Scientific paper

Docking of Selected Natural Polyphenols to ARF Activated A1 Subunit of Cholera Toxin

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Dedicated to Professor Josef Barthel on the occasion of his 80th birthday

Abstract

Natural products containing polyphenols are traditional drugs to treat diarrheal diseases such as cholera. One of the suggested mechanisms of action of these compounds is inhibition of ADP-ribosylation induced by cholera toxin. In this study we used docking methods to explore the ability of polyphenols to bind to the binding site for NAD^+ of the CTA_1:ARF_6-GTP complex (*Science* 2005*, 309*, 1093). Docking results show that catechins may enter into the binding site of CTA_1. Epigallocatechin gallate, theaflavin-3,3'-digallate, 1,2,3,6-tetra-O-galloyl-D-glucose are the best binders among studied compounds.

Keywords: Cholera Toxin, ADP-ribosylation, natural polyphenols, molecular docking

1. Introduction

Cholera Toxin (CT) produced by *Vibrio Cholerae* and Heat-Labile Toxins (LTs) produced by *Escherichia coli* are enterotoxins that have different effects on human populations, from relatively mild travellers' diarrhea caused by LT-I to life-threatening cholera caused by Cholera Toxin. The World Health Organisation (WHO) reports that diseases caused by various enterotoxins are responsible for hundred thousand deaths in the third-world each year. [1]

Cholera toxin belongs to a class of AB_5 bacterial toxins, that are known for their characteristic architecture comprising a single active unit A and a non-toxic receptor binding component, a pentamer of B subunits.[2,3] The B pentamer is responsible for binding to gangliosides at the cell surface. Its recognition function is retained even in the absence of the A subunit. A lot of effort has been taken to develop metabolically stabile compounds that competitively inhibit binding of the B pentamer of Cholera toxin to a natural GM_1-ganglioside receptor.[3–11]

The threatening action of CT is initiated by binding of the B subunits to the GM_1 ganglioside on intestinal epithelial cell membranes. This binding event is followed by nicking of the A chain, and Cys187 – Cys199 disulphide bond reduction, which yield the two fragments A_1 and A_2.[3,12] A_1 is then translocated across the membrane to the cytosol of the host cell, where it catalyses the covalent transfer of an ADP-ribose moiety from NAD^+ to Arg201 of the signalling protein $G_{s\alpha}$ (a component of an adenylate cyclase system). This modification of the adenylate cyclase system results in a series of events that lead to enormous loss of water from the epithelial cells into the intestinal lumen, causing water diarrhea characteristic for cholera. It has been shown that A_1 by itself has relatively low enzymatic activity *in vitro*. The interaction of A_1 with ADP-ribosylation factor (ARF) protein from human host increases the enzymatic activity of A_1.[13–15] Numerous studies *in vitro* and *in vivo* have indicated importance of tight interaction between A1 fragment and ARF. Recent structural investigations of a CTA_1:ARF_6-GTP complex pointed out that binding of ARF_6-GTP causes dramatic changes in the CTA_1 loop regions that open binding site for NAD^+.[16]

The main aspect of our work was to find out if there is any possibility for inhibiting CTA_1 by displacement of NAD^+. The main reason why we selected natural polyphenols as possible inhibitors is that these substances are very abundant in nature and they are frequently present as an active compound in numerous traditional medicaments. Several investigations indicate that natural polyphenols disturb biological activity of CT *in vivo,* be-

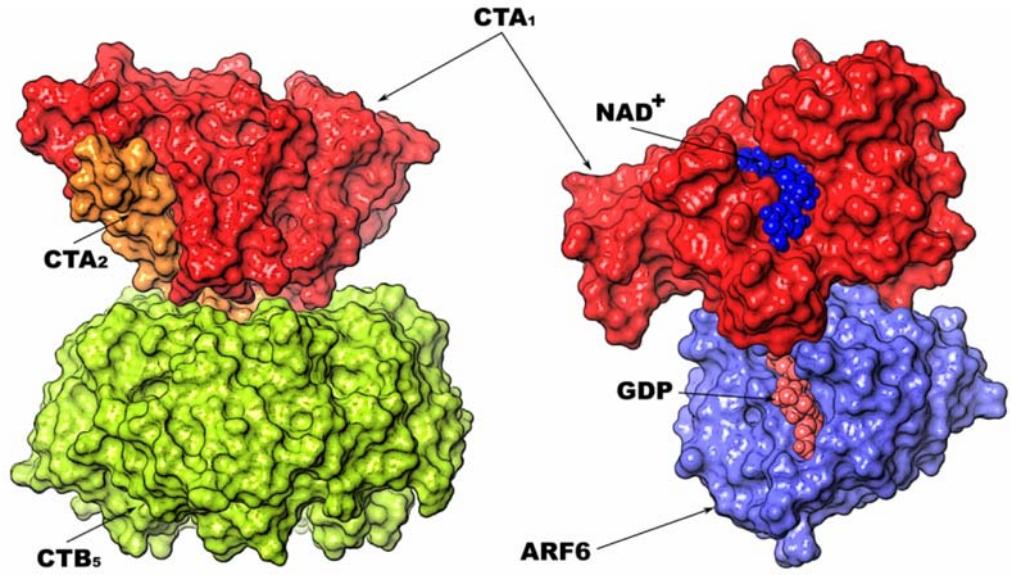

Figure 1. Crystallographic structures of Cholera Toxin (AB_5 holotoxin – left) and complex between enzymatic active unit A_1 of Cholera Toxin and ARF_6 – ADP ribosylation factor from human host (right).

