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Impact of structural differences in carcinopreventive agents indole-3-carbinol and 3,3′-diindolylmethane on biological activity. An X-ray, ^{1}H–^{14}N NQDR, ^{13}C CP/MAS NMR, and periodic hybrid DFT study

Jolanta Natalia Latosińska [a,*], Magdalena Latosińska [a], Marek Szafrański [a], Janez Seliger [b,c], Veselko Žagar [c], Dorota V. Burchardt [d]

[a] Faculty of Physics, Adam Mickiewicz University, Umultowska 85, 61-614 Poznań, Poland
[b] Faculty of Mathematics and Physics, University of Ljubljana, Jadranska 19, 1000 Ljubljana, Slovenia
[c] "Jozef Stefan" Institute, Jamova 39, 1000 Ljubljana, Slovenia
[d] Department of Paediatric Dentistry, Poznan University of Medical Sciences, Bukowska 70, 60-812 Poznań, Poland

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ABSTRACT

Three experimental techniques ^{1}H–^{14}N NQDR, ^{13}C CP/MAS NMR and X-ray and Density Functional Theory (GGA/BLYP with PBC) and Hirshfeld surfaces were applied for the structure–activity oriented studies of two phyto-antioxidants and anticarcinogens: indole-3-carbinol, I3C, and 3,3′-diindolylmethane, DIM, (its bioactive metabolite). One set of ^{14}N NQR frequencies for DIM (2.310, 2.200 and 0.110 MHz at 295 K) and I3C (2.315, 1.985 and 0.330 MHz at 160 K) was recorded. The multiplicity of NQR lines recorded at RT revealed high symmetry (chemical and physical equivalence) of both methyl indazole rings of DIM. Carbonyl ^{13}C CSA tensor components were calculated from the ^{13}C CP/MAS solid state NMR spectrum of I3C recorded under fast and slow spinning. At room temperature the crystal structure of I3C is orthorhombic: space group $Pca2_1$, $Z = 4$, $a = 5.78922(16)$, $b = 15.6434(7)$ and $c = 8.4405(2)$ Å. The I3C molecules are aggregated into ribbons stacked along [001]. The oxygen atoms are disordered between the two sites of different occupancy factors. It implies that the crystal is built of about 70% *trans* and 30% *gauche* conformers, and apart from the weak O—H···O hydrogen bonds (O···O = 3.106 Å) the formation of alternative O′—H···O bonds (O′···O = 2.785 Å) is possible within the 1D ribbons. The adjacent ribbons are further stabilised by O′—H···O bonds (O′···O = 2.951 Å). The analysis of spectra and intermolecular interactions pattern by experimental techniques was supported by solid (periodic) DFT calculations. The knowledge of the topology and competition of the interactions in crystalline state shed some light on the preferred conformations of —CH_2OH in I3C and steric hindrance of methyl indole rings in DIM. A comparison of the local environment in gas phase and solid permitted drawing some conclusions on the nature of the interactions required for effective processes of recognition and binding of a given anticarcinogen to the protein or nucleic acid.

1. Introduction

The increased incidence of cancer observed in last decades (Ferlay et al., 2010) has been a strong motivation to search for both new methods of cancer treatment and effective methods of its prevention (Antonio et al., 2014; Kelloff, 2000; Lee and Park, 2003). Therefore a rapid progress in drug design is accompanied with the discovery of new compounds, able to inhibit, delay or even reverse carcinogenesis (Landis-Piwowar and Iyer, 2014). The invaluable source of carcinopreventive agents are green plants (Latosińska and Latosińska, 2013). These agents can prevent the early stages of carcinogenesis (pre-initiation, initiation or promotion), but often have less impact on the later stages (progression or malignancy) (Landis-Piwowar and Iyer, 2014). Epidemiological studies and experiments on animals have permitted identification of certain active components associated with cancer chemoprevention in cruciferous vegetables from *Brassicaceae* family like cabbage, radishes, cauliflower, broccoli, Brussels sprouts, kale and daikon (Bradlow et al., 1999; Hecht, 2000; Higdon et al., 2007;

* Corresponding author.
E-mail address: Jolanta.Latosinska@amu.edu.pl (J.N. Latosińska).

