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ABSTRACT

Human cytochromes present in lungs, plays an important role in the metabolic activation of chemical ca.rcinogens, and in particular, is thought to be linked to lung cancer. The mechanism of carcinogenesis is related to the enzyme's ability to oxidize highly toxic compounds, such as polycyclic aromatic hydrocarbons (PAHs), to their carcinogenic derivatives. In order to better understand Cytochrome P450 (CYP) function, a homology model of this enzyme has been constructed with its isoforms CYP 1A1, CYP 1A2, and CYP 1B1. CYP substrates, such as benzo[a]pyrene [B(a)P], benzo[a]pyrene 7,8 dihydrodiol [B(a)P diol] and Retinoic acid (RA) were docked into the active site of the model, binding interactions and key amino acid residues able to interact with the substrate, have been identified. The analysis of enzyme-substrate interactions indicated that Vander waals, hydrogen and hydrophobic interactions are mainly responsible for binding of these substrates in the active site. CYP 1A1 and 1A2 shows the binding similarities comparing with CYP1B1. Key residues Ala and Gly in the position 317 and 318 play an important role both in procarcinogen activation and RA binding. Additionally, the binding free energy calculations were performed for the three substrates. Lower binding energy required for RA binding than procarcinogen activation. The obtained values were similar to those observed experimentally, which suggests that this approach might be useful for prediction of binding constants.

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INTRODUCTION

Carotenoids have been reported to have multiple biological activities such as anticarcinogic, and as a chemopreventive agent against cancer in various organs like lung, stomach, colon, breast and prostate. Also carotenoids can play important role

their ability to form vitamin A in the body **(1)**. Carotenoids are considered potential membrane antioxidants due to the way they react with oxygen FR and singlet oxygen - a non radical pro oxidant found in biological system and capable of damaging protein, lipids and DNA **(2).**

Carotenoids strongly interact with ROS and thus act in plant and animal organisms as potent FR quenchers, singlet oxygen scavengers, lipid antioxidants and chain breaking antioxidants and some of them also serve as precursors for retinoids. Consequently carotenoids have been instantly studied by organic chemists, food chemists, biologists, physiologists, medical doctors and recently also by environmentalists and great demands have been placed on their identification and determination **(3, 4)**. The nutritional and therapeutic relevance of dietary carotenoids is attributed to their ability to act as provitamin A, they can be converted into vitamin A and vitamin A analogue by the human body. Moreover, vitamin A plays a protective role by preventing the formation of ROS **(5)**.

In many animals, the most important metabolic product of carotenoids is the retinoids and the metabolic reactions of carotenoids in animals are essentially oxidative. However, pathways of reductive metabolism have been discovered and this has opened up the possibility that xanthophylls could be precursors of retinoids - retinol, 3-dehydroretinol and 3-dehydroretinal. From the structural point of view, retinol precursors must have at least one unsubstituted β-ring attached to a conjugated polyene structure that is intact from C-7 to C-15. Reports confirmed that there was an increase in levels of retinol and 3-dehydroretinol after feeding the retinoid depleted diet supplemented with xanthophylls such as astaxanthin, canthaxanthin, zeaxanthin, lutein, and tunaxanthin **(6)**.

Retinoids [vitamin A and its derivatives, which include all-trans retionic acid (atRA)] play a central

obtained in the diet in the form of carotenoids (provitamin A), or preformed retinoids. Retinol (vitamin A) is the major retinoid absorbed after complex metabolic reactions in the intestines, and is stored in esterified form in the liver. After ester hydrolysis, retinol is then transported by a plasma protein (retinol binding protein) to the tissues where it can exert its activity. A small fraction of the plasma retinoids is available in the form of atRA bound to albumin, which is rapidly taken up by tissues **(9).** The conversion of retinol to retinal by the P450s is considered to be the rate-limiting step for the biosynthesis of atRA, and, in addition to retinol dehydrogenases, P450s 1A1, 1A2, 1B1, and 3A4 have recently been shown to be the major human P450s that catalyze this reaction **(10).**

Biological responses to retinoids may therefore be modulated by the availability of a specific ligand, and also by the type of nuclear receptors available. In addition to the nuclear receptor-mediated responses to retinoids, it has also been suggested that retinoylation, or covalent binding of retinoids to specific proteins, may also play a role in cellular response to atRA **(11).** AtRA is considered the active form of vitamin A and is involved in gene regulation, leading to differentiation and apoptosis of normal and cancer cells **(12).** AtRA undergoes P450-mediated metabolism to oxidized compounds that include 4-OH-RA, 4-oxo-RA, 18-OH-RA, and 5,6-epoxy-RA **(13).** Although the metabolism of atRA has been considered to be a catabolic process, some of its oxidized metabolites have been shown to display biological activity in the modulation of genes expressed in apoptosis, cellular growth and differentiation, embryonic development, and in the inhibition of proliferation of several normal and neoplastic cells *in vitro* **(14)**.