cause of the inhibition of the enzymatic activity of the A subunit.[17–20] Saito and co-workers have shown the biological activity of natural polyphenols extracted from immature apples.[17] They described that the inhibitory effect of apple polyphenols extract (APE) on CT-catalyzed ADP-ribosylation of agmantine is dose-dependent and it is due to the inhibition of the enzymatic activity of the A subunit of CT. The concentration of APE at which 50% of the enzymatic activity of CT (15 μg/ml) is inhibited was approximately 8.7 μg/ml. Bioassay oriented fractionation of APE indicated that the highly polymerized catechins, also named procyanidine polymers, are the major inhibitory components of this apple extract. Other constituents like the non-catechin-type polyphenols (chlorogenic acid, phloridzin, phloretin, caffeic acid, and p-coumaric acid) and the monomeric catechins (catechin and epicatechin) have shown week inhibitory activity (See Table 1). The structures of selected polyphenols used in our study are presented in Figure 2.

Monomeric catechins are also major CT inhibitory substances found in unfermented green tea (*Camelia sinesis*).[16,17] Theaflavins and thearubigins are products of enzyme oxidation of green tea. They are found as active compounds in black tea.[19–23]

Table 1: Experimental data of inhibitory effect of polyphenols .[17]

Sample	[a]Inhibition [%]	Components
Control	0.0 ± 4.4	–
APE	95.4 ± 0.7	–
FAP1	3.5 ± 5.2	non-catechin
FAP2	39.4 ± 5.8	mono to trimeric catechins
FAP3	98.3 ± 1.5	oligomeric catechins
FAP4	95.5 ± 0.3	polymeric catechins
catechin, **1**	0.1 ± 5.3	mono catechin
epicatechin, **2**	2.4 ± 3.3	mono catechin
procyanidin B1, **6**	17.4 ± 12.7	dimeric catechin
procyanidin B2, **7**	9.8 ± 0.5	dimeric catechin
procyanidin C1, **8**	25.5 ± 5.5	trimeric catechin
p-coumaric acid, **16**	5.3 ± 10.1	non-catechin
phlorizidin, **17**	8.4 ± 6.6	non-catechin
phloretin, **18**	2.8 ± 5.0	non-catechin
chlorogenic acid,**19**	4.6 ± 3.8	non-catechin

[a] CT at 15 μg/ml is inhibited with 25 μg/ml of APE and FAPs. APE – Apple Polyphenol Extract. FAPs – Fractions from Apple Polyphenol. The values represent the mean and standard deviation from three determinations. Data are collected from Ref: 17.

