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Sweeteners

Saccharin Inhibits Carbonic Anhydrases: Possible Explanation for its Unpleasant Metallic Aftertaste

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Saccharin is the oldest artificial sweetener, and was discovered by serendipity in 1879 by Fahlberg and Remsen.[1] About 450 times as sweet as sucrose, it was a very important discovery, particularly for diabetics. Commercialization started shortly after its discovery and it has become increasingly popular in recent years as a calorie-free sugar surrogate. Blended with cyclamate, it has been hugely successful and sold, for example, as "Sweet 'n Low". Saccharin is not only known for its extreme sweetness, but also for a bitter and metallic aftertaste. Even though there have been worries about its safety since its introduction, particularly in the 1960s and 1970s, only speculations about its putative carcinogenicity have been put forward. At normal doses, no clear evidence for a causal correlation between saccharin consumption and health risks for humans could be demonstrated.[2]

Saccharin is used as its sodium salt. Chemically it consists of a sulfimide with a lactam and cyclic sulfonamide moiety. The latter functionality is responsible for the acidic character of the molecule and suggests its potential to interact with the zinc ion at the floor of the binding pocket of carbonic anhydrases. The carbonic anhydrases are an important family of ubiquitously present proteins distributed over many compartments and organs of our body. To date, 15 isozymes have been characterized.[3] They all catalyze the same reaction: the fixation of carbon dioxide by water to form bicarbonate. As such, the different carbonic anhydrases (CAs) are involved in various essential tasks, for example, CO_2 transportation, pH regulation, or delivery of C_1 building blocks in biosynthesis.[4]

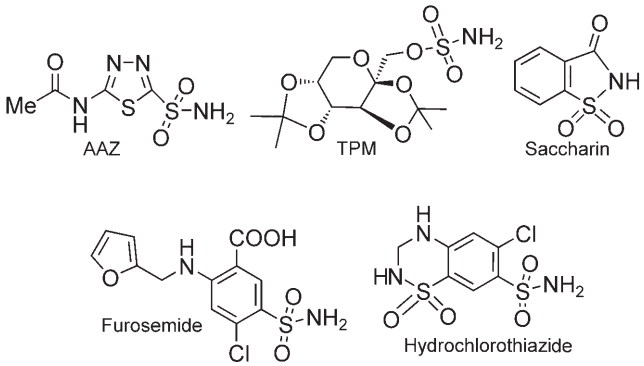
We determined the inhibition profile[5] of saccharin against a panel of different carbonic anhydrase isoenzymes. Surprisingly enough, the compound shows inhibition of some members of this protein family at the nanomolar level (Table 1). Compared to the classical and therapeutically well-established CA inhibitor acetazolamide (AAZ) and other sulfonamides, such as furosemide, hydrochlorothiazide, and topiramate (TPM), saccharin shows remarkable selectivity discrimination among the different isoforms. This fact is particularly impressive as all the CAs possess largely conserved catalytic centers, and medicinal chemists have to fight hard to equip their development compounds with a sufficient selectivity profile. Saccharin shows low micromolar inhibition towards the ubiquitously distributed CAII. This made us confident to embark on the structure determination of saccharin in a complex with carbonic anhydrase II (Figure 1 and the Supporting Information).[6]

Saccharin most likely coordinates in a deprotonated state through its nitrogen atom to the catalytically active zinc ion, thereby producing a flattened distorted tetrahedral environment at the metal center. The local environment around the

Table 1: Inhibition data of AAZ, TPM, furosemide, hydrochlorothiazide, and saccharin against isozymes I–XIV (stopped-flow, CO_2 hydration method[5]).

Isozyme[a]	K_i[b] [nM]				
	AAZ	TPM	furosemide	hydrochlorothiazide	saccharin
hCAI[c]	250	250	62	328	18540
hCAII[c]	12	10	65	290	5950
hCAIV[c]	74	4900	564	427	7920
hCAVa[c]	63	63	499	4225	10060
hCAVb[c]	54	30	322	603	7210
hCAVI[c]	11	45	245	3655	935
hCAVII[c]	2.5	0.9	513	5010	10
hCAIX[d]	25	58	420	367	103
hCAXII[d]	5.7	3.8	261	355	633
mCAXIII[c]	17	47	550	3885	12100
hCAXIV[c]	41	1460	52	4105	773

[a] h = human; m = murine isozyme. [b] The errors are in the range of 5–10% of the reported value (from 3 different assays). [c] Human (cloned) isozymes, by the CO_2 hydration method. [d] Catalytic domain of human, cloned isozyme, by the CO_2 hydration method.[5]