Studies using animal material have shown P450s to be important in the oxidation of atRA **(15-16)**, and several P450s have been identified in rabbits **(17).** In humans, only a few P450s have been ascribed to

enzymes in toxicant biotransformation and drug metabolism. The CYP family is responsible for the metabolic activation of some known procarcinogenic environmental chemicals, toxins, and toxic drugs **(21).** It has been shown that CYP1A1 plays a predominant role in the oxidation of benzo[a]pyrene and other polycyclic aromatic hydrocarbons (PAHs) to mutagenic epoxides and diols **(22)**. For example, dibenzo[a]pyrene, considered the most potent carcinogen among all PAHs, is oxidized almost exclusively by 1A1 to highly mutagenic diol-epoxides **(23)**. This CYP is essentially an extrahepatic enzyme, present in the liver at only very low levels and found primarily in lungs, where it is strongly induced by cigarette smoking and thereby associated with lung cancer **(24)**. High activity of CYP1A1 is suspected to increase cancer risk, and all substances resulting in enzyme induction may also increase the risk of tumor initiation **(25).** The elucidation of the structural basis of P450 1A1 specificity is of great importance in understanding enzyme function and mechanism, and may provide a foundation for the rational design of drugs and inhibitors.

MATERIALS AND METHODS

The docking procedure consisted of four main steps: preparation of target protein, preparation of ligand, docking, and post docking analysis.

Preparation of target protein

To initially validate the pharmacological interactions, three therapeutic protein targets, including cytochrome P_{450}1A1 (CYP1A1), cytochrome P_{450}1A2 (CYP1A2), and cytochrome P_{450}1B1 (CYP1B1) were prepared. The three dimensional structures were obtained from Protein Data Bank (PDB) **(26).**

Preparation of ligand

To check the binding interactions of the carcinogen with the target protein, the procarcinogen B(a)P, its primary product B(a)P 7,8 dihydrodiol and the cleavage product of carotenoids (carotene and xanthophylls) retinoic acid (RA) were selected as

*i*GEMDOCK that provides interactive interfaces to prepare both the binding site of the target protein and the screening ligand library. Each ligand in the library was docked into the binding site by using the in-house docking tool **(27).**

Post docking analysis

Subsequently, *i*GEMDOCK generates protein-ligand interaction profiles of electrostatic (E), hydrogen (H), and Vander Waals (V) interactions. Based on these profiles and ligand structures *i*GEMDOCK infers the pharmacological interaction and the clusters of the screening compounds for post docking analysis. Finally *i*GEMDOCK ranks and visualizes the docking compounds by combining the pharmacological interactions and energy based scoring function of *i*GEMDOCK.

RESULTS AND DISCUSSION

Molecular simulation such as molecular docking has become an important approach to elucidate between ligand and macromolecular target, which is a rapid low cost detection system that has been successfully used in the macromolecule - ligand interactions. The analysis was carried out to study the possible relationship between lung damage and cytochrome expression. The binding effect of B(a)P and retinoic acid with CYP the similarities and dissimilarities in the binding sites were observed by the docking studies.

Docking of B(a)P with Cytochrome P_{450}

The molecular docking was aimed to find out the interactions between the enzymes CYP isoforms CYP1A1, 1A2, 1B1 and B(a)P. Docking results that showed the interactions between the CYP with B(a)P are depicted in **Figure 1**. The packets for B(a)P binding possibly exist in the active centre of CYP that were identified by molecular docking. The present results indicate that the Vander Waals interaction was observed to be the characteristic binding of B(a)P. The best position for B(a)P binding was determined. Residues defining the binding

other amino acids that existed in the active centre were Asp and Gly. Moreover, molecular docking showed that the predicted average energy for B(a)P binding were -100.22 for CYP1A1 and CYP1A2 and -111.46 for CYP1B1, which have the values higher than other PAHs. In order to further explore binding characteristics of substrate, the binding

experiment. The results are indicated in **Table 1.** The isoforms CYP1A1 and CYP1A2 show similar amino acid residues in the binding cavity of B(a)P. CYP1B1 is another form which shows the binding interaction involved three Phe residues (Phe 134, Phe 231 and Phe 268) within the binding packet.