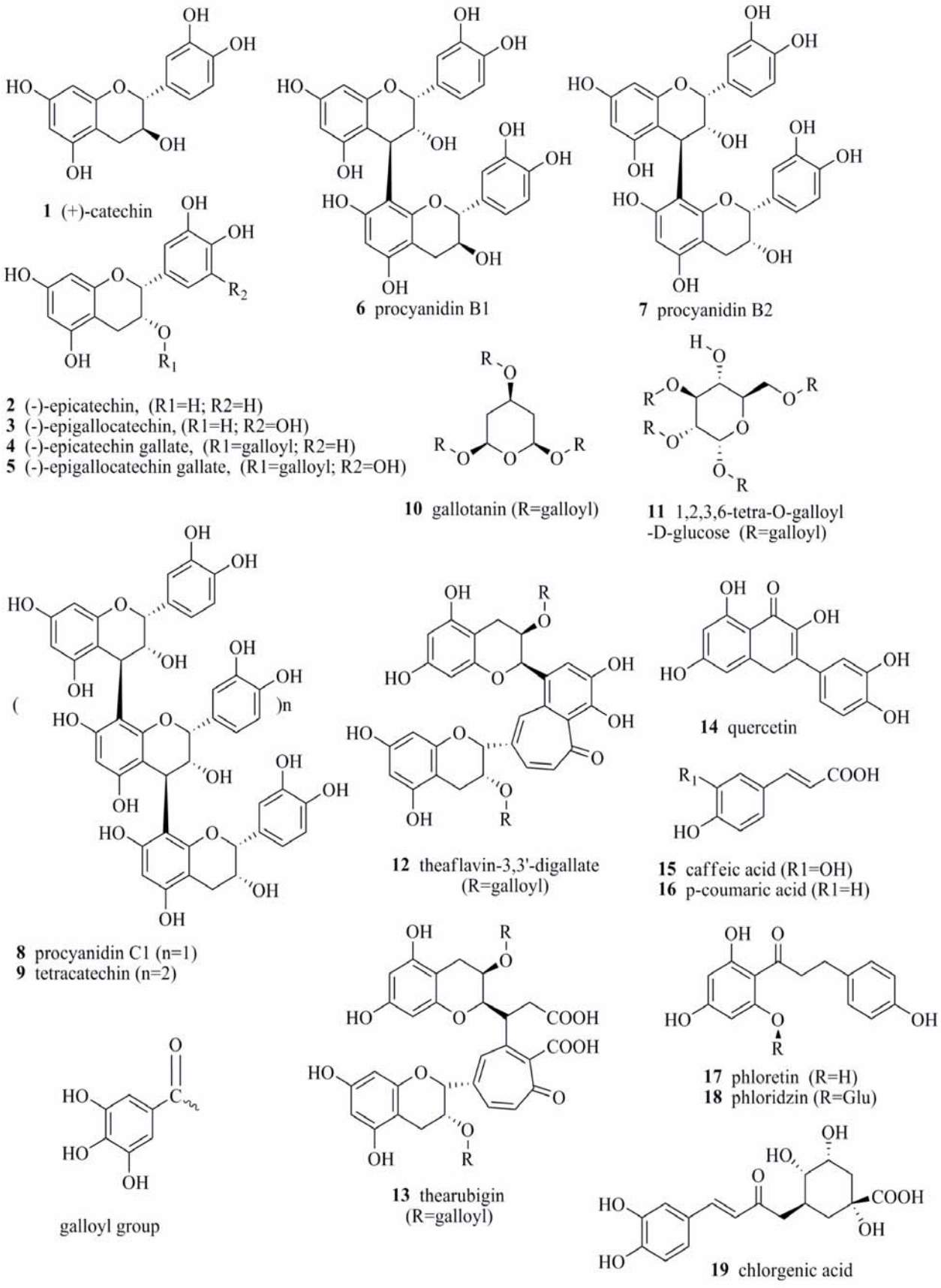

Figure 2. Structures of studied polyphenols.

With molecular docking and subsequent molecular mechanics minimisation we tried to check if some of the selected polyphenols could fit into activated CTA_1 receptor site and thus consequently inhibit ADP ribosylation activity of cholera toxin. An initial pool of compounds for virtual screening was formed from natural polyphenols and their structures were taken from different sources.[17–23]

2. Methods

The docking protocol involves several steps: preparation of protein target structure, preparation of ligands with LigPrep[24] and finally docking of ligand using docking program Glide[24–28]. The co-crystallized complex between NAD^+ and activated A_1 unit of CT bound to Human ARF_6-GTP (PDB ID: 2A5F) has been used for the preparation of a receptor model. In first step we have deleted all water molecules and glycerol added in the process of crystal growth. We have also corrected some mistakes in PDB file (correction of bond order for several atoms of GTP). Then we have applied hydrogens to all atoms. The multi step Schrödinger's Protein preparation tool[24] (PPrep) has been used for final preparation of receptor model. PPrep neutralizes side chains that are not close to binding cavity and do not participate in salt bridges. This step has been then followed by restrained minimization of co-crystallized complex, which reorients side chain hydroxyl groups and alleviates potential steric clashes.

The library consists of polyphenol structures that have been selected from different sources (Figure 2). The ligands of the library have been sketched and converted to 3D structures using Chemaxon Marvin[28]. LigPrep[24] has been used for final preparation of ligands for docking. The LigPrep is an utility in Schrödinger software suite that combines tools for generating 3D structures from 1D (Smiles) and 2D (SDF) representation, searching for tautomers and steric isomers, and geometry minimization of ligands.

For docking studies we used Glide 5.0 docking program incorporated in Schrödinger suite. The Macromodel's eMBraCe minimization that is also a part of Schrödinger suite has been used to study association of polyphenols with the CTA_1 receptor. All atom OPLSAA force field have been used for all eMBraCe minimisations. To speed up calculations we have used substructure based on three shells to allow the ligand to relax in its new environment. The protein has to be flexible enough to allow this relaxation. The first shell consisted of the ligand and the residues within 8 Å distance from the ligand. The second shell consisted of all the atoms within 12 Å from the ligand that did not belong to the first shell. A constraint of 200 kJ mol^{-1} $Å^{-2}$ has been applied to maintain these atoms in place. The third shell consisted of all atoms within 16 Å from the ligand that have not been already included. Those atoms have been completely frozen, though they have participated in the force field. All other atoms have been ignored. The Polak-Ribiere Conjugate Gradient (PRCG) algorithm has been used with a limit of 5000 steps or less, when the energy gradient ion converged below 0.05 kJ mol^{-1}.

In addition we have also done docking with Autodock 4.0.[31] We have used the same protein ligand complex for preparation Autodock 4.0 grids that we have used in case of Glide grid generation. The Autodock grids have been generated using center of NAD^+ ligand as a center of $60 \times 60 \times 60$ grid, with spacing of 0.375 Å. The Lamarckian genetic algorithm with translation step of 0.2 Å, and quaternion and torsional step of 5.0° has been used. We have done 100 independent docking runs due to convergence check of docking results.