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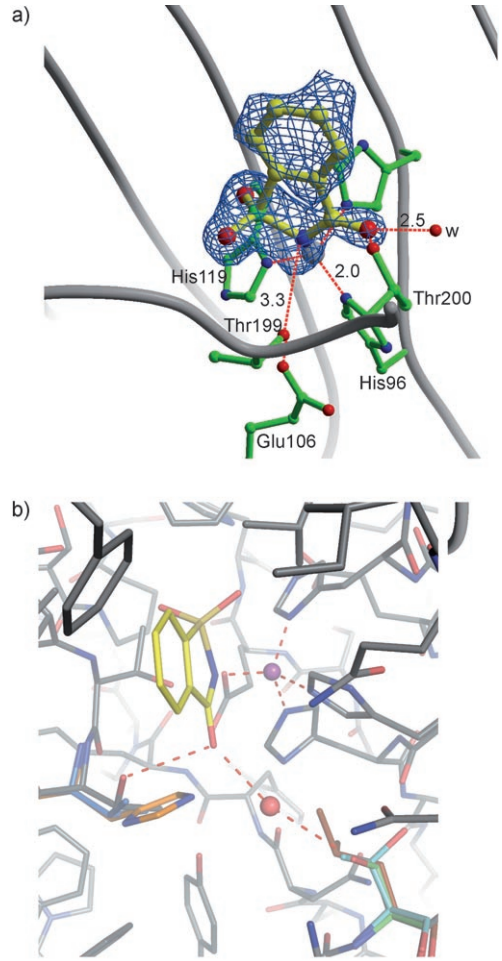

Figure 1. a) Binding site of carbonic anhydrase II (catalytic residues in green) with bound saccharin (yellow, difference electron density (blue) contoured at 3 σ). The picked-up water molecule in the active site is shown to the right as a red sphere. b) Crystal structure from (a) (protein residues, gray; saccharin, yellow; active-site water molecule, red sphere; zinc, violet) with superimposed residues mutated in the other isoforms: His200 (orange, CAI), Val200 (blue, mCAXIII), Ser65 (dark green, CAVII), Leu65 (brown, CAVa), and Thr65 (cyan, CAXIV).

nitrogen atom itself is pyramidal, with the oxygen atom Oγ of the Thr199 side chain found along the assumed direction of the lone pair of electrons. The separation of 3.3 Å advocates for the formation of a weak hydrogen bond; however, the latter oxygen atom is also in hydrogen-bonding distance of one oxygen atom of Glu106 (2.5 Å). One oxygen atom of the neighboring sulfonyl group increases the coordination at the zinc center towards a distorted bipyramidal geometry (3.1 Å), while the second oxygen atom forms a weak hydrogen bond to the backbone NH group of Thr199. The lactam carbonyl oxygen atom is in hydrogen-bonding distance to Thr200Oγ. Furthermore, it forms a hydrogen bond to a water molecule in the active site.

Another peculiarity indicated by the present structure is the incorporation of a putative second zinc ion in the active site. His64, which is involved in shuttling protons in and out of the catalytic site, is found distributed over two conformations. The first conformer is superimposed by a high electron density that could be refined as a second partially populated zinc ion (67%). It coordinates to His64Nδ in the second conformer, the backbone NH group of the same residue, and the backbone carbonyl group of Asn62. Furthermore, two water molecules complete the coordination sphere of this second zinc ion. Zinc ions are prevalent, usually in micromolar concentration, and thus they can be picked up from the buffer conditions and incorporated into the structure.

On the basis of the present structure determination, it is tempting to speculate about the impressive selectivity discrimination of saccharin among the different isozymes (a sequence alignment table can be found in the Supporting Information). Almost all the well-established and clinically approved CA inhibitors exhibit a terminal exocyclic sulfonamide, sulfamate, or sulfimine functionality.[7] In contrast, saccharin has a cyclic structure and its lactam carbonyl group in the five-membered heterocycle is an exclusive feature not present in the skeletons of other compounds usually considered as inhibitors. Saccharin shows only moderate inhibitory potency towards CAII, but nanomolar binding is recorded for CAVII.

As no crystal structure is available for this isoform, a homology model retrieved from the Swiss-Model Repository[8] was used. Interestingly, an alanine residue is replaced by a serine residue at the far end of the binding pocket in the vicinity of the lactam carbonyl oxygen atom (Figure 1b). In the crystal structure of the saccharin–CAII complex, a water molecule is observed which is ideally placed to mediate a contact between the lactam carbonyl group and the serine Oγ atom present in the homology model of CAVII. This additional contact is likely to enhance ligand binding. In a recent study on aldose reductase we showed that an inhibitor which picks up a contact-mediating water molecule gains a significant affinity advantage over a closely related ligand that lacks a similar contact.[9]

Interestingly, a sequence alignment shows that this Ala/Ser replacement also occurs in CAI, CAIV, CAVb, CAIX, CAXII, and murine CAXIII. CAIX and XII also show enhanced saccharin binding, whereas a dramatic drop in affinity is observed for CAI and CAXIII. In the latter two isoforms Thr200 is replaced by either His200 or Val200, both of which require significantly more space next to the lactam carbonyl group. Thus, they are expected to produce steric conflicts with the bound saccharin, and a dramatic drop in affinity can be expected. Weak inhibition is also observed in CAVa. Here, the crucