J Chem Biol (2011) 4:109–116
DOI 10.1007/s12154-011-0055-9

ORIGINAL ARTICLE

Hill coefficients of dietary polyphenolic enzyme inhibitiors: can beneficial health effects of dietary polyphenols be explained by allosteric enzyme denaturing?

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Received: 30 November 2010 / Accepted: 7 January 2011 / Published online: 29 January 2011
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Abstract Inspired by a recent article by Prinz, suggesting that Hill coefficients, obtained from four parameter logistic fits to dose–response curves, represent a parameter allowing distinction between a general allosteric denaturing process and real single site enzyme inhibition, Hill coefficients of a number of selected dietary polyphenol enzyme inhibitions were compiled from the available literature. From available literature data, it is apparent that the majority of polyphenol enzyme interactions reported lead to enzyme inhibition via allosteric denaturing rather than single site inhibition as judged by their reported Hill coefficients. The results of these searches are presented and their implications discussed leading to the suggestion of a novel hypothesis for polyphenol biological activity termed the insect swarm hypothesis.

Keywords Polyphenols · Enzyme inhibition · Human diet

Introduction

Numerous epidemiological studies have shown that a diet rich in fruit and vegetables is linked to a considerable reduction of chronic multifactorial diseases including cancer, cardiovascular disease, diabetes and osteoporosis [1, 2]. Further evidence of these health effects has been obtained through human intervention studies and detailed in vitro and in vivo testing of selected polyphenolic compounds [3, 4]. As a result of these numerous studies producing overwhelming evidence, there is little debate among the scientific community about the fact that phenolic plant secondary metabolites, otherwise referred to as dietary polyphenols, are responsible for these beneficial health effects [2–4].

However, the molecular mechanisms leading to the beneficial health effects of dietary polyphenols are still under intense discussions. Generally, two lines of arguments are given to rationalize the health effects of dietary polyphenols.

Firstly unspecific interactions, based on the general chemical properties of polyphenols, have in the previous decades been suggested to be the likely reason behind beneficial health effects. The most important property of any dietary polyphenol, as an electron-rich aromatic compound, is their ability to act as a radical scavenger and as a reducing agent [5]. This property leads to two biological effects that have been termed the antioxidant effect and the pro-oxidant effect [6, 7]. As antioxidants, polyphenols are able to scavenge free radicals, mainly reactive oxygen species, and reduce metal ions in high oxidation states, which otherwise react to produce free radicals. Hence, polyphenols are able to limit the cellular damage caused by oxidative stress triggered by these highly reactive species. More recently, further unspecific modes of actions rationalizing the beneficial health effects of dietary polyphenols have been suggested that include the interaction with the redox-sensitive NF−κB signaling pathway, the activator protein-1, or the phase II enzyme modulation via the activation of the Nrf2 pathway [8].

Secondly a series of highly specific enzyme interactions and inhibitions have been reported for a considerable number of selected compounds investigated. For some highly abundant polyphenols, such as epigallocatechin gallate (EGCG), quercetin, or resveratrol, close to a

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hundred biological targets inhibited by these compounds at the milli- and even micromolar level or below have been identified. All these biological targets have, hence, been linked to the beneficial health effects of dietary polyphenols. The following literature survey and discussion will focus on such specific enzyme inhibitions by dietary polyphenols.

In two recent publications, Prinz has thoroughly analyzed dose–response curves from high-throughput screening data of enzyme inhibition assays [9, 10]. An important parameter in such dose–response curves is the Hill coefficient $n_{H,}$ which was initially defined by the equation below assuming simultaneous binding of n agonist molecules (A) to a receptor (R).

$$n\,A + R \rightleftharpoons A_nR$$

When n=1, only a single binding site of a receptor or enzyme is occupied. With values of n>1, more than one molecule interacts with the receptor or enzyme. The value of n_H corresponds to the reaction stoichiometry in supramolecular chemistry obtained through JOB plots [11]. Surprisingly for the large majority of dose–response curves analyzed by Prinz, a value of n>1 was observed. Prinz suggested two different rationales for this behavior. Firstly, he introduced the concept of transient binding patches, where more than one inhibitor molecule transiently interacts with a binding patch in spatial proximity of the enzyme or receptor binding site [9, 10]. In a more revolutionary second rationale, he introduced the concept of allosteric mechanisms [10]. Here, binding of several inhibitor molecules to any conformationally sensitive locations of the enzyme or receptor leads to loss of activity. In other terms, binding of several inhibitors leads to inactivation by breaking secondary and tertiary structures and simply denatures the enzyme or receptor.