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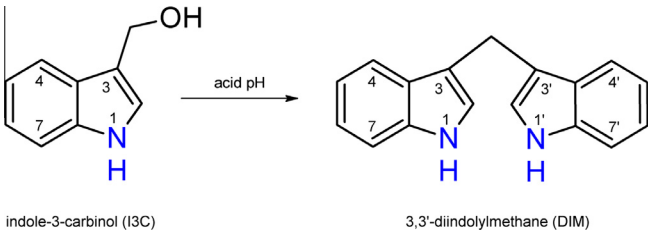

Fig. 1. Structural formula of I3C and DIM (atoms numbered as shown).

Kristal and Lampe, 2002; Kwon et al., 2007; Murillo and Mehta, 2001). Two indoles – 3,3′-diindolylmethane (DIM, $C_{17}H_{14}N_2$) and its precursor, indole-3-carbinol (I3C, C_9H_9NO), Fig. 1, discovered in the 1970s belong to the most popular cancer prevention dietary supplements promoting the healthier estrogen metabolism. I3C in contrast to DIM has been found highly reactive and less stable (Arnao et al., 1996). In water acidic environment, I3C undergoes complicated reactions and is metabolized to a number products, including DIM, indolocarbazole (ICZ), ascorbigen (ASG), Linear Trimer (LTR), Cyclic Trimer (CTR), Indole-3-acetonitrile (IAN), and many oligomers (linear and cyclic) (Brandi et al., 2003). The major, most active and effective metabolite and natural autolytic product in plants, DIM, has many biological properties common with I3C. I3C can suppress the proliferation of certain cancers, especially estrogen-dependent reproductive cancers: breast (Wong et al., 1997), cervical (Bell et al., 2000; Jin et al., 1999), ovarian (Raj et al., 2008), endometrial, prostate (Bradlow, 2008; Chinni et al., 2001; Kristal and Lampe, 2002; Sarkar and Li, 2004; Singh and Agarwal, 2006; Zhang et al., 2003) but also head and neck (Fowke, 2007), colon (Bonnesen et al., 2001), melanoma (Aronchik et al., 2014) or blood cells cancer–leukemia. Some authors (Hsu et al., 2006; Machijima et al., 2009; Singh and Agarwal, 2006) proved that I3C induces a G1 cell cycle arrest of human reproductive cancer cells, while the other (Raj et al., 2008) described inhibited cell proliferation, cell contraction and apoptosis. I3C anti-cancer properties result from its ability to modulate multiple signalling pathways which control DNA repair, hormonal regulation, inflammation, cell division and growth, apoptosis, angiogenesis, and multiple drug resistance (Aggarwal and Ichikawa, 2005; Weng et al., 2008).

