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Dictyostelium: A Nucleotide Regulated Life-Cycle of Relevance to the Human Condition

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Abstract

Aspects of Dictyostelium cell biochemistry are sufficient to provide a model for investigating deficiencies in human cell function, of relevance to neurological disorders, mitochondrial syndromes and cancers. Some rapid drug toxicity tests are also based on a Dictyostelium model. Nucleotide modulation is of critical importance in each life-cycle stage of Dictyostelium. In Dictyostelium cells, nucleotide modulators include exclusive eukaryotic compounds, cytokinins, mammalian vasoactive amines and steroids. Many of these modulator compounds do not appear to resemble nucleotide molecular structure. Computational software is used in this study to explore molecular similarity within the molecular structures of nucleotides and compounds demonstrated to influence Dictyostelium development. Results reveal how a disparate range of compounds in the above chemical groups has evolved to modulate the nucleotide chemistry and life-cycle stages of Dictyostelium.

Keywords

Dictyostelium, Life-Cycle, Purine Nucleotides, Molecular Similarity

1. Introduction

The branching of slime moulds from the main evolutionary pathway, shortly after separation of the plant and animals kingdoms, endowed Dictyostelium with biochemical and physiological characteristics common to both. This social amoeba possesses mitochondria and an extensive metabolic repertoire based on the activity of cytokinins, neurotransmitter amines, peptides and proteins [1]. Signal transducing G-proteins, phosphodiesterases and kinase enzymes are involved in regulating biochemical cascades [2] [3]. Several types of the adenyl cyclase enzyme promote aggregation, terminal differentiation and spore germination [4]. This degree of biochemical complexity and a sequenced genetic code make Dictyostelium

a useful laboratory model for drug development, toxicity testing and the investigation of disease processes [5]-[9].

Changes in the amoeboid form and motility of Dictyostelium are primarily dependent on the effects of cAMP/ATP nucleotides, in respect of intra- and intercellular signaling [10] [11]. The dipeptide glorin is the chemoattractant (acrasin) in some Dictyostelium species [12]. Cytokinins and signaling molecules such as discadenine and cyclic di-GMP modulate changes in adenine nucleotide levels [3]. Nitric oxide (NO) pulses inhibit cAMP initiated aggregation and differentiation via a mechanism which remains obscure [13]. Cell polyketides, including MPBD (4-methyl-5-pentylbenzene-1,3-diol) and differentiation inducing factors (DIFs) impact different life-cycle stages [6]. GABA and glutamate neurotransmitters have functions that differ in an organism without a central nervous system. GABA participates in the sporulation of Dictyostelium, and enters the TCA cycle contributing to nitrogen metabolism [14]. GABA release is initiated by spore differentiation factor SDF-3, a steroid of undetermined structure [15]. Spore formation is also induced experimentally by mammalian steroid structures and plant cytokinins [2] [15]. Functional change in the Dictyostelium life-cycle is underpinned by nucleotide modulation of Ca^{2+} influx. The equivalent of the mammalian IP3 receptor regulates Ca^{2+} release from endoplasmic reticulum stores [16]. P2X ATP-gated ion channels, sited on intracellular vacuoles, release Ca^{2+} in response to luminal ATP accumulation [17].

Mechanisms of cell death in Dictyostelium are of interest in medical disciplines relating to the pathogenesis and treatment of cancer and neurodegenerative disorders [9]. Autophagic cell death through autophagosome formation contributes to the differentiation of Dictyostelium in respect of stalk-cell formation; necrotic cell death is associated with mitochondrial uncoupling [18]. Mitochondria maintain homeostasis in Dictyostelium cells, whereas a G-protein linked surface cAMP receptor regulates cell development through the process of cAMP signaling [19]. This study explores mechanisms relating to cell development and cell death by reviewing published *in vitro* findings using a computational approach. The methodology focuses on relative molecular similarity within the structures of small molecular weight compounds modulating purine nucleotide levels. The established nucleotide dependency of Dictyostelium, if evident within the structures of compounds regulating life-cycle stages of the amoeba, should facilitate understanding of the mechanisms involved.

2. Materials and Methods

2.1. Compound Structures

The investigated compound structures include endogenous acrasins, cytokinins, polyketides, pteridine derivatives, teratogens and growth factors investigated in previous studies [6] [7] [12] [13] [20]. Flavonoid, steroid, neurotoxin and synthetic drug compounds with effects on Dictyostelium development are included, as are inactive comparator structures identified in the studies [15] [21]-[23]. The

specific compounds are listed in Table 1. Compound structures are taken from Pubchem (https://pubchem.ncbi.nih.gov/) and the previous references.