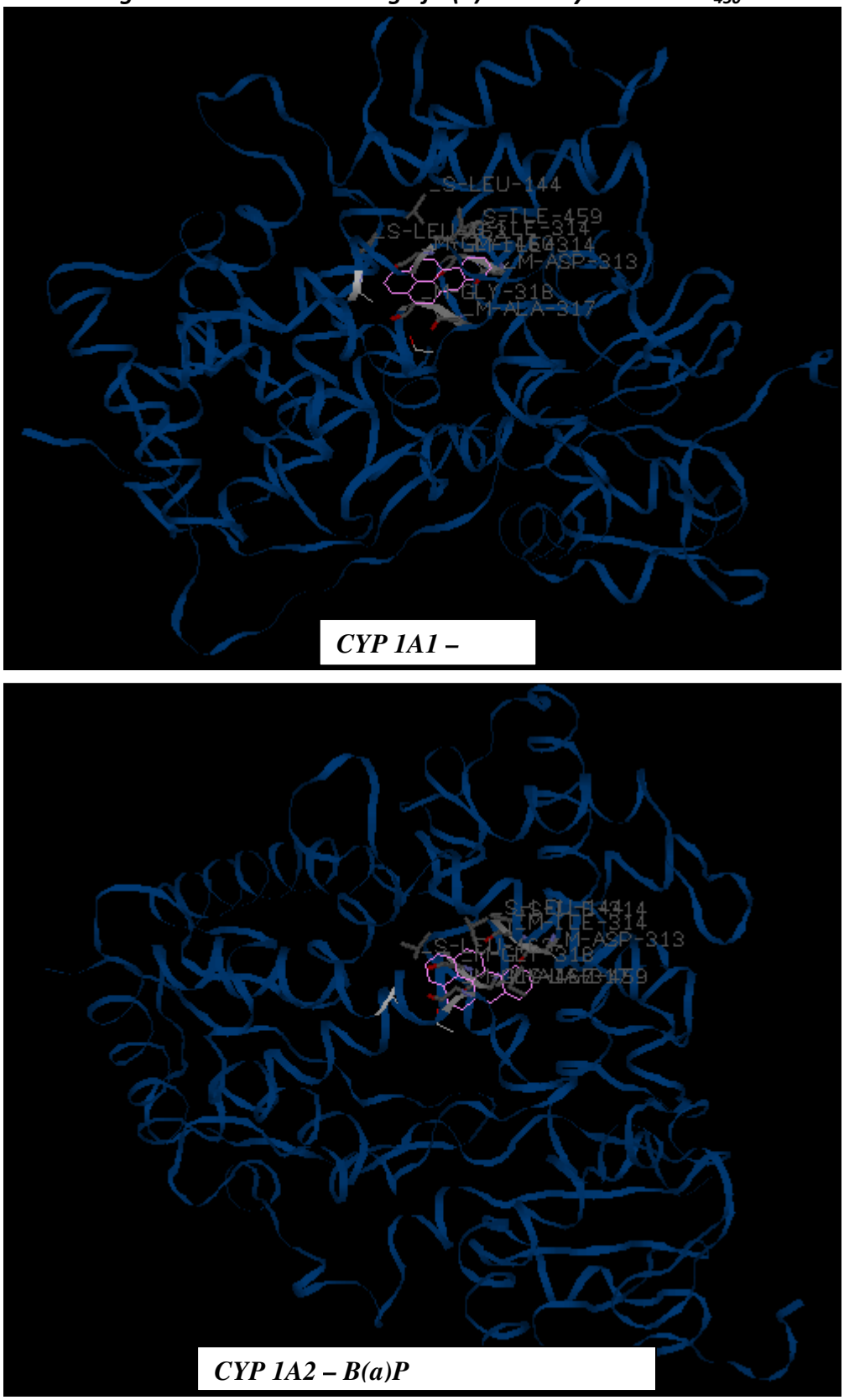
Table 1

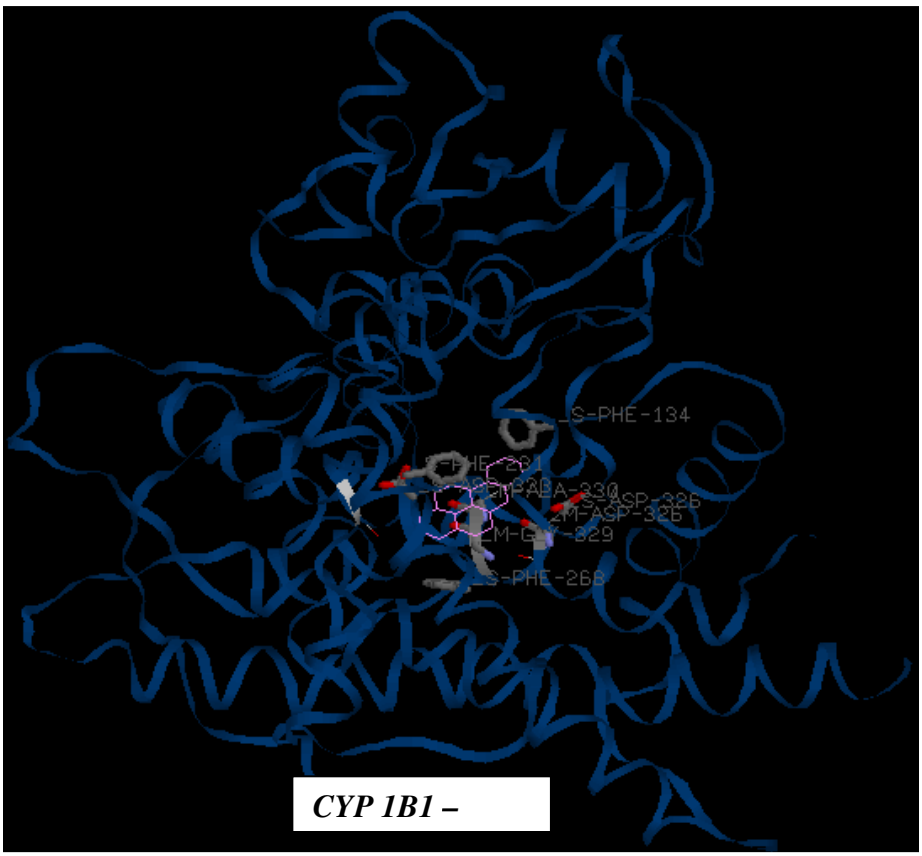
Energy levels and bonding interactions on molecular docking with Cytochrome P_{450}