Such allosteric denaturing mechanisms appear attractive for dietary polyphenols, since this class of compounds is notorious for nonspecific protein interactions through OH–O=C and NH–OH amide phenol hydrogen bonds and aromatic interactions [12, 13]. The subclass of plant tannins for example has been for centuries used in the process of leather tanning, where nonspecific phenol protein interactions lead to precipitation of proteins in animal skin producing leather [14–16]. The phenomenon of astringency has been rationalized in a similar way. The nonspecific interactions between dietary polyphenols and intrinsically unstructured saliva proteins, leading again to protein precipitation on the tongue, produce the sensory property or mouthfeel of astringency [8, 17]. For such interactions, stoichiometries of up to 15:1 have recently been demonstrated experimentally [18–20]. In the production of beer tannoids, another class of polyphenols interacts in a nonspecific way with malt and yeast proteins to produce a haze in the beverage negatively affecting shelf life time and sensory properties. Polyphenols are removed industrially from beer by filtration taking advantage of their nonspecific interactions with synthetic protein like amides such as polyvinylpyrrolidinone [21]. These few examples serve to highlight the nonspecific interactions between polyphenols and proteins, typically with several phenol molecules binding to a single protein [22], and there are many others also relevant to the food sector.

Combining the knowledge about the nonspecific polyphenol protein interactions with the Prinz hypothesis prompted us to address this issue in more detail. According to Prinz, nonspecific or allosteric multiple interactions leading to protein denaturation should be detectable through an analysis of Hill coefficients from dose–response curves in reported polyphenol protein and receptor binding studies.

The question we like to address here is the following: from the available literature data, is there any evidence that dietary polyphenols are single binding site inhibitors or multiple binding site allosteric denaturing agents? Following an analysis of literature data, a further question will arise: what are the biological consequences of any such finding?

Literature search

As discussed above, we decided to carry out literature searches to identify reported Hill coefficients from dose–response curves reported for dietary polyphenol enzyme and receptor inhibition studies. In a first search, we decided to focus on a series of selected polyphenols, which are most relevant to the human diet. For these compounds, strong evidence exists that their consumption is beneficial for human health to a degree that the compounds have been featured regularly in the press and compounds are available as dietary supplements. Furthermore these compounds, due to their popularity among the scientific community and due to their commercial availability have been included frequently in high-throughput and targeted screening campaigns. The compounds include 5-caffeoyl quinic acid (chlorogenic acid) **1**, catechin **2**, epicatechin **3**, EGCG **4**, caffeic acid **5**, galloyl glucose **6**, resveratrol **7**, kaempferol **8**, quercetin **9**, rutin **10** (one of the most abundant phenolics in dietary plants, however, hydrolyzed by gut floral metabolism prior to absorption), myricetin **11**, luteolin **12**, and genistein **13**. Their chemical structures are shown in Fig. 1.

We carried out several searches using both ISI Web of Science and Scifinder, including as keywords the name of the compound in question along with the keywords “enzyme inhibition,” “enzyme kinetics,” and “receptor inhibition.” From the resulting publications, we extracted and compiled Hill coefficients whenever they were given. Table 1 summarizes the findings of these searches.