Table 1. Values for fitting compound structures to adenine and guanine nucleotide templates.

Compounds	Fitting points	Interatomic distances (Å)	RMS (Å)
2-en-VPA	O9C1'C8	0.09, 0.11, 0.04	0.0092
2-ethyl-pentaic acid	C2'C1'C8	0.06, 0.08, 0.04	0.0181
2-methylheptanoic	N9C1'O8	0.07, 0.10, 0.14	0.0047
4-BCCA	C8C1'O7	0.08, 0.08, 0.05	0.0137
4-EOA	O3C2'C8	0.09, 0.13, 0.04	0.0234
(R)-4-yn-VPA	C2'C1'C8	0.06, 0.08, 0.04	0.0182
(S)-4-yn-VPA	C2'C1'C8	0.06, 0.08, 0.04	0.0183
aldosterone	O6C6O8	0.11, 0.11, 0.17	0.0045
AR-12	C1'N9C3	0.07, 0.07, 0.03	0.0140
biopterin	C8N3C4'	0.04, 0.05, 0.01	0.0082
biopterin	O2C2'C2	0.01, 0.05, 0.05	0.0048
biotin	C3'C2'O6	0.02, 0.01, 0.03	0.0004
biotin	N3C4O7	0.05, 0.05, 0.05	0.0005
cerulenin	N3C4C4'	0.04, 0.02, 0.04	0.0044
cortisol	O6C6O8	0.05, 0.06, 0.02	0.0034
cortisol	C2'C1'C6	0.02, 0.14, 0.15	0.0126
curcumin	C4'C3'N7	0.06, 0.08, 0.09	0.0009
cyclic-di-GMP	N3O3C4'	0.02, 0.05, 0.06	0.0036
decanoic acid	C6C5O8	0.12, 0.04, 0.13	0.0005
diethylstilbestrol	C1'C4C2	0.06, 0.06, 0.10	0.0133
DIF-1	N7C8'C2'	0.09, 0.11, 0.06	0.0144
DIF-1	O3'C2'C4'	0.07, 0.07, 0.06	0.0040
discadenine	N7C8O7	0.09, 0.08, 0.05	0.0080
domoic acid	C5C4C1'	0.13, 0.05, 0.11	0.0003
estradiol	C2'C1'C5	0.07, 0.15, 0.07	0.0251
estradiol	C3'C1'C4	0.07, 0.06, 0.05	0.0221
EGCA	C4'O3C5	0.04, 0.04, 0.02	0.0083
folic acid	C6N1C4'	0.11, 0.10, 0.03	0.0078
GABA	N6C6N1	0.06, 0.04, 0.10	0.0036
glorin	O8C4'N6	0.08, 0.05, 0.05	0.0118
glutamic acid	N1C6C5	0.10, 0.06, 0.07	0.0137

Continued

isopentenyladenine	C8N9C2'	0.03, 0.03, 0.04	0.0042
mifepristone	C2'C1'C6	0.03, 0.13, 0.11	0.0182
MPBD	C6C4C2	0.02, 0.06, 0.07	0.0110
MPBD	N6C4C2	0.02, 0.06, 0.07	0.0184
MPBD	C4'C2'O3	0.04, 0.05, 0.06	0.0123
naringenin	O9C1'C4	0.05, 0.08, 0.03	0.0163
neopterin	O3N1C5	0.02, 0.05, 0.05	0.0009
phenytoin	C8C4C5	0.06, 0.06, 0.12	0.0054
progesterone	C6C5C2'	0.06, 0.06, 0.01	0.0067
testosterone	O6C6O7	0.10, 0.10, 0.07	0.0065
thalidomide	C8C1'C4	0.00, 0.01, 0.01	0.0042
valproic acid	O3C3'C4'	0.10, 0.14, 0.04	0.0237
zeatin	N1C2O2	0.07, 0.06, 0.08	0.0112

2.2. Molecular Modeling

Compound structures are built from contents of the Nemesis software program fragment file (Oxford Molecular version 2.1) and minimised by conformational analysis. The stereochemistry and isomerism requirements of structures are addressed during construction (Table 1). Nemesis allocates partial charges to the molecular structures and the those used for fitting are all minimum energy conformers in an uncharged form. The conformation of the cGMP structure is described by the torsion angle (bond angle described by 4 adjacent atoms) C8N9C1'O9 −33˚ (Figure 1). The same torsion angle in the GTP and ATP structures are respectively −47˚ and −38˚. The Nemesis program fits paired molecular structures on a three-point basis. Fitting-points, comprised of atoms of similar type and partial charge within compound and nucleotide structures, are identified in the text and table with respect to the nucleotide labels. Colour-coded atoms in the figures identify ligand fitting-points: carbon-green, nitrogen-blue, oxygen-red, sulphur-yellow. To improve on figure presentation of the fitted compounds, bond order within molecular structures is not shown and the nucleotide triphosphate chain is cropped after fitting. The Nemesis program computes goodness-of-fit values, in respect of inter-atomic distance at each fitting-point and root mean square (RMS) value. The sequence of fitting points for each structure (given in Table 1, left to right) provides the fit with the lowest RMS value.