Enzyme	Ligand	Energy	VDW interaction	Hydrogen Bonding	Electrostatic interaction	Amino acids
CYP1A1	B(a)P	-100.23	-100.23	-	-	Leu-144, Asp-313, Ile-314, Ala-317, Gly-318, Ile-459, Gly-460, leu-463
CYP1A2	B(a)P	-100.21	-100.21	-	-	Leu-144, Asp-313, Ile-314, Ala-317, Gly-318, Ile-459, Gly-460, leu-463
CYP1B1	B(a)P	-111.46	-111.46	-	-	Phe-134, Phe-231, Phe-268, Asp-326, Gly-329, ala-330, Asp-333
CYP1A1	B(a)P 7,8 dihydrodiol	-106.09	-100.09	-6.0	-	Thr--124, Ile-459, Leu-144, Ile-314, Ala-317, Gly-318, Cys-458, Ile-459, Gly-460, leu-463
CYP1A2	B(a)P 7,8 dihydrodiol	-106.39	-99.09	-7.3	-	Asp-313, Leu-144, Asp-313, Ile-314, Ala-317, Gly-318, Ile-459, ly-460, Leu-463
CYP1B1	B(a)P 7,8 dihydrodiol	101.65	-99.15	-2.5	-	Asp-326, Ile-327, Ala-330, Ser-331, Ile-471, Gly-472
CYP1A1	RA	-77.96	-62.26	-12.71	-2.99	His-388, Met-453, Gly-318, Ala-317, Asp-320, Thr-385, Ile-386, Arg-456, Leu-497
CYP1A2	RA	-86.04	-86.29	-	0.25	Leu-124, Phe-125, Phe-226, Phe-260, Ala-317, Gly-318, Leu-382, Ile-386, Phe-451
CYP1B1	RA	81.14	-78.78	-1.23	-1.13	Arg-194, Ser-226, His-227, Asn-228, Arg-233, Gly-236, Ser, 239, Asn-504, Tyr-507, Gly-508, Lys-514

CYP- Cytochrome $P_{450,}$ B(a)P- Benzo (a) Pyrene, RA- Retinoic acid, VDW interaction- Vander waal's interaction

Figure 1 Molecular docking of B(a)P with Cytochrome P_{450}





CYP 1B1 –

CYP1A1, a phase I metabolizing enzyme that is involved in the bio activation of carcinogenic PAH has been strongly implicated in lung cancer. Exposure of PAH induces the production of lung and liver CYP isoforms, CYP1A1 and CYP1A2 **(28)**. Beta carotene supplementation determining a substantial accumulation of beta carotene itself in the considered organs was able to act on cytochrome isoforms in all tissues **(29)**. Indeed if PAH is incorporated to humans, similar increase in cytochrome levels could raise in the risk of lung cancer in heavy smokers, due to the immense range of procarcinogen.

CYP1A2, CYP2A1 and CYP3A2 are reported to be responsible for metabolic activation of tobacco in rats. On the other hand, CYP1A1 and 1A2 are known to be selectively involved in metabolic activation in humans **(30)**. Present result established the direct correlation between the predicted and the experimentally measured binding energy **(31)**. Human CYP1A1 and 1A2, the two major isoforms in the P_{450}1A sub family, share 72% amino acid sequence identity but display different substrate specificities. CYP1A1 prefers to metabolize B(a)P, where as CYP1A2 favours the oxidation of heterocyclic aromatic amines **(32)**.

Human CYP1A1 which is present in lungs plays an important role in the metabolic activation of chemical carcinogens and in particular is thought to be linked to lung cancer. The mechanism of carcinogenesis is related to the enzyme's ability to oxidize highly toxic compounds such as PAHs to their carcinogenic derivatives **(33)**. Molecular docking of CYP1B1 based on crystal structure suggested that there are clear differences in the PAH inhibitors with CYP1B1 and CYP1A isoforms and that these differences may explain why PAH inhibitors inhibit CYP enzymes by different mechanisms **(34).**

The present analysis of enzyme substrate interactions indicated that Vander Waals interactions are mainly responsible for the binding of these substrates in the active site. Thus in the present study a step was undertaken to examine the docking of a planer B(a)P to ensemble models would yield distributions of conformations that agree with experimentally determined region specific patterns of oxidation. In the case of B(a)P,

the Vander Waals contribution was similar to that of calculated results, but there were no electrostatic contribution which indicates more favourable binding in case of B(a)P. As shown in **Table 1**, predicted binding energies for B(a)P examined in the different isoforms were very close to the observed values. This close agreement strongly suggests that binding free energy calculations may have a predictive value.