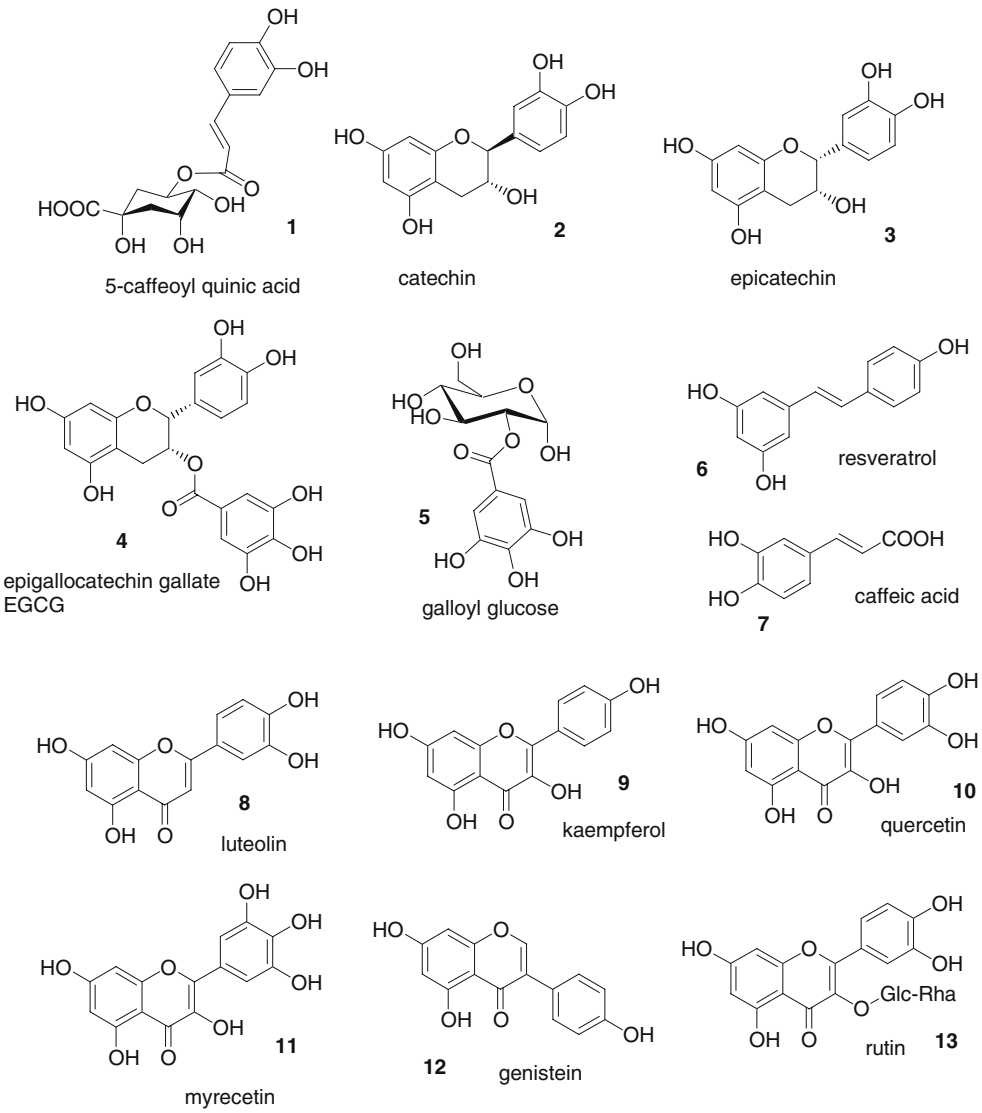

Fig. 1 Selected dietary polyphenols for which literature searches for Hill coefficients were carried out

Surprisingly, only around 15% of the publications found here quoted explicitly numerical values for Hill coefficients in their experimental dose–response curves. Around 30% of all Hill coefficients stated gave a value of $n=1$, whereas 70% of Hill coefficients stated were giving a value of $n>1$ with an average value of $n=4.6$. None of the publications discussed the significance of the Hill coefficients obtained.

In a second search, we consulted the NIH high-throughput screening database [23]. Direct searching for the required information using a substructure search turned out difficult so we took the following detour. The compounds above **1–12** were searched in the NIH database and high-throughput screening data identified that included these compounds. In the majority of high-throughput campaigns, the authors of the data serendipitously had an affinity for dietary polyphenols and included a nice selection of several polyphenolic compounds in their screen. We extracted available Hill coefficients for all compounds with a polyphenolic substructure shown in Fig. 2. From these manual searches, we obtained a selection of 200 representative Hill coefficients. The frequency of Hill coefficients found in both searches is shown in a histogram in Fig. 3.

Around 8% of all Hill coefficients stated gave a value of $n=1$, whereas 92% of Hill coefficients stated were giving a value of $n>1$ with an average value of $n=4.2$.

Discussion

From our literature searches compiling Hill coefficients of polyphenol enzyme interactions, the following observations were made:

1. It becomes obvious that in the majority of publications consulted, no numerical values for Hill coefficients were stated.

Table 1 Results of searches for Hill coefficient for thirteen selected dietary polyphenols

Number	Compound	Papers identified	Papers reporting Hill coefficient	$n_H=1$	$n_H>1$
1	5-CQA	9	0	NA	NA
2	Catechin	42	8	2	6
3	Epicatechin	38	6	3	3
4	EGCG	32	15	6	9
5	Caffeic acid	14	2	1	1
6	Galloyl glucose	11	0		
7	Resveratrol	33	3	1	2
8	Kaempferol	34	2	1	1
9	Quercetin	29	4	1	3
10	Rutin	16	1	0	1
11	Myricetin	8	1	0	1
12	Luteolin	7	4	1	3
13	Genistein	21	1	0	1
Total		294	47	16	31