3. Results

The compound structures in Figure 1 regulate Dictyostelium development and are primarily based on benzene, adenine and pterine ring systems. The plant-like cytokinins, isopentenyladenine (1) and zeatin (2) have fitting-points on the purine

and ribose moieties of the ATP template. Biopterin (3) and neopterin (4) have similar structures but differ in their nucleotide fits. Several fits to the cAMP template are given for the MPBD and DIF-1 conformers; one of each structure has the same fitting-points (7 and 9). Discadenine (8) and DIF (10) structures differ in influencing the purine and cyclised rings of cAMP, as does cerulenin (11) an antibiotic and inhibitor of polyketide synthesis. Goodness-of-fit values for the above compounds (Table 1) range from 0.01 - 0.11Å (RMS values 0.0009 - 0.0184Å).

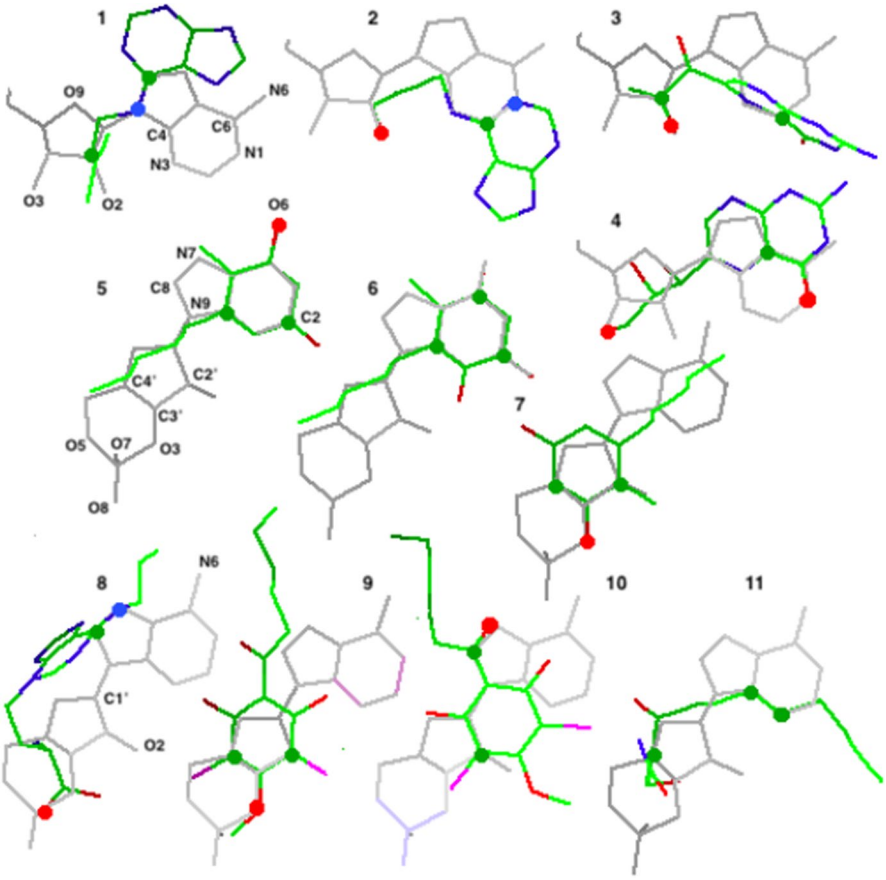

Figure 1. Fits of compound structures to ATP (1 - 4), cGMP (5 - 6) and cAMP (7 - 11) templates (grey). 1 isopentenyladenine, 2 cis-zeatin, 3 threo-biopterin, 4 threo-neopterin, 5 - 7 MPBD, 8 (S)-discadenine, 9 DIF-1, 10 DIF-1, 11 cerulenin.

The diverse compounds in Figure 2 have slightly larger and more elaborate structures than those of the previous figure and fit to the cyclic nucleotide templates. Glorin (2), folic acid (3), curcumin (4) and AR-12 (7) superimpose over the length of the cAMP structure, whereas the cyclic-di-GMP structure (1) exceeds it. Of these structures, glorin provides the best match for the more common acrasin cAMP, with fitting points at N6 and O8. Naringenin (5), biopterin (6) and EGCA (8) have fitting-points on the purine and ribose-phosphate rings. Goodness-of-fit values for these structures are less than 0.12Å (RMS 0.0009 - 0.0163Å).