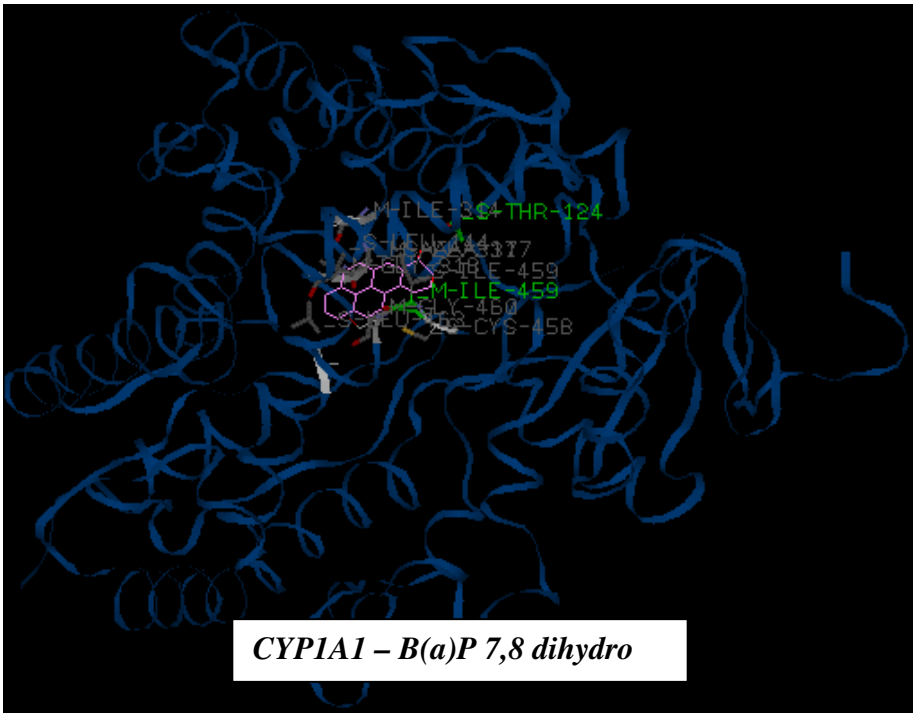
Docking of B(a)P 7,8 dihydrodiol with Cytochrome P_{450}

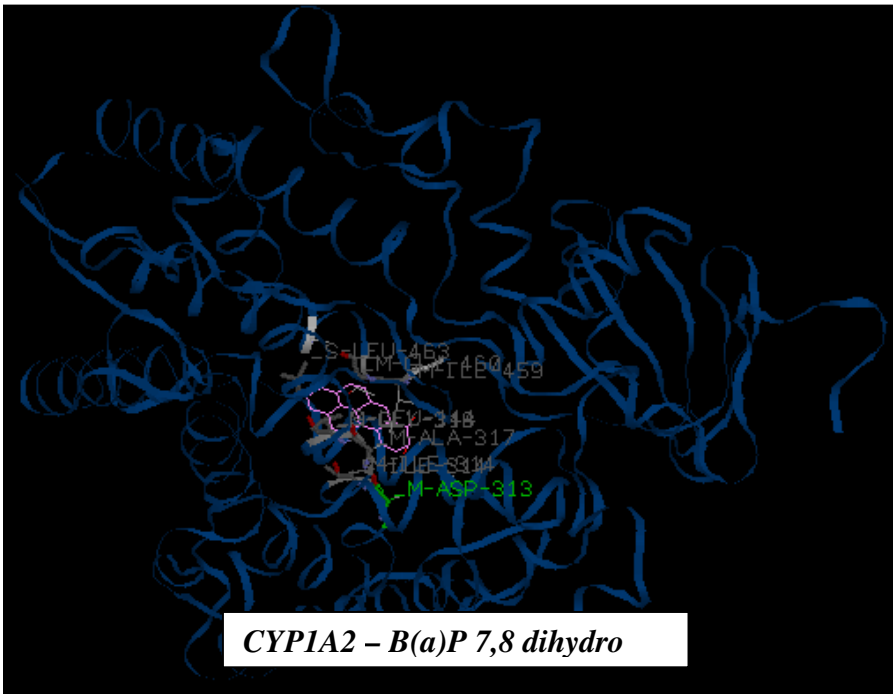
Benzo(a)Pyrene 7,8 dihydrodiol is the primary activation product of B(a)P by the action of CYP. Benzo(a)Pyrene 7,8 dihydrodiol docked with all the cytochrome iso enzymes which show the similar binding packet occupied by the B(a)P 7,8 dihydrodiol for the further activation. From the **Figure 2,** it was confirmed that the amino acids Leu, Ile, Ala, Gly are found as common amino acids that involve both the B(a)P and B(a)P 7,8 dihydrodiol interactions with CYP1A1 and CYP1A2 which further confirms these amino acid residues are residing in the active centre of the enzyme. In case of CYP1B1 Asp and Ala play the major role in the bonding interaction with B(a)P and B(a)P 7,8 dihydrodiol. The average energy required for the bonding interactions is quite higher than the B(a)P interaction that is found to be -106.24. The required overall energy is needed for the formation of Vander Waals interaction and hydrogen bonding between CYP and B(a)P 7,8 dihydrodiol **(Table 1)**.