3. Results and Discussion

In first experiment we have validated the docking protocol by re-docking of native ligand NAD^+ to receptor model of the activated CTA_1. We have docked NAD^+ into binding site using Glide SP. Best pose has been then redocked using Glide XP. RMSD between native conformer of NAD^+ and docked NAD^+ conformation has been used as a measure of docking fitness. We have found that the best docked conformation of NAD^+ obtained by Glide XP

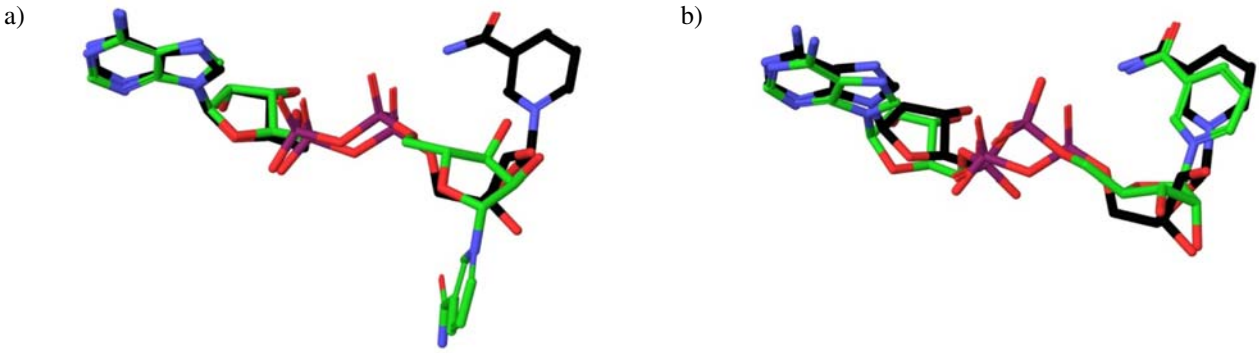

Figure 3: Superposition of crystal structure of NAD^+ from 2A5F (black carbons) and the best pose of NAD^+ obtained with Glide docking (green carbons). A: The best pose from Glide SP; B: The best pose from Glide XP.

Table 2: RMSD for superposition of native crystal structure of ligand and their moieties.

Docking protocol	*RMSD* (adenosine ribose)	*RMSD* (nicotinamide)	*RMSD* (NAD^+)
Glide XP	0.50 Å	0.70 Å	1.49 Å
Glide SP	0.24 Å	9.23 Å	4.43 Å

protocol is quite similar to the native one found in crystallographic complex. On the other hand the best scored conformation obtained by Glide SP has big deviation from crystallographic structure in place of nicotinamide moiety. Superpositions of native conformation and best scored Glide SP and Glide XP poses are presented in Figure 3, RMSD between native NAD^+ conformation and given poses are collected in Table 2.

In the second part of our study we have docked a library of selected polyphenols to the receptor model of

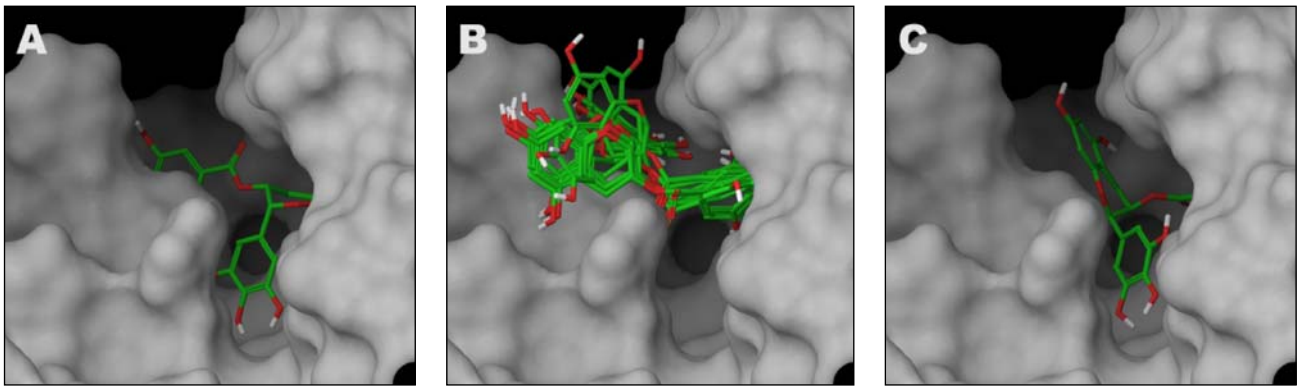

Figure 4: Docking poses of epigallocatechin gallate, **5** within CTA_1 receptor. (A) members of the best Autodock scored cluster; (B) members of the best populated Autodock cluster; (C) pose obtained with GlideXP.

Table 3: Docking scores (Glide), results from eMBraCe minimization, and Autdock 4.0 results for compounds **1–19** and NAD^+.