The fit of the GABA structure (1) to the cAMP nucleotide template (Figure 3) utilises the carboxyl group, which comprises over 40% of the compound's

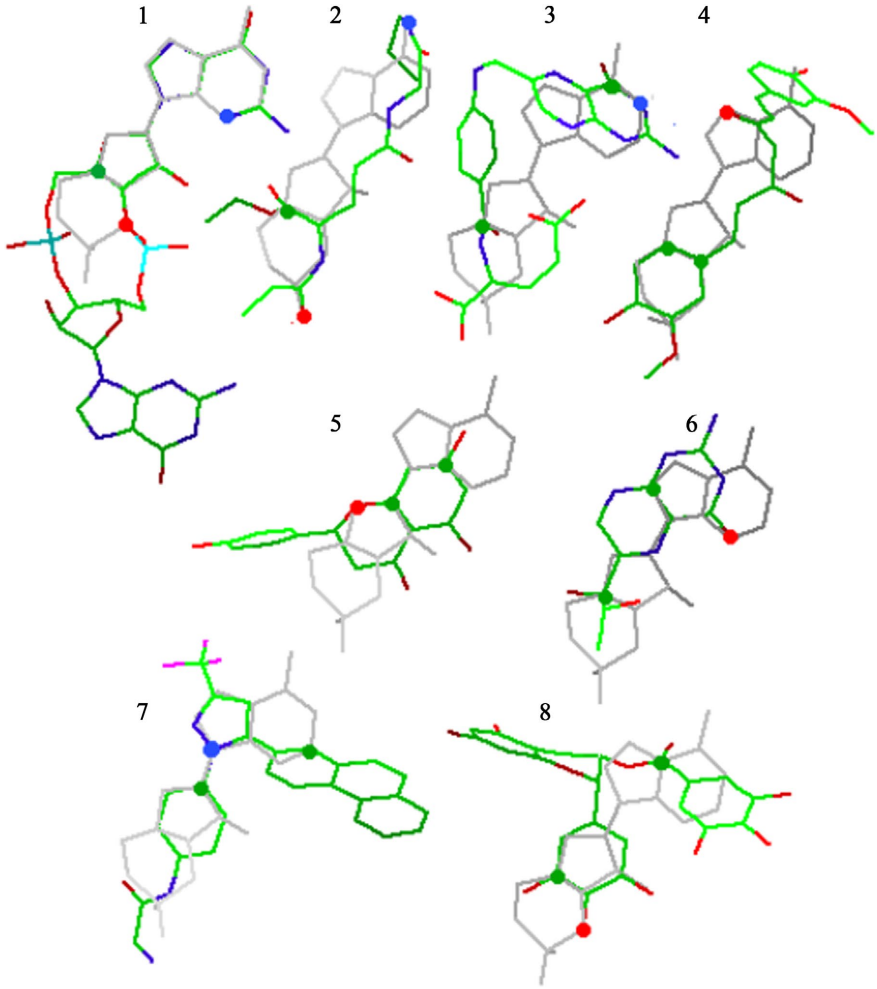

Figure 2. Fits of compound structures to cAMP template (grey). 1 cyclic-dI-GMP, 2 glorin, 3 folic acid, 4 curcumin, 5 naringenin; 6 biopterin, 7 AR-12; 8 epigallocatechin gallate (EGCA).

molecular weight. Fitting-point atoms of the GABA structure match positive and negative partial charges represented within the nucleotide template. Glutamic acid (3) provides a similar fit on the nucleotide structure. Valproic acid (VPA), analogues of VPA (4-BCCA, 4-EOA) and decanoic acid initiate autophagy in Dictyostelium. The fit of VPA (2) is confined to the cyclised ribose-phosphate ring, whereas decanoate (6) superimposes over the length of the cAMP template. 2-methylheptanoic acid (7) a compound that does not induce autophagy in Dictyostelium, demonstrates least overlay of the ribose-phosphate ring. The above VPA analogue structures use O3/O7/O8 fitting-points on the nucleotide cyclised ring. Biotin, an essential growth factor for Dictyostelium, provides fits to cGMP (8) and GTP templates (9). Structures 10 - 17 represent compounds with teratogenic properties: VPA analogues (11 - 13) and phenytoin (17) are not teratogenic, in contrast to thalidomide (14), domoic acid (15) and stilbestrol (16) which are apoptotic and teratogenic. The difference in the nucleotide fits of phenytoin and the teratogenic compounds is evident, in that the fit of the former structure is