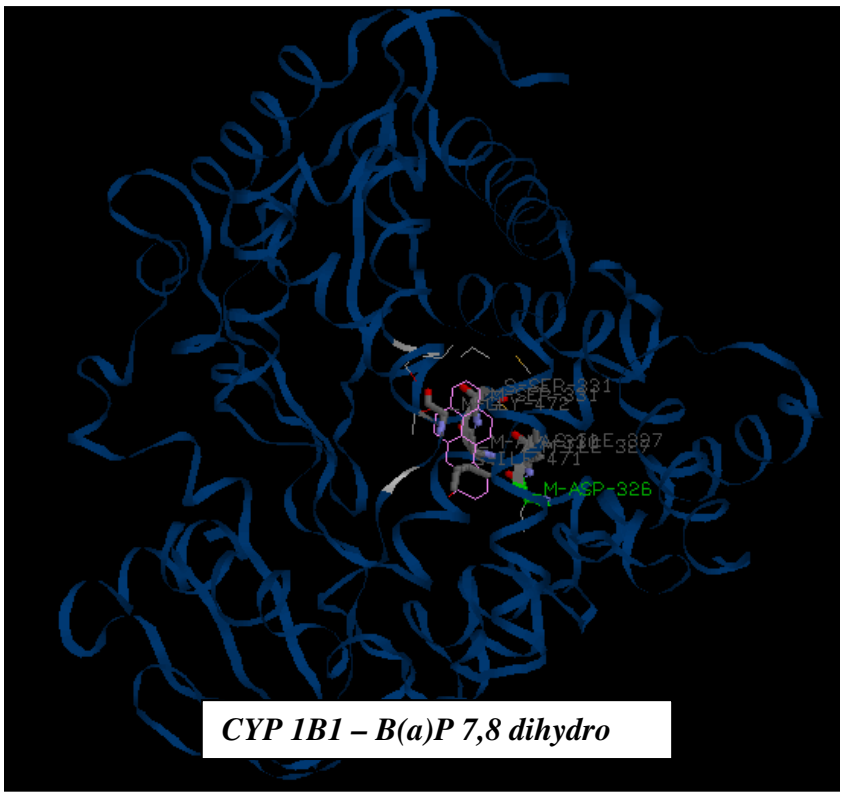
B(a)P, a promutagenic and procarcinogenic prototype PAH requires metabolic activation by CYP1A1 to exert toxicity. The required metabolic activation of B(a)P to 7,8 dihydrodiol to the ultimate carcinogen B(a)P 7, 8 diol 9, 10 epoxide is analogous to the bio activation mechanism. Several different metabolites of B(a)P are derived from P_{450} pathways represented by CYP1A1 and CYP1B1 produces the 7,8 dihydrodiol of B(a)P. B(a)P 7,8 diol can enter another round of metabolism by CYP1A1 or CYP1B1 to form 7,8 diol 9,8 epoxide of B(a)P. The proven key carcinogenic product B(a)P 7,8 dihydrodiol 9,8 epoxide, interacts rapidly with DNA base pairs to form a complex, which undergoes protonation to yield an intercalated triol carbonium ion intermediate **(35).** The results of the previous studies indicate that CYP1B1 also carries out metabolism of B(a)P along the pathway to the postulated ultimate carcinogen, the diol epoxide formation at rates much higher than CYP1A2 but less than CYP1A1. This is in agreement with the other reports that CYP1B1 appears to be very active for B(a)P and 7,8 diol activation process. CYP1B1 may significantly contribute to B(a)P and 7,8 dihydrodiol metabolism and carcinogenesis in human tumour models **(36).** Similar analysis was carried out using CYP1A2 B(a)P adapted to CYP1A2 in other orientation than CYP1B1. The carbon at 8 or 9 positions of B(a)P was easy to approach to the iron of heme in CYP1A2 **(34)**.

The increase of carotenoid concentration increases the tendency toward the safe detoxification of the carcinogen B(a)P. The presence of carotenoid concentrations in hepatic B(a)P metabolism leads to a selective in 7,8 dihydrodiol. These carotenoids put together not only inhibit the chemical carcinogenesis of B(a)P but also facilitate the natural removal of the parent carcinogen. Beta carotene is the strongest of the all carotenoids in the anticarcinogenic and detoxification activities.