Compounds	Glide Score (SP)	[a]Glide Score (XP)	eMBraCe (OPLSAA/GBSA) kJ/mol	Autodock 4.0 (Lowest Score)	Autodock 4.0 (Best pop. clust.)
1	–6.18	–6.07	–90.94	–4.91 (8)	–4.46 (29)
2	–5.76	–5.73	–47.03	–5.16 (5)	–4.98 (22)
3	–5.89	–6.71	–69.01	–5.66 (17)	–4.99 (24)
4	–6.84	–7.80	–137.43	–7.00 (5)	–6.07 (12)
5	–7.00	–9.31	–147.06	–6.32 (1)	–6.20 (8)
6	–4.44	–6.33*	–92.68	–4.85 (6)	–4.84 (36)
7	–4.52	–5.19*	–92.62	–7.76(13)	–7.19 (18)
8	–4.86	–7.13*	–112.40	–8.00(3)	–4.65 (15)
9	–4.33	–9.66*	–70.20	–2.55 (6)	–2.55(6)
10	–5.61	–8.42	–109.01	–6.75 (1)	–6.36 (4)
11	–8.67	–14.02	–99.49	–8.71 (1)	–7.09 (2)
12	–7.51	–8.76	–178.76	–9.74 (2)	–8.93 (3)
13	–7.91	–10.41	–107.18	–7.99 (1)	–7.57 (5)
14	–6.34	–6.89	–87.83	–5.74 (54)	–5.74 (54)
15	–5.59	–4.14	–67.80	–4.52 (6)	–4.12(71)
16	–3.81	–2.84	–32.57	–3.55 (15)	–3.29(60)
17	–6.17	–5.45	–91.33	–4.48 (8)	–3.99 (12)
18	–5.64	–7.73	–165.85	–5.80 (3)	–5.06 (5)
19	–5.88	–8.84	–36.70	–6.59 (11)	–6.59 (11)
NAD+	–9.91	–11.81	–98.18	–5.66 (1)	–4.34 (2)

[a] The pose with best Glide Score SP is selected as entry for Glide XP docking. [b]The best GlideXP pose is selected as entry point for the eMBraCe calculations. *For these scores we have not taken into account penalty terms for intramolecular contacts, please check table 4 for uncorrected results. The numbers in brackets in columns of Autodock results represent the numbers of conformations in each individual cluster.

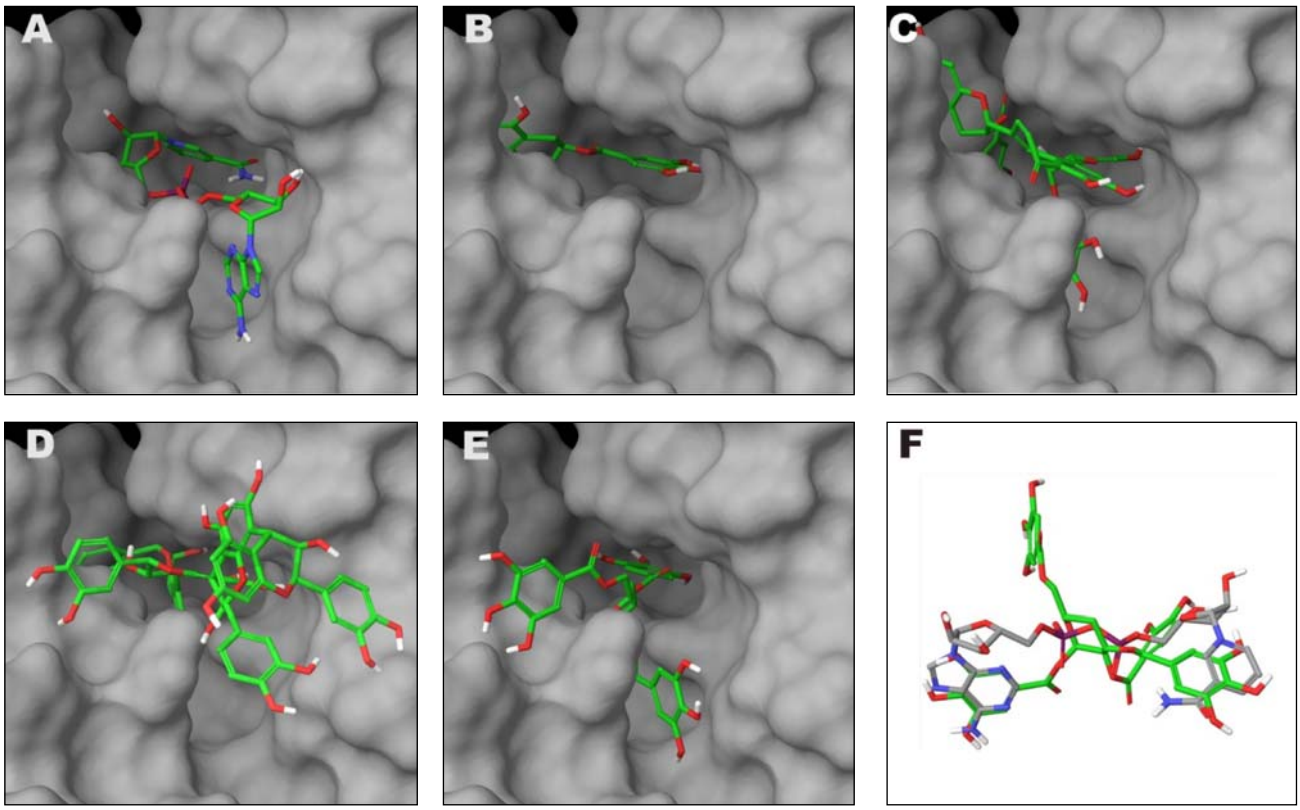

Figure 5: Glide XP poses for selected ligands. Legend: Gray surface – Molecular surface of CTA_1 receptor site; (A) NAD^+; (B) epigallocatechin gallate, **5**; (C) theaflavin-3,3'-digallate, **12**; (D) tetrameric catechin, **9**; (E) 1,2,3,6-tetra-O-galloyl-D-glucose, **11;** (F) Superposition of NAD^+ (gray carbons) and compound **11** (green carbons).