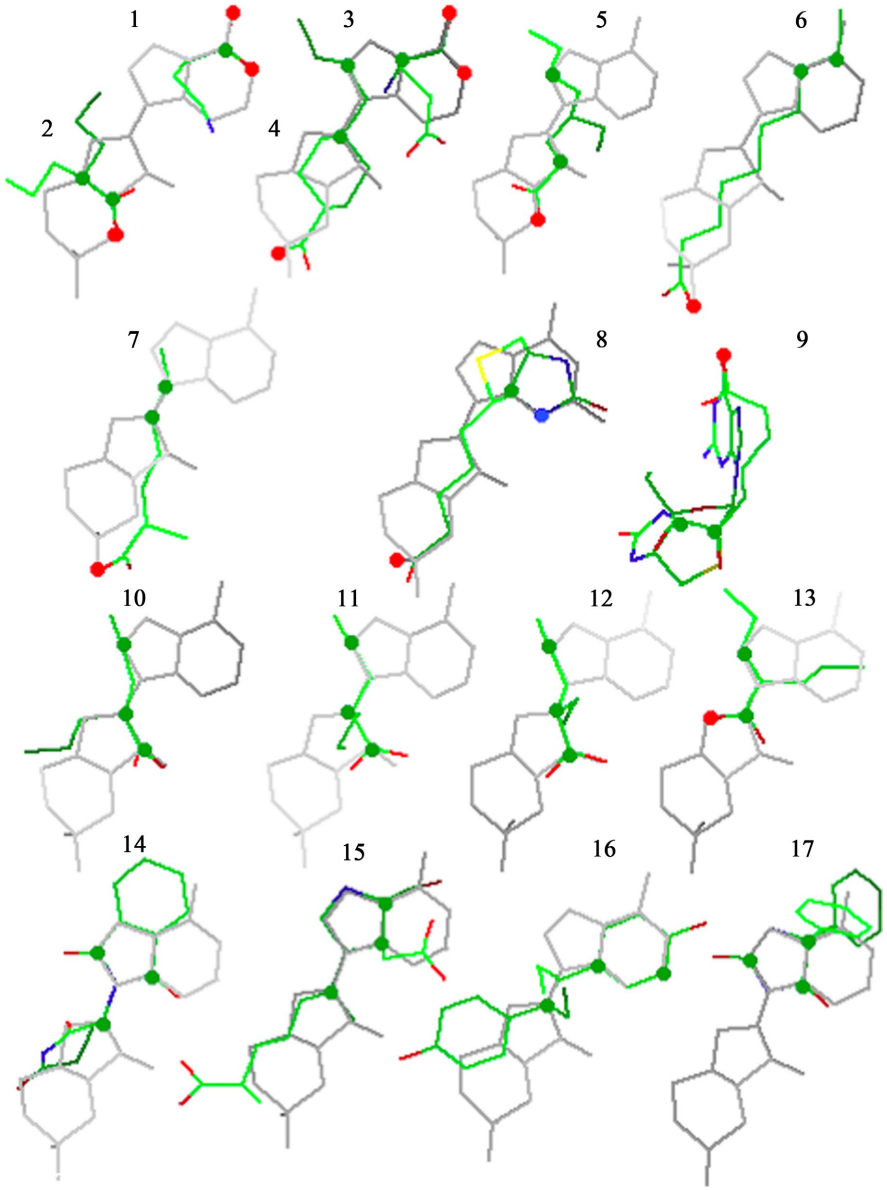

Figure 3. Fits of compound structures to cAMP template (grey). 1 GABA, 2 valproic acid, 3 glutamic acid, 4 4-BCCA, 5 4-EOA, 6 decanoic acid, 7 2-methylheptanoic acid, 10 (S)-4-yn-valproic acid, 11 (R)-4-yn-valproic acid, 12 2-ethyl-4-pentaic acid, 13 2-en-valproic acid, 14 thalidomide, 15 domoic acid, 16 diethylstilbestrol, 17 phenytoin. 8 biotin fit to cGMP template (grey), 9 biotin fit to GTP template (grey).

confined to the purine ring. The VPA analogues, in common with the teratogenic compounds, have fitting-points on the purine and ribose rings. Lack of teratogenicity in the VPA analogue group may be attributable to a less substantive structure, and in particular lack of substituent groups leading from the C1' fitting-point. The teratogens valproic acid (2) and (S)-4-yn-VPA (10) demonstrate an alkyl substituent that mimics the phenol ring position of diethylstilbestrol, which is absent in the non teratogenic enantiomer (R)-4-yn-VPA (11). Fitting-values of the Figure 3 structures (Table 1) are not greater than than 0.14Å (RMS values 0.0003 -

0.0237Å).