2. On no occasion was this important parameter discussed. In the light of Prinz's work, we hope that in the future, Hill coefficients will attract the attention they deserve.
3. In the large majority of polyphenol enzyme or receptor interactions investigated, Hill coefficients of the dose–response curves are significantly larger than one. Only in few cases is the Hill coefficient $n=1$. Selected examples of such a Hill coefficient of $n=0.9$ to 1.6 indicating the interaction of a single polyphenol molecule with the active site of the target enzyme include the inhibition of EGCG of Ca^{2+}-dependent myofibrillar ATPase [24], glutamate dehydrogenase [25], ATP-sensitive potassium channels [26], rat brain Kv 1.5 potassium channels [27], DNA methyltransferase 1a [28], apigenin binding to GABA receptors [29], phloridzin inhibiting SGLT-1 [30], Genistein-inhibiting calf uterus estrogen receptor [31] and various tyrosine kinases [32–34], Luteolin binding to the Benzodiazepin receptor [35], and finally, 5-caffeoyl quinic acid inhibiting a series of non-human enzymes such as Helianthinin [36], sunflower 11S protein [37], diphenol oxidase [38], and phenylalanine ammonia lyase from potato [39].
4. Additionally, the available data suggest that if several structurally distinct polyphenols were investigated in a high-throughput screening campaign, several of these compounds displayed inhibition constants in the upper micromolar region with Hill coefficients larger than 1, indicating that independent of their unique chemical structure the combination of phenolic functionalities present, lead to allosteric inhibition.
5. A significant number of chemically distinct dietary polyphenols interact with a significant number of human enzymes with Hill coefficients larger than 1.
6. Single site inhibition appears to be the exception rather than the rule.

From these observations, it can be concluded that if Prinz's interpretation of a Hill coefficient of larger than one is taken at face value, the majority of polyphenols denature enzymes and receptors by allosteric mechanisms. In most cases, the interactions of four and more polyphenol molecules with one enzyme or receptor must be assumed, judging by the numerical values of their Hill coefficients. Similar to leather tanning or astringency, dietary polyphenols interact in the majority of cases in a nonspecific way with proteins and affect their activity in this way [40]. Their basic structure is designed by nature to carry out such nonspecific interactions, and these are clearly reflected in the experimental data available. The data additionally suggest that certain proteins possess on average 4–5 distinct polyphenol binding sites. Obviously, these nonspecific interactions have dramatic biological consequences, which are loss of activity of many enzymes and proteins.

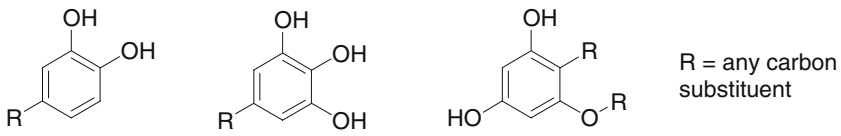

Fig. 2 Basic structural units of polyphenols, for which NIH database searches were carried out

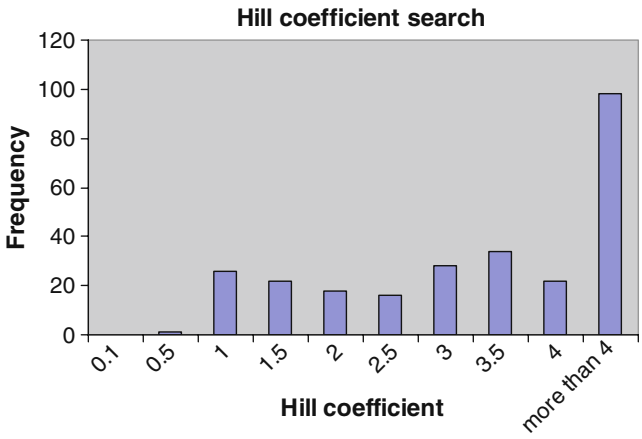

Fig. 3 Frequency of Hill coefficients compiled from NIH high-throughput database

Relevance of allosteric inhibition for and biological activities including human health benefits

From the above, the biological activity of dietary polyphenols resulting in human health benefits can be rationalized in terms of single site inhibition; however, as we propose here for the first time, it could as well be rationalized in terms of allosteric denaturing of enzymes resulting in loss of enzyme function.

To estimate the relative value of these two alternatives, we would like to argue as follows: in order for any compound to show a physiologically relevant biological effect by single site inhibition, the blood plasma concentration of the compound must reach a level equivalent to its inhibition constant IC_{50} [41]. This notion is undisputed, constituting a key element in pharmaceutical chemistry. Recent review on pharmacokinetic data of dietary polyphenols suggests that this is rarely the case [4, 42]. Whereas experimental IC_{50} values are generally in the lower micromolar region, C_{max} values tend to be in the nanomolar region. These findings suggest that single site inhibition is not only a rare event among the literature data searched here but is also a rare event in a physiologically relevant human setting, with most dietary polypenols not reaching the required plasma concentration required for single site inhibition.