activated CTA_1. The SP and XP Glide scores, results of eMBraCe minimization and Autodock results for different polyphenols and NAD^+ are collected in table 3. We have run 100 independent dockings using Autodock for each ligand. Results of Autodock calculations are collected in two columns: in first column the lowest score and number of conformations in that cluster is presented; while in second column we can find the lowest score of best populated cluster with the number of conformations in that cluster. Number of members in the best populated cluster could be used as criteria for convergence of Autodock computations. If the total number of clusters is small and one or two clusters with low Autodock score are well populated then we may trust to the Autodock result. In figure 4 are presented docking poses of epigallocatechin gallate, **5** obtained with Autodock and Glide XP.

From Figure 4 we can observe that Autodock best scored pose (Figure 4A) for compound **5** is very similar to one obtained with Glide XP (figure 4C), compound **5** is entered deep into the binding site in both cases. Figure 5B represents poses of members of the best populated Autodock cluster, we can see that in this case poses are not docked so deep as in previous two cases.

In Table 3 good convergence of Autodock calculations for smaller compounds **1–8** and **14–17** is observed, the convergence for larger compound are in contrast not so pretty. It is interesting that convergence is worst for NAD^+, where the best populated cluster has only two members and total number of clusters is 96.

The Glide score can be used as a semi-quantitative descriptor for the ability of ligands to bind to a specified conformation of the protein receptor. Generally speaking, for low Glide score good ligand affinity to the receptor target may be expected. Some interesting poses between polyphenol ligands and CTA_1 receptor obtained by Glide XP are displayed in Figure 5.

According to the Glide XP and Autodock 4.0 results the catechins could be ordered in following manner: epigallocatechin gallate < epicatechin gallate < epigallocatechin < epicatechin ≈ catechin. Epigallocatecin gallate has the lowest Glide XP score among mono catechins (–9.67), this compound is also main catechin component of green tea extract (59% w/w) used as a effective cure against experimental infection with *Vibrio cholerae*[18], also study of Oi et al.[20] indicates high affinity of epigallocatechin gallate against CT-catalyzed ADP-ribosylation. The concentration of epigallocatechin gallate at which 50% of the enzymatic activity of CT (1 μg/ml) is inhibited was approximately 10 μg/ml.[20] It can be seen from figure 5B that galloyl group of epigallocatechin gallate nicely fits in pocket for nicotina-

mide in receptor site of CTA_1. We can also observe that di- and trimeric catechins (procyanidins) could also fit to this binding site, the docking scores (Glide XP) are for all procyanidins higher than score that we obtained for epigallocatechin gallate. The Glide XP docking results (scores and poses) show that tetrameric catechin, **9** may interact with the receptor and consequently inhibits ADP-ribosylation activity of CTA_1. Pose of compound **9** within CTA_1 receptor site is presented in figure 5D. We have observed that binding of **9** is less specific than binding of mono catechins, big tetrameric catechin cannot penetrate deeply into receptor site such as epigallocatechin gallate. At this point, we may suggest that higher oligocatechins and polycatechins from apple polyphenol extract interact with CTA_1 receptor site in similar way than tetrameric catechin. Oligocatechins (polycatechins) may partially enter into the receptor cavity and the rest of the catechin may interact non-specifically with the protein surface. There is also experimental evidence that polyphenols not only inhibit the transferase activity of cholera toxin but also modify the function of intestinal mucosa, protecting it from secretory effects of bacterial toxin. [17]

Oi et al. have tested traditional herbal medications and synthetic gallate derivates against cholera toxin induced fluid accumulation. They have found that the most active compound among traditional medications studied is Rhubarb galloyl (RG) tannin – procyanidinic polymer.[20] We have included to our virtual library the most simple Oi's synthetic gallate, **11**, that is compound in which D-glucose is esterified with four galloyl groups. Compound **11** is according the Oi's study good inhibitor of CT ADP-ribosyltranferase activity. The study of experimental inhibitory activity of galloyl derivates of di- and tri- glucopyranose done by Oi et al.[20] shows that activity increases with number of galloyls attached to scaffold. Unfortunately galloyl derivates of di and tri- glucopyranose are too big to get docked by Glide or Autodock software. The compound **11** has the lowest Glide XP score (–14.02) among all compounds of our library. From Figure 5E we can observe that galloyls form compound **11** are involved in specific interactions with receptor site. It seems that D-glucose scaffold is optimal solution to orient galloyls in right position to get it ideally superimposed with nicotinamide and adenosine ribose from NAD^+ (Figure 5F).

Typical catechin consists of numerous hydroxyl groups attached to its core, these hydroxyls are able to form numerous hydrogen bond contacts with partner amino acids residues from CTA_1 binding pocket. Additional support for binding of catechins to the CTA_1 are hydrophobic interactions between apolar part of catechins (phenyl rings) and hydrophobic amino acid residues from CTA_1 binding site.

Docking study performed by Glide has confirmed that theaflavin-3,3'-digallate, **12**, and thearubigin, **13**, compounds from black tea that poses beneficial effects against cholera and other diarrheal infections[19], fits into binding pocket of CTA_1 receptor. Glide XP docking score for compounds **12** (–8.76) and **13** (–10.41) is in the range of score for epigallocatechin gallate, **5** (–9.67). It is interesting that compound **12** has the lowest Autodock score among all studied compounds.

In Table 4 the contributions of individual term to total Glide XP score for selected ligands and NAD^+ are presented. From the presented results we may observe that for successful docking of polyphenols ligands to CTA_1 receptor Glide XP terms that describe intermolecular hydrogen bonding and lipophilic interaction between ligand and receptor are very important. We can also explain why the Glide XP score of procyanidins (**6–9)** are much higher than we have expected, the main reason for this are penalties for close intra-ligand contacts.

We performed OPLSAA/GBSA minimisation of complexes obtained with Glide XP using Macromodel eMBraCe to allow the relaxation of ligand within the receptor site and to calculate ligand/complex interaction energy obtained by eMBraCE. The eMBraCe energies of

Table 4: The contributions of individual Glide score terms to total Glide XP score for selected ligands and NAD^+.

Compound/ Score Contrib.	2	5	6	7	8	9	11	12	NAD^+
Glide Score	–5.73	–9.24	–3.81	–2.70	–4.63	–7.16	–14.02	–9.46	–11.81
Lipo	–1.84	–2.99	–2.50	–1.26	–2.23	–1.60	–4.87	–3.69	–3.20
Hbond	–2.51	–4.98	–3.19	–3.20	–4.36	–6.80	–8.13	–5.29	–6.56
Estatic	–0.99	–1.49	–0.97	–0.94	–1.13	–1.85	–1.18	–1.33	–2.00
Sitemap	0	0	0	0	0	0	–0.07	0	–0.40
Low MW	–0.50	0	0	0	0	0	0	0	0
Intramol. Cont.	0	0	2.52	2.50	2.50	2.50	0	0	0.09
Phobic	0.02	0.06	0	0.17	0.53	0.53	0.12	0.51	0.11
Rot	0.09	0.16	0.03	0.04	0.06	0.03	0.12	0.01	0.14

Legend: Glide Score – Total Glide score, sum of XP terms; Lipo – lipophilic pair term and fraction of the total protein-ligand vdW energy; Hbond – H-bond pair term; EStatic – electrostatic rewards; Sitemap – ligand/receptor non H-bonding polar/hydrophobic and hydrophobic/hydrophilic complementary terms; Low MW – Rewards for ligands with low molecular weight; Intramol. contacts – penalty for intra-ligand contacts; Phobic – penalty for exposed hydrophobic ligand groups; Rot – Rotable bond penalty.

complexes between selected ligands and activated CTA_1 are collected in Table 3. We can see that the complex between CTA_1 and theaflavin-3,3´-digallate, **12** has the lowest eMBraCe energy among all compounds that has been taken into our study. The hydrogen bonding maps for the interaction of NAD^+, epigallocatechin gallate, **5**, Oi's synthetic gallate, **11** and theaflavin-3,3´-digallate, **12** with CTA_1 are presented in Figure 6.

It can be seen from Figure 6 that CTA_1 binding site is rich with Arg and Asp residues that have tendency to form intermolecular hydrogen bonds with catechin like polyphenols. The topology of hydrogen bonding network is different from case to case, but we may find some similarity between these networks. For example Arg7B, Ala8B and Arg25B are involved in intermolecular networks of CTA_1 and NAD^+, compound **11** and compound **12.**

4. Conclusions

Using results obtained with Glide software provided by Schrödinger Inc. we have described a structure based model of inhibition of ADP-ribosyltransferase activity of Cholera Toxin by polyphenols. We can emphasize that inhibitory activity of polyphenols generally increases with their complexity. In our case the complexity is determined with the number of hydroxyl groups attached to the scaffold that could form hydrogen bond contacts with the CTA_1 receptor, and the number of phenyl rings that have capacity to form hydrophobic contacts with the same receptor. Docking results show that mono catechins (eg. epigallocatechin gallate) may penetrate deeply into binding site and thus compete with NAD^+. Study of inhibition of CTA_1 by fractions of apple polyphenols extracts[18] indicate