Mineralocorticoid and gonadosteroid structures demonstrate general and specific fits to the nucleotide templates (Figure 4), the latter providing better fitting-values. The general fits are represented by 17-β-estradiol (1) and cortisol (2). 17-β-estradiol and testosterone structures have a C5 fitting-point, whereas cortisol and steroids with a side-chain (dexamethasone, corticosterone, progesterone, deoxycorticosterone) fit at C6 and also at C5. The template 4 fit is specific for 17-β-estradiol, as is the template 6 fit for testosterone. Progesterone, cortisol and aldosterone fit to the cGMP template at O6C6O8 and to the cAMP template at C6C5C2' by respectively fitting the acetyl group oxygen at O6, or the carbon subtending the steroid acetyl group at C6. Progesterone (7) and cortisol (5) structures demonstrate these fits to the cGMP and cAMP templates. The fit of aldosterone (8) using C6C5C2' fitting-points (0,07Å, 0.04Å, 0.03Å; RMS 0.0008Å) is a better alternative to the fit listed in Table 1.

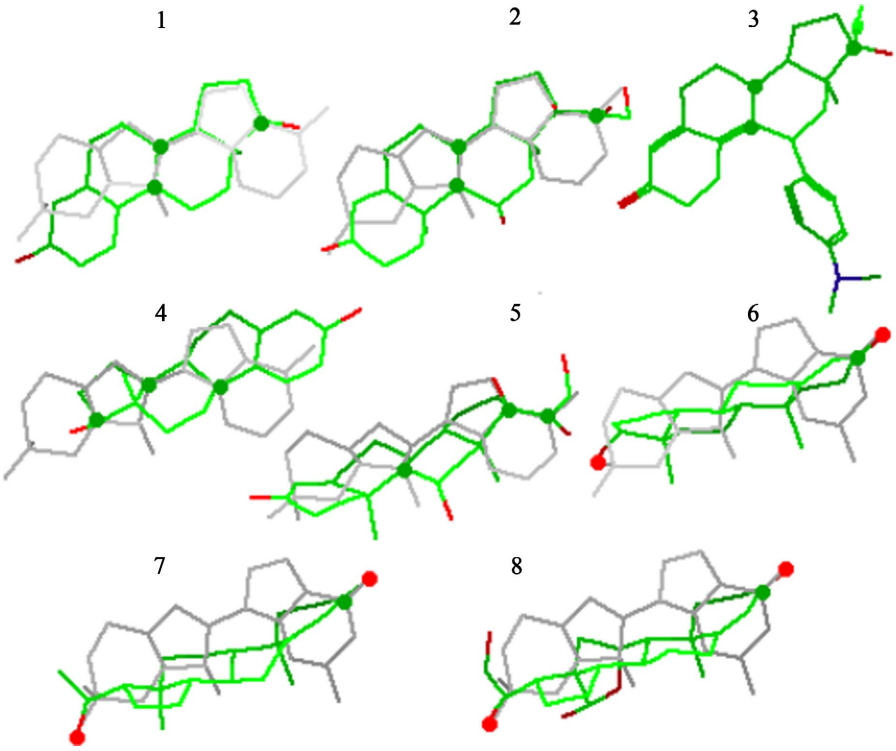

Figure 4. Fits of compound structures to cAMP template (1 - 5) and cGMP (6 - 8) templates (grey). 11-17 β-estradiol, 2 cortisol, 3 mifepristone, 4 17 β-estradiol, 5 cortisol; 6 testosterone, 7 progesterone, 8 aldosterone.

4. Discussion

MPBD, one of the simplest of organic compounds promoting differentiation in Dictyostelium, is a benzene structure with alkyl and hydroxyl substituents. Structure-activity studies indicate that these substituents differentially effect aggregation and spore maturation [24]. The properties of the oxidised derivative, dictyoquinone, are restricted to aggregation and would relate better to the guanine nu-

cleotide. MPBD has significant *in vitro* effects on mitochondrial function, promotes Dictyostelium differentiation and suppresses human cancer cell growth [5] [25]. An opposing action of MPBD on cAMP function in regard to protein kinase action is reported in Dictyostelium [12]. Isopentenyladenine has proliferative effects on Dictyostelium cells [26] and shows better molecular similarity to the ATP structure. Dictyostelium contains several types of adenyl cyclase and responds mitogenically to isoproterenol [27]. The nucleotide template fits of isopentyladenine, zeatin, biopterin and neopterin would support the hypothesis that these compounds influence cAMP formation in a manner similar to the effects of catecholamines on mammalian adenyl cyclase.

