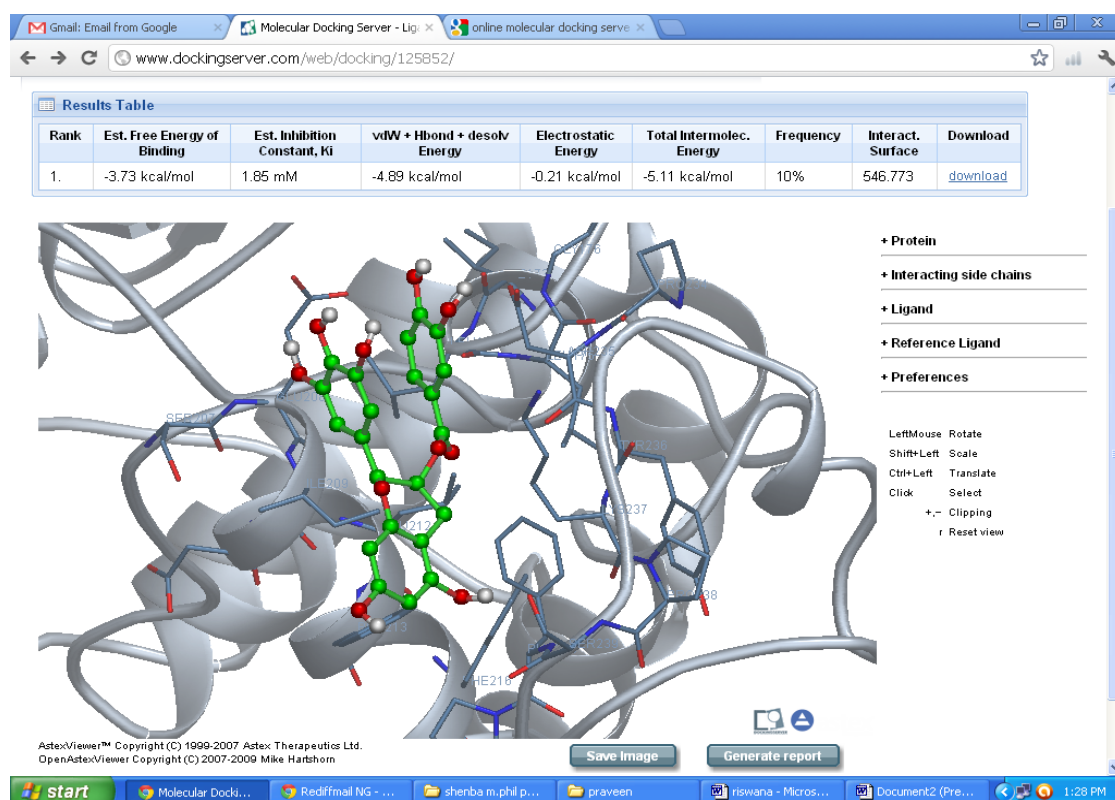
Figure 1. Phylogenetic analysis of CDK protein among various organism (Fungi, Plants, animals, Protozoa and Metazoa).

Figure 2 and 3 shows the secondary structure of *Chlamydomonas reinhardtii* and *Volvox carteri f. nagariensis* were predicted by using the GOR IV method. In this the sequence length of *Chlamydomonas reinhardtii* is 387. Then the secondary structure predicted result shows that it contained 49.61%, alpha helix region, 12.92% of Extended strand, 37.47% of random coil and 310 helix, pi helix, beta bridge, beta turn bend region, Ambiguous states and other states were found to be absent and for *Volvox carteri f. nagariensis* possess the sequence length is 392. It contained 43.88% of alpha helix region, 14.8% of extended strand and 41.33% of random coil. But the 310 helix, pi helix Beta bridge, beta turn, Bend region, Ambiguous states and other states shows 0% result which indicates their absence in the secondary structure prediction.

3.3 Interaction of CDK2 protein with Epigallocatechin gallate compound in *Solanum nigrum*

Docking of Epigallocatechin gallate with CDK2 protein is shown in the figure 4. Based on the information available it has been shown clearly that Epigallocatechin gallate compound in *Solanum nigrum* has been used as ligand for the CDK2 protein. Epigallocatechin gallate compound on interaction with CDK2 protein produced an bond energy value -4.89 Kcal/mol, Estimated free energy of binding -3.73 kcal/mol, the interaction surface area of 546.773 and the estimated inhibition constant (ki) 1.85 mM. Active groups present in the CDK2 protein were the site of binding to the ligand EGCG. The energy values were calculated using by using molecular modeling software how the pharmacophoric part of the drug was initially identified. The specificity and affinity between enzyme and its inhibitor depend on directional hydrogen bonds and ionic interactions, as well as on shape complementarity of the contact surfaces of both partners (Canduri et al., 2001; De Azevedo, 2001; Kim, 1996). CDK2 crystal structures identified to date shows that ligands binding at the ATP binding- site should form a hydrogen bond. Based on the information available in the literature it has been shown clearly that (-) - Epigallo catechin3- gallate compound in *Solanum nigrum* has been used to interact with CDK2 protein.

Fig. 4 Interaction of CDK2 protein with Epigallocatechin gallate compound in *Solanum nigrum*



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