Figure 2 Molecular docking of B(a)P 7,8 dihydrodiol with Cytochrome P_{450}







CYP 1B1 – B(a)P 7,8 dihydro

Docking of Retinoic acid with Cytochrome P_{450}

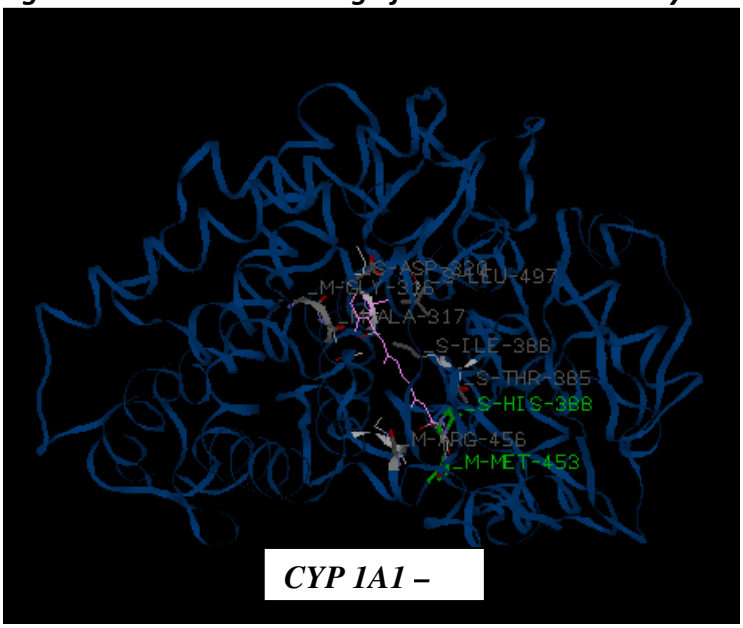
In the present study, to understand whether the anticarcinogenic effects of carotenoids on B(a)P metabolism are related to its possible carcinogen inactivating mechanism, the carotenoid cleavage product retinoic acid was docked with the carcinogen activating enzyme and the binding interactions are depicted in **Figure 3** and **Table 1**. According to the presented results, there are three different binding interactions (Vander Waals, hydrogen and electrostatic interactions) identified between cytochrome and RA. The overall binding energy was comparatively less than B(a)P and B(a)P 7,8 dihydro diol binding energy obtained for the same cytochrome enzymes. To elaborate the amino acid involvement in the interaction the results indicate that there were a variety of amino acids such as His, Met, Gly, Ala, Asp, Thr, Ile, Arg, Leu directly involved in the bonding interaction of RA. In the helix, the backbone of CYP Ile 386 established an inter H bond with the backbone carbonyl oxygen of Ala 317, keeping the helical secondary structure stable. The ligand binding packet in the present model is more compact, surrounded mainly by the other residues. The side chains were all pointing towards binding packet, indicating structurally their direct involvement. Additional hydrogen bonding interactions were noted between the carboxylate group of RA and Arg 456 of CYP1A1. In these interactions Gly and Ala in the position 317 and 318 were directly involved in the bonding interaction of B(a)P and B(a)P 7,8 dihydrodiol with CYP1A1 and CYP1A2 and were also utilized by RA for its interaction in the both isoforms. These findings clearly indicate that RA also interacts through the active centre of the procarcinogen B(a)P and B(a)P 7,8 dihydrodiol in CYP, and interferes with the binding of procarcinogen. Through that RA inhibits the activation of procarcinogen to ultimate carcinogen. Present investigation clearly shows that these two residues were in close proximity neither to the substrate nor to any other residue essential for the activity.

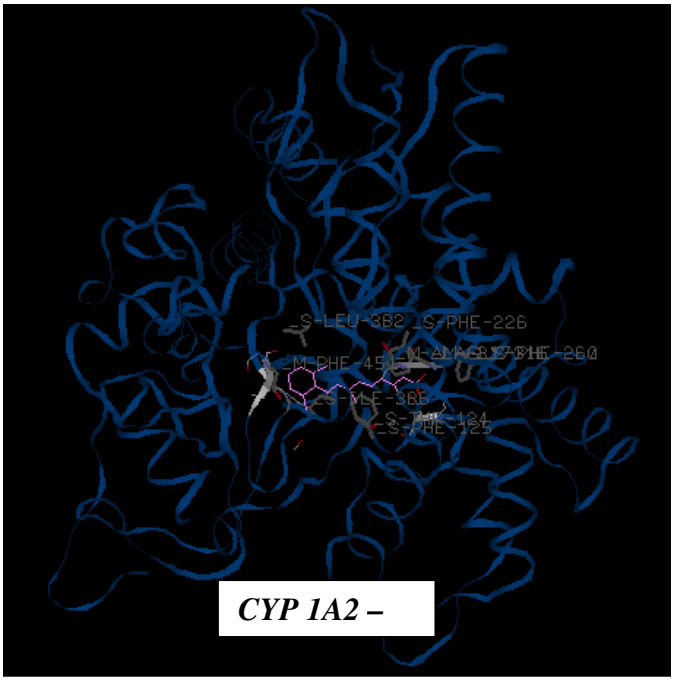
But in contrast no such similarity was found in B(a)P and RA interactions in CYP1B1. The docking experiments support the identification of the binding sites in CYP with the previous findings. In this respect it is interesting to compare the structural model of binding presented here with