One might ask the question whether at these low plasma concentrations binding stoichiometries with $n > 1$ can be realized, which must be strongly negated. Because of these facts, the biological activity of dietary polyphenols must be rationalized in other ways based on the assumption that in order to show any biological effects reasonably, high plasma concentration of active species must be achieved. Three alternatives for achieving sufficiently high plasma concentrations for biological activity appear worthy of consideration. The first option was recently introduced by Crozier and Clifford, and we would like to term it the "limited metabolite hypothesis" [2]. Although the dietary polyphenols are present in the diet in considerable concentrations, both gut microflora and human metabolism convert the dietary compounds into a large set of metabolites, thereby reducing the individual concentration of the parent compound dramatically. However, Crozier and Clifford suggested that the actual number of metabolites obtained from the large diversity of polyphenols consumed in the human diet is actually rather small, much smaller if compared to the vast number of different compounds ingested. Independent of the structure of the original plant secondary metabolite the same few metabolites have been observed experimentally, suggesting that such metabolites, mainly phenolic acids, reach sufficient plasma concentrations, and could act as single site inhibitors on so far unidentified biological targets.

As a second alternative, partition of polyphenols or their metabolites with a hydrophobic phase present in plasma such as membranes or serum albumin could be used to rationalize this observation. The final alternative can be derived from the observations made in our literature search, and we would like to term it the insect swarm hypothesis, since there are no single insects similar in single site inhibition but rather a large number of structurally diverse insects in a swarm affecting the activity of the biological target. From the literature search, it was observed that polyphenols of identical structure are able to inhibit several enzymes of different structure with $n \geq 4$ at micromolar IC_{50} and several polyphenols of different structure inhibit an enzyme of identical structure with $n \geq 4$ at micromolar IC_{50}. So it might be conceivable, however, requiring urgent experimental verification that several polyphenols of different structure allosterically denature a single enzyme with $n \geq 4$ at micromolar IC_{50}. We propose that polyphenols are able to act additively or even synergistically on the same biological target leading to loss of activity by nonspecific interactions. Summing up the individual C_{max} values of many structurally diverse polyphenols would

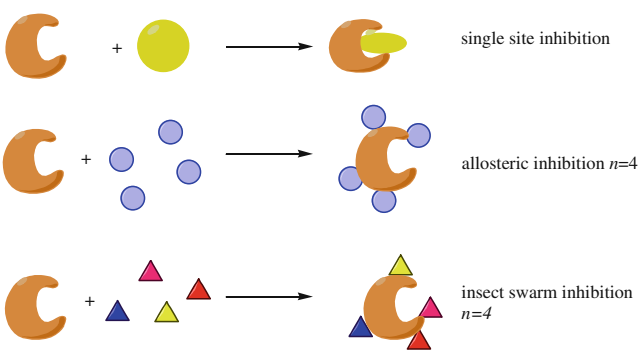

Fig. 4 Principle of single site, multiple allosteric, and insect swarm inhibition

result in an overall plasma concentration in the micromolar range required for physiologically relevant biological activity. The principle is illustrated in Fig. 4.

In a second line of arguments, we would like to turn to the role of polyphenols in nature itself. Like most secondary metabolites, it must be assumed that polyphenols play a pivotal function for the organisms biosynthesizing them, giving the organism in question an evolutionary advantage [43]. The main mechanism of realizing evolutionary fitness is inhibition of the enzymatic functions of any pest organisms. Similar to drug development, the paradigm in natural product chemistry states that such inhibition is usually achieved by specific single active site inhibition of the pest organism enzymes by any natural product. It has been suggested by Firn and Jones that nature tries to achieve such a single site inhibition by producing diverse compounds and thereby using an approach termed the "screening hypothesis of natural products" [44–46].

Here, we propose that nature's more efficient and successful strategy might involve the design and biosynthesis of highly efficient allosteric denaturing agents that act additively to achieve protection against pest organisms using an insect swarm approach. The observation of frequent Hill coefficients larger than one additionally suggests that many enzymes might possess within their primary and tertiary structure distinct polyphenol binding motives. Polyphenols due to their unique arrays of functional groups should be considered as such compounds, designed and biosynthesized to fulfill the purpose of allosteric denaturation of pest enzymes. Supporting evidence for this notion comes from a look at the number of individual polyphenols produced by plants.

Within poylphenol biochemistry, plants can be generally classified in three groups:

1. Plants that biosynthesize a relatively small number of distinct polyphenolic molecules (around 5–30).
2. Plants that biosynthesize a relatively small number of distinct polyphenolic molecules (around 5–30), however, possess oxidative enzymes that upon damage of the cell in their defense strategy, convert these reactive poylphenolic molecules into a vast array of diverse polyphenolic compound libraries (this process is generally referred to as fermentation or vegetable browning, dietary plants of this type include apple, pear, banana, tea, chocolate, etc.) [47–50].
3. Plants that biosynthesize a large number of structurally diverse polyphenolic molecules (larger than 30, with the most diverse set found in coffee with more than 100 chlorogenic acids) [51–54].

In particular, plants in groups 2 and 3 seem to follow a cunning strategy by producing a vast number of structurally diverse polyphenolic compounds (e.g., at least 30,000 in the case of the thearubigins in tea *Camellia sinensis*) each in small quantities (femtomolar to nanomolar), quantities too small to show even after significant absorption any biological effect that could be rationalized in terms of single active site enzyme inhibition [41, 55]. However, if one assumes that these compounds are produced with a single aim of nonspecific allosteric enzyme denaturation by the insect swarm hypothesis, this plot of nature suddenly seems to make sense. Additionally, such a strategy would add to the evolutionary fitness of any organism, since the biosynthesis of few single site inhibitors increase the risk of non-survival after development of resistance by the pest organisms. However, using structurally diverse nonspecific allosteric inhibitors minimizes the risk of submission to resistance.

At this point, we briefly like to return to our initial starting point of this discussion the health effects of dietary polyphenols. If the novel concepts and ideas developed here are true, it is a highly puzzling conundrum that the daily consumption of gram quantities of nonselective allosteric enzyme denaturing agents results in any beneficial health effect. Only in the case of cancer chemoprevention does it appear an attractive hypothesis to assume that a large number of polyphenolic metabolites inhibit the growth of rapidly proliferating cancer cells by multiple allosteric denaturation of cancerous enzymes and receptors. In all other areas of health benefits, an intense discussion is required to rationalize current results in the light of the observations made here, which could be rationalized in terms of a sophisticated training exercise for the human molecular defense system. It is also worth noting that certain drug synergisms could be explained by the insect swarm hypothesis.

In his seminal publication, Prinz asked the question whether there had ever been a successful drug developed with a Hill coefficient different from one. Dietary polyphenols are not drug molecules in the strictest sense [2], however treated by the human body as xenobiotics similar to drugs and frequently marketed with drug-like applications in mind such as food additives or neutraceuticals. In any case, to conclude, compounds with a Hill coefficient different from one are consumed on a daily basis in gram quantities by humans and display undoubtedly beneficial health effects.

References

1. Hertog MGL, Feskens EJM, Hollman PCH, Katan MB, Kromhout D (1993) dietary antioxidant flavonoids and risk of coronary heart-disease - the zutphen elderly study. Lancet 342:1007–1011
2. Crozier A, Jaganath IB, Clifford MN (2009) Dietary phenolics: chemistry, bioavailability and effects on health. Nat Prod Rep 26:1001–1043