I3C belongs to antiestrogens, negative regulators of oestrogen receptor-alpha signalling in human tumour cells. Preclinical and phase I trials have shown some efficacy and very low toxicity of I3C in breast and prostate cancers (Reed et al., 2005) treatment and beneficial activity for the suppression of currently incurable Human Papilloma Virus (HVP) (Jin et al., 1999) induced cervical cancers (e.g. cervical intraepithelial neoplasia (Bell et al., 2000)). It is also beneficial in the treatment of autoimmune diseases (Auborn et al., 2003), including systemic lupus erythematosus, SLE (Yan et al., 2009). Most findings suggests that many cancer-fighting effects of I3C are in fact a result of co-operation of I3C with DIM (Licznerska et al., 2013; Poornima and Mirunalini, 2014). Amongst them are anti-angiogenesis, anti-inflammation, anti-viral, anti-biotic, anti-cancer, anti-androgen effects as well as cytostasis, apoptosis and hormone control. The main difference between both compounds is the ability to DNA repair (*in vitro*) in DIM and the I3C ability to inhibit DNA adduct formation (*in vivo*). But some sources indicate that I3C is considered more active (Cover et al., 1998) but less consumer safe than DIM. In the mild pH environments, I3C may convert to toxic 3-methylindole leading to DNA-damaging adducts (Markushin et al., 2003). DIM contributes to the growth of breast cancer cells *in vitro* but in the absence of estrogen (Anderton et al., 2004). Pre-initiation exposure to I3C resulted in the reduction of hepatocellular carcinomas, while post-initiation strongly enhanced the tumour incidence (Bailey et al., 1987). Little is known about the differences between both compounds on molecular level and the modes of their action are still under study (Ahmad et al., 2010; Landis-Piwowar and Iyer, 2014).

Although the knowledge of structure is the basis for research and determination of the relationship between structure and function, only the crystalline structure of DIM (Maciejewska et al., 2005) has been resolved. The factors responsible for the large differences in water solubility (insoluble DIM versus soluble, 7 mg/ml, I3C) and melting point (436–439 K, DIM versus 369–372 K, I3C) are also not known. In our current study we combined different techniques: ^1H–^{14}N NQDR, ^{13}C CP/MAS NMR, X-ray and DFT calculations often (Latosińska, 2005, 2007; Latosińska et al., 2014a,b,c) used separately in the studies of pharmaceutically relevant solid samples. The X-ray diffraction method provided the information on the crystalline packing including the positions of all atoms and the effects of atomic vibrations (e.g. isotropic or anisotropic atomic motions resulting from internal static or dynamic disorder, lattice defects). Two non-destructive spectroscopies ^1H–^{14}N NQDR and ^{13}C CP/MAS (direct probes of the short-range order) delivered description of the neighbourhood of the vicinity of all nitrogen and carbon atoms, respectively and provided information on the nature of interactions from local single atoms perspective (Latosińska, 2005). To analyse crystalline packing 3D Hirshfeld surface approach was applied. The assignment and interpretation of the experimental solid spectra was performed within periodic DFT calculations. The correlation between spectral parameters obtained in the experiment and the calculations was used as a test of the quality of the wave function (important factor for further reliable description of the inter- and intra-molecular interactions). Both I3C and DIM are capable of forming complexes with nucleic acid or proteins. The susceptibility of the groups/atoms in the reactive region to different intermolecular interactions is important from the point of view of binding of a small ligand to a large pocket of an enzyme. Geometrical descriptors like volume and surface describe stereochemistry of the potential ligand, while topological descriptors like bonds or contacts reveal structural information and bonding abilities. The presence of weak bonds or contacts can be substantial for the binding with proteins or nucleic acid, similarly as it is for polyhalobenzimidazoles. (Latosińska et al., 2014a) Therefore hydrogen atoms, which participate in hydrogen bonds, are often omitted in classical QSAR analysis. We hope that our study will contribute to the explanation of differences between the two compounds I3C and DIM, (Aggarwal and Ichikawa, 2005) important for understanding of the effective processes of recognition and binding them by many target proteins. The results of our study can be used for the prediction of the ability to form intermolecular interactions by I3C- and DIM-inspired compounds of great practical importance, for instance potential anticancer agents.

2. Experimental

2.1. Material

High purity polycrystalline samples of I3C and DIM (commercial, 98%) were purchased from Sigma–Aldrich, Germany and used without further recrystallisation or any additional purification. The purity of the I3C and DIM samples verified by high performance/pressure liquid chromatography (HPLC) was even higher than declared by Sigma–Aldrich.

2.2. Methods

2.2.1. X-ray data

The single-crystal X-ray diffraction studies of I3C were performed at room temperature with a Gemini A Ultra diffractometer