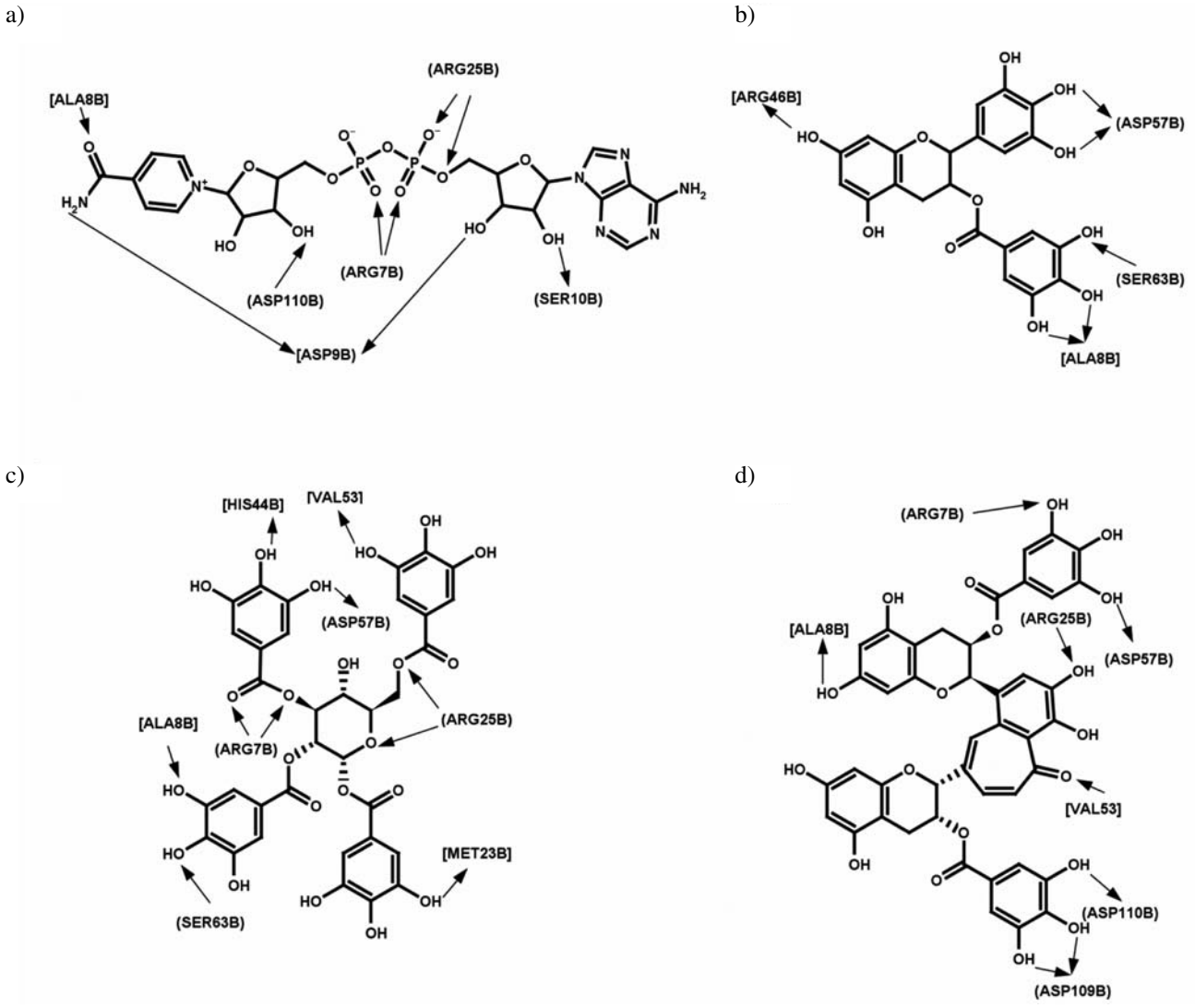

Figure 6: Hydrogen bonding network of several compounds that interact with CTA_1. Complexes are results of eMBraCe minimisation. Legend: (A) NAD^+; (B) epigallocatechin gallate, **5**; (C) Oi's synthetic gallate, **11**; (D) theaflavin-3,3´-digallate, **12**; direction of arrows are from donor to acceptor of hydrogen bond; [RES] – interaction with residue backbone; (RES) – interaction with residue side chain; [RES] – interaction with residue backbone and also side chain.

that higher molecular weight fractions containing polymeric catechins are very effective against action of CTA_1. Due to limitation of docking software we docked only tetrameric catechin from the family of oligocatechins. We found tetrameric catechin cannot penetrate into binding site in whole extension, this catechin may form numerous nonspecific interactions with the protein surface. Theaflavin-3,3'-digallate from black tea and Oi's synthetic gallate[20] are the most effective inhibitors taking into account docking results. It is interesting that Oi in his study has also indicated that his synthetic gallate derivates of di and tri saccharides are much better inhibitors of ADP-ribosylation than compound **11** presented in our study. Unfortunately mentioned ligands are too complex to get docked. The presented study of docking polyphenols to the binding site of activated subunit A_1 of cholera toxin could help in further attempts to develop a potent synthetic inhibitor of ADP-ribosylation activity that is the key mechanism in action of numerous enterotoxins, such as Cholera Toxin, Heat-Labile toxins and Pertusiss toxin.

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Povzetek

Polifenole pogosto najdemo v tradicionalnih zdravilnih pripravkih zoper kolero. Eden izmed mehanizmov delovanja polifenolnih spojin je zaviranje procesa ADP-ribozilacije induciranega s toksinom kolere. V tej študiji smo z uporabo metod molekulskega *dockinga* raziskali možnost vezave nekaterih polifenolnih spojin v vezavno mesto NAD^+ znotraj kompleksa CTA_1-ARF_6 (*Science* 2005, *309*, 1093). Rezultati molekulskega *dockinga* so pokazali, da se katehini lahko vgnezdijo v vezavno mesto katalitske enote A_1 toksina kolere. Glede na rezultate *dockinga* lahko sklepamo, da so ligandi epigalokatehin galat, teaflavin-3,3´-digalat in 1,2,3,6-tetra-O-galoil-D-glukoza dobri inhibitorji katalitskega delovanja enote A_1 toksina kolere.