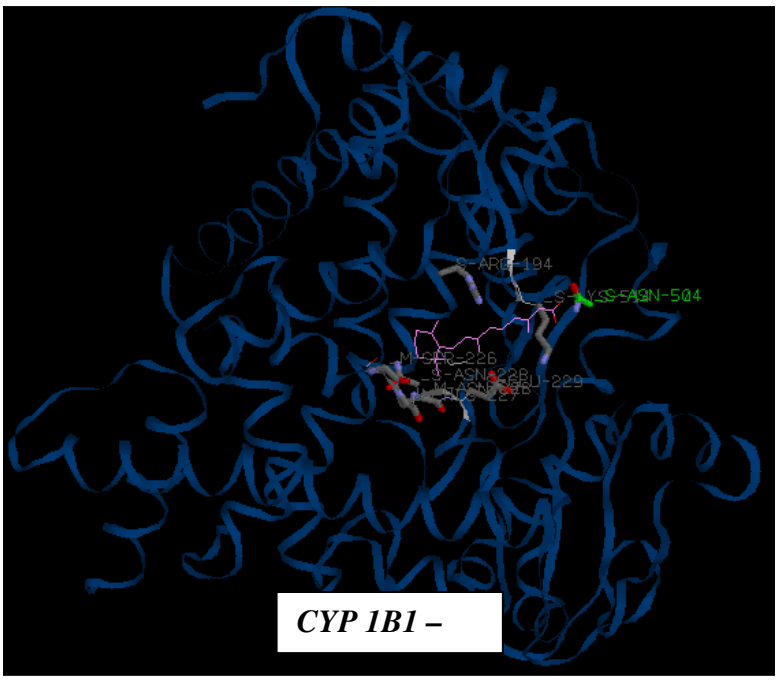
the experimental data. CYP has been shown to be active with RA in trans configurations. Cis configuration is identical to trans except in the position of double bond and the rest of the molecule is identical. This is consistent with the dimeric model, which in the active form of cytochrome contains the cavity for RA. Several inter chain interactions further support the dimeric model **(37)**.

Retinoic acid is among the 99 molecules consistently predicted to bind best within the CYP1201A1 active site. A more detailed analysis described that the binding models form a cluster where the reactive carbon is predicted to be the carbon atom on the β-ionone ring cycle located between the hydrophobic residues Ala94, Phe253, Leu20, Thr258 with the reactive carbon 3.6 away from the iron atom **(38)**.

Figure 3: Molecular docking of Retinoic acid with Cytochrome P_{450}







CYP 1B1 –

Findings from the present study suggest that the administration of carotenoids enhance retinoic acid catabolism through the induction of CYP1A1 and CYP1A2 in lungs, because β-carotene supplementation affects molecular markers in human lungs and it was measured and compared **(28, 39)**. Reports confirm that several carotenoids including LU, AX exert their antioxidant and anticarcinogenic activity through the production of RA analogue metabolites **(40-42).** Increased dietary carotenoids and xanthophylls through the CYP mediated protection alter the steady state concentration of carotenoids in the body fluids or tissues and serve as localized substances for retinoid formation. RA which exerts striking effects on diverse precursors such as growth, development and differentiation can be produced from carotenoids during intestinal metabolism both in animals and humans **(43)**.

From the present findings it is evident that vitamin A is a member of retinoid families, obtained from βC - a preliminary carotenoid and from other xanthophylls. It exists in several forms, such as retinol, retinal and retinoic acid. Inter conversions between these chemical forms readily occur in the body. Administration of RA and its analogue producing carotenoids, to animals, was reported to delay, arrest and even reverse the progression of premalignant and malignant characteristics. Available online on www.ijprd.com

Multiple possibilities exist: certain carotenoids can be converted to retinoids that can modulate the enzymatic activities of lipoxygenases and can have antioxidant properties by activating the proteins. On the other hand, it should be recognized that carotenoids were acting as an effective agent. However there is increasing evidence that oxidation products of carotenoids, especially RA analogues, significantly contribute to this biological property.

CONCLUSION

Docking studies were carried out with PDB structures of cytochrome P_{450} isozymes CYP1A1, 1A2, and 1B1. The procarcinogens B(a)P and B(a)P 7,8 dihydrodiol and the metabolic product of carotenoids, RA were used as ligands. The docking results confirm that the cytochrome isoforms 1A1 and 1A2 share similar amino acids which were involved in the interaction of B(a)P and B(a)P 7,8 dihydro diol activation with similar binding energy, whereas 1B1 shows quite different set of amino acids with difference in binding energy indicating their structural variation. The RA docking in the CYP 1A1 and 1A2 shows that a variety of amino acids were involved in the binding interaction among which Ala and Gly in the position 317 and 318 were directly involved in binding of B(a)P and B(a)P 7,8 diol. The results confirms that RA interacts through

B(a)P binding site with lowest binding energy, suggesting RA and RA analogue producing carotenoids interfere the carcinogen in the CYP binding sites and blocking the procarcinogen activation thereby establish their chemopreventive action.

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