Other cytokinin cell targets include the cyclin-dependent kinase (Cdk) group [28]. Cdk inhibitors, such as isopentenyladenine and the diaminopurine drug roscovitine, compete with ATP at the catalytic binding-site [29] [30]. Cytokinin regulation of cAMP is also influenced by several types of Dictyostelium membrane histidine kinase (DhK) which participate in autophagic cell death [31] [32]. The conversion of cytokinin nucleotides into active free-bases by phosphoribohydrolases is another contributory factor in the modulation of cAMP [33]. As a potent inducer of stalk-cell differentiation in Dictyostelium, DIF-1 participates in programmed cell death. DIF-1, a chlorinated alkylphenone of similar structure to MPBD, shares the same fit to cyclic nucleotide templates. The mechanisms responsible for the wide ranging effects of DIF compounds, including those on rat pancreatic cells, are not known [6]. DIF-1 and nucleotide compounds in Dictyostelium cultures interact both positively and antagonistically [32] [34]. It may be reasonable to assume that nucleotide relative molecular similarity within cytokinin-like compounds is able to contribute to our understanding of the Dictyostelium life-cycle.

In comparison to the compound cyclic-di-GMP, the occurrence of discadenine is limited to some eukaryotic species [33] [35]. Discadenine, an inhibitor of spore germination restricted to Dictyostelids responsive to the cAMP acrasin, functions in the presence of adenyl cyclase and kinase DhKB [13]. Cyclic-di-GMP enables Dictyostelium to form stalks and complete fruiting body formation and induces cell death in the presence of DIF-1 [35] [36]. The nucleotide template fits of DIF-1, cyclic-di-GMP and cerulenin relate to the interaction of these compounds in Dictyostelium cultures. The template fitting of the cerulenin structure has more in common with cyclic-di-GMP than with the DIF-1 structure. Cerulenin blocks the production of DIF-1 by Dictyostelium and impairs vacuolisation (initiation of cell death) by cyclic-di-GMP but not by DIF-1. Furthermore, cyclic-di-GMP initiated death requires preincubation with cAMP [36]. These experimental observations are further complicated by reports that cAMP brings cells to a DIF-responsive state and is a potent inhibitor of stalk differentiation in the DIF-1 dependent stage [34]. Data from the nucleotide template model generates a hypothesis for the sequential displacement of ligand by cyclic-di-GMP and DIF at a cAMP receptor. Downstream mechanisms of autophagic cell death in Dictyoste-

lium involve Ca^{2+} flux from the endoplasmic reticulum mediated by the inositol 3-phosphate receptor [35]. The apoptotic properties of cerulenin result from deregulation of adenine nucleotide synthesis [36] [37].

The flavonoids naringenin, curcumin and EGCA have similar inhibitory effects on Dictyostelium cell development and migration [38]-[40]. The effects of naringenin and curcumin are attributed to inhibition of phosphodiesterase or kinase activity. EGCA block of essential Dictyostelium cAMP gradients and signal relay activity is recognised by McQuade *et al.* [40]. The cAMP template fits of these flavonoid structures suggests a common mechanism for their inhibitory effects. The naringenin fit supports the observation that this compound inhibits phosphodiesterases in bovine aorta [41]. There are several phosphodiesterases within Dictyostelium cells with unique structures and roles [42]. AR-12, a protein kinase inhibitor, induces ROS and autophagic flux in Dictyostelium [43].

Folic acid has the property of an acrasin, although biopterin and neopterin are considerably more potent [44]. A combination of the non chemotactic remnants of the folic acid structure (lumazine and aminobezoylglutamate or deaminopteroic acid) with various amino acids initiates chemotaxis in Dictyostelium [45]. Interpretation of these observations as evidence of separate receptors for the folic acid non chemotactic moieties is apposed by Devreortes, in favour of a mechanism based on the potentiation of the cAMP response by folic acid [46]. The present results demonstrate relative molecular similarity within the pteridine and glutamic acid moieties of folic acid and structural commonality with cAMP. Reported increases in cAMP following the addition of folic acid to cultures and the cell density dependency of this observation [46] may be influenced by the displacement of nucleotide from cell receptors. Van Haastert has reported on the use of folic acid or cAMP in the desensitisation of Dictyostelium cells to the initiation of cGMP responses [47]. The template fit of folic acid applies to both cAMP and cGMP nucleotides. Glorin, the endogenous non nucleotide alternative acrasin, has a cAMP-like character in respect of molecular dimensions and nucleotide template fitting-points.