3. Williamson G, Manach C (2005) Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. Am J Clin Nutr 81:243S–255S
4. Scalbert A, Williamson G (2000) Dietary intake and bioavailability of polyphenols. J Nutr 130:2073S–2085S
5. RiceEvans CA, Miller J, Paganga G (1997) Antioxidant properties of phenolic compounds. Trends Plant Sci 2:152–159
6. Halliwell B, Gutteridge JMC, Cross CE (1992) Free-radicals, antioxidants, and human-disease: where are we now? J Lab Clin Med 119:598–620
7. Middleton E, Kandaswami C, Theoharides TC (2000) The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. Pharmacol Rev 52:673–751
8. Baeuerle PA, Henkel T (1994) Function amd activation of NF-kappa-B in the immune-system. Annu Rev Immunol 12:141–179
9. Prinz H, Schönichen A (2008) Transient binding patches: a plausible concept for drug binding. J Chem Biol 1:95–104
10. Prinz H (2010) Hill coefficients, dose response curves and allosteric machanisms. J Chem Biol 2:37–44
11. Kuhnert N, Le-Gresley A (2005) Synthesis and capsule formation of upper rim substituted tetra-acrylamido calix 4 arenes. Org Biomol Chem 3:2175–2182
12. Haslam E (1974) Polyphenol-protein interactions. Biochem J 139:285–288
13. McManus JP, Davis KG, Lilley TH, Haslam E (1981) The association of proteins with polyphenols. J Chem Soc Chem Comm 7:309–311
14. Haslam E (1988) Plant polyphenols (syn vegetable tannins) and chemical defense - a reappraisal. J Chem Ecol 14:1789–1805
15. Hagerman AE, Butler LG (1978) Protein precipitation method for quantitative-determination of tannins. J Agr Food Chem 26:809–812
16. Hagerman AE, Butler LG (1980) Determination of protein in tannin-protein precipitates. J Agr Food Chem 28:944–947
17. Haslam E, Lilley TH (1988) Natural astringency in foodstuffs - a molecular interpretation. Crc Crit Rev Food Sci 27:1–40
18. Baxter NJ, Lilley TH, Haslam E, Williamson MP (1997) Multiple interactions between polyphenols and a salivary proline-rich protein repeat result in complexation and precipitation. Biochemistry 36:5566–5577
19. Boze H, Marlin T, Durand D, Perez J, Vernhet A, Canon F, Sarni-Manchado P, Cheynier V, Cabane B (2010) Proline-Rich Salivary Proteins Have Extended Conformations. Biophys J 99:656–665
20. Canon F, Pate F, Meudec E, Marlin T, Cheynier V, Giuliani A, Sarni-Manchado P (2009) Characterization, stoichiometry, and stability of salivary protein-tannin complexes by ESI-MS and ESI-MS/MS. Anal Bioanal Chem 395:2535–2545
21. Charlton AJ, Baxter NJ, Khan ML, Moir AJG, Haslam E, Davies AP, Williamson MP (2002) Polyphenol/peptide binding and precipitation. J Agr Food Chem 50:1593–1601
22. Hagerman AE, Rice ME, Ritchard NT (1998) Mechanisms of protein precipitation for two tannins, pentagalloyl glucose and epicatechin(16) (4 ->8) catechin (procyanidin). J Agr Food Chem 46:2590–2595
23. NIH database at http://www.ncbi.nlm.nih.gov/sites/entrez (search PubChem bioassay).
24. Liou YM, Kuo SC, Hsieh SR (2008) Differential effects of a green tea-derived polyphenol (-)-epigallocatechin-3-gallate on the acidosis-induced decrease in the Ca^{2+} sensitivity of cardiac and skeletal muscle. Pflug Arch Eur J Phy 456:787–800
25. Li M, Allen A, Smith TJ (2007) High throughput screening reveals several new classes of glutamate dehydrogenase inhibitors. Biochemistry 46:15089–15102
26. Baek WK, Jang BC, Lim JH, Kwon TK, Lee HY, Cho CH, Kim DK, Shin DH, Park JG, Lim JG, Bae JH, Yoo SK, Park WK, Song DK (2005) Inhibitory modulation of ATP-sensitive potassium channels by gallate-ester moiety of (-)-epigallocatechin-3-gallate. Biochem Pharmacol 70:1560–1567
27. Choi BH, Choi JS, Min DS, Yoon SH, Rhie DJ, Jo YH, Kim MS, Hahn SJ (2001) Effects of (-)-epigallocatechin-3-gallate, the main component of green tea, on the cloned rat brain Kv1.5 potassium channels. Biochem Pharmacol 62:527–535
28. Lee WJ, Zhu BT (2006) Inhibition of DNA methylation by caffeic acid and chlorogenic acid, two common catechol-containing coffee polyphenols. Carcinogenesis 27:269–277
29. Campbell EL, Chebib M, Johnston GAR (2004) The dietary flavonoids apigenin and (-)-epigallocatechin gallate enhance the positive modulation by diazepam of the activation by GABA of recombinant GABA(A) receptors. Biochem Pharmacol 68:1631–1638
30. Kottra G, Daniel H (2007) Flavonoid glycosides are not transported by the human Na^+/glucose transporter when expressed in Xenopus laevis oocytes, but effectively inhibit electrogenic glucose uptake. J Pharmacol Exp Ther 322:829–835
31. Picherit C, Dalle M, Neliat G, Lebecque P, Davicco MJ, Barlet JP, Coxam V (2000) Genistein and daidzein modulate in vitro rat uterine contractile activity. J Steroid Biochem 75:201–208
32. Tao J, Zhang Y, Li SN, Sun WH, Soong TW (2009) Tyrosine kinase-independent inhibition by genistein on spermatogenic T-type calcium channels attenuates mouse sperm motility and acrosome reaction. Cell Calcium 45:133–143
33. Molokanova E, Kramer RH (2001) Mechanism of inhibition of cyclic nucleotide-gated channel by protein tyrosine kinase probed with genistein. J Gen Physiol 117:219–233
34. Kusaka M, Sperelakis N (1996) Genistein inhibition of fast Na^+ current in uterine leiomyosarcoma cells is independent of tyrosine kinase inhibition. Biochim Biophys Acta 1278:1–4
35. Coleta M, Campos MG, Cotrim MD, de Lima TCM, da Cunha AP (2008) Assessment of luteolin (3′, 4′, 5, 7-tetrahydroxyflavone) neuropharmacological activity. Behav Brain Res 189:75–82
36. Suryaprakash P, Prakash V (1995) Interaction of 3′-O-caffeoyl D-quinic acid with multisubunit protein helianthinin. J Biosci 20:531–549
37. Sastry MCS, Rao MSN (1991) Effect of chemical modification of sunflower 11 S protein on the binding of chlorogenic acid. J Agr Food Chem 39:63–66
38. Goodenough PW, Kessell S, Lea AGH, Loeffler T (1983) Monophenolase and diphenolase activity from fruit of *Malus-pumila*. Phytochemistry 22:359–363
39. Tanaka Y, Uritani I (1977) Purification and properties of phenylalanine ammonia-lyase in cut-injured sweet-potato. J Biochem 81:963–970
40. Spencer CM, Cai Y, Martin R, Gaffney SH, Goulding PN, Magnolato D, Lilley TH, Haslam E (1988) Polyphenol complexation - some thoughts and observations. Phytochemistry 27:2397–2409
41. Hanlon N, Coldham N, Gielbert A, Kuhnert N, Sauer MJ, Kingi LJ, Ioannides C (2008) Absolute bioavailability and dose-dependent pharmacokinetic behaviour of dietary doses of the chemopreventive isothiocyanate sulforaphane in rat. Brit J Nutr 99:559–564
42. Manach C, Williamson G, Morand C, Scalbert A, Remesy C (2005) Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am J Clin Nutr 81:230S–242S
43. Haslam E (1986) Secondary metabolism - fact and fiction. Nat Prod Rep 3:217–249