The pharmacological effects of the biotin compound in animals are considerable and along with lipoate, folate and thiamin appear to be essential growth-factors for Dictyostelium [48] [49]. Biotin may operate via guanylate cyclase/protein kinase G mechanisms and deficiency may contribute to teratogenic defects in animal and human foetuses [50]. This study provides some evidence for the interaction of biotin with both forms of the guanine nucleotide. Biotin may also interact with adenine nucleotides, as biotin carboxylase (involved in long chain fatty acid synthesis) has low ATPase activity in the absence of the compound [51]. The analogue tetrahydrodictyopterin competitively inhibits cAMP stimulated binding of GTP gammaS and GDP/GTP exchange in membrane fractions of Dictyostelium, resulting in loss of adenyl cyclase function [52].

The teratogenic effects attributable to valproic acid are cognitive as well as physical [53]. Warren *et al.* have established that decanoate and a range of fatty acids

with seizure control activity induce autophagy in Dictyostelium [20]. The nucleotide template model demonstrates differences between active fatty acid structures (VPA, 4-EOA, 4-BCCA, decanoate) in comparison to 2-methylheptanoic acid, a compound with no seizure control activity. Thalidomide, domoic acid and diethylstilbestrol structures demonstrate quite different template fits in comparison to the non teratogenic drug phenytoin. Oxidative stress and apoptosis are common properties of neurotoxins and teratogens such as thalidomide and domoic acid [21]. Baines has further developed a Dictyostelium model for developmental and reproductive toxicity testing with the characteristics of high-throughput, genomic screening and broad prediction of mammalian toxicity [7] [8] [54].

Molecular similarity within GABA and glutamic acid structures enable glutamate to share and antagonise the GABA receptor of Dictyostelium during early development. Glutamate inhibits the spore-inducing precursor SDF-2 if present at a much higher concentration than GABA [55]. Tillner and co-workers [22] report on the correlation between the teratogenic potential of VPA analogues in mouse and dictyostelium models of development. In the dictyostelium model, (S)-4-yn-VPA strongly inhibits spore cell differentiation, whereas (R)-4-yn-VPA and 2-ethyl-4-pentaic acid have lesser effects. The nucleotide template fitting characteristics of the VPA analogue structures with teratogenic and non teratogenic properties demonstrate some interesting differences.

Steroid compounds are far from absent in the metabolome of Dictyostelium. In stimulating the release of GABA, the steroid compound SDF-3 initiates a signaling cascade that amplifies encapsulation [15]. Inhibition of steroidogenesis, or the presence of the hydrocortisone inhibitor mifepristone, blocks SDF-3 action, whereas mifepristone fails to block spore formation by GABA or SDF-2. Steroid compounds mimic the sporulation effects of SDF-3: cortisol, dexamethasone, corticosterone, 17-alpha progesterone and deoxycorticosterone induce spore formation at 50 nM or less**,** whereas aldosterone, cholesterol, estradiol and testosterone are ineffective [15]. In regard to the growth and aggregation of Dictyostelium cells, inhibition requires much higher steroid concentrations and inhibitory activity is attributed to liposolubility; estradiol and testosterone are less potent [56]. This computational study finds that all steroid structures were able to provide good fitting values to the nucleotide template. The structures of dexamethasone, corticosterone, 17-alpha progesterone and deoxycorticosterone (not listed in **Table 1**) mimic the nucleotide template fits of cortisol and progesterone.

The limitations of this study reside in the methodology, in that data on compound structures are generated in a non physiological environment. Molecules within living organisms carry charges which influence conformation and properties. It is not an easy matter, however, to decide on which molecules are charged and which atoms within a molecule carry a charge. The approach adopted, of using uncharged structures, has consistency and simplicity. Fitting data on compounds demonstrating relative molecular similarity cannot be extrapolated to provide values of receptor affinity or evolutionary significance.

5. Conclusion

In conclusion, molecular evolution has provided Dictyostelium with some unique compounds, in addition to the capacity to respond functionally to non endogenous compounds which modulate purine nucleotide gradients. Molecular modeling assists in establishing relationships between structures modulating the life-cycle of Dictyostelium, which are otherwise difficult to establish in two-dimensional format. Molecular similarity within the structures of nucleotides and compounds which impact development and autophagy in Dictyostelium, supports a structural and functional link in these processes. Research findings from experimental studies on Dictyostelium provide some insight in regard to competition between endogenous compounds at receptor sites. Ratio changes in the concentrations of compounds; GABA/glutamate, cAMP/cyclic-di-GMP/DIF-1, initiate life-cycle changes. These in vitro findings have implications for research studies which focus on the administration or measurement of a single substance. Physiological changes in human-kind could be expected from altered ratios in nucleotides/steroid concentrations during ageing, which may be significant in respect of ageing-related diseases.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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