44. Firn RD, Jones CG (2003) Natural products - a simple model to explain chemical diversity. Nat Prod Rep 20:382–391
45. Jones CG, Firn RD (1991) On the evolution of plant secondary chemical diversity. Philos T Roy Soc B 333:273–280
46. Firn R (2004) Plant intelligence: An alternative point of view. Ann Bot 93:345–351
47. Kuhnert N (2010) Unraveling the structure of the black tea thearubigins. Arc Biochem Biophys 501:37–51
48. Kuhnert N, Drynan JW, Obuchowicz J, Witt M, Clifford M (2010) On the chemical characterization of black tea thearubigins using mass spectrometry. Rapid Commun Mass Spetrom 24:3387–3404
49. Kuhnert N, Müller A, Clifford (2010) Analysis of black tea thearubigins: Evidence for oxidative cascade reactions forming the thearubigins. Food Funct 1:180–199
50. Drynan JW, Clifford MN, Obuchowicz J, Kuhnert N (2010) The chemistry of low molecular weight black tea polyphenols. Nat Prod Rep 27:417–462
51. Jaiswal R, Patras MA, Eravuchira PJ, Kuhnert N (2010) Profile and characterization of the chlorogenic acids in green Robusta coffee beans by LC-MS^n: Identification of seven new classes of compounds. J Agr Food Chem 58:8722–8737
52. Kuhnert N, Jaiswal R, Eruvichera P, El-Abassy R, von der Kammer B, Materny M (2010) Scope and limitations of principal component analysis of high resolution LC-MS data: The analysis of the chlorogenic acid fraction in green coffee beans as a case study. Anal Meth. doi:10.1039/c0ay00512f
53. Jaiswal R, Sovdat T, Vivan F, Kuhnert N (2010) Profiling and characterization by LC-MS^n of the chlorogenic acids and hydroxycinnamoylshikimate esters in mate (*Ilex paraguariensis*). J Agr Food Chem 58:5471–5484
54. Clifford MN, Kirkpatrick J, Kuhnert N, Roozendaal H, Salgado PR (2008) LC-MS^n analysis of the cis isomers of chlorogenic acids. Food Chem 106:379–385
55. Haslam E (1996) Natural polyphenols (vegetable tannins) as drugs: Possible modes of action. J Nat Prod 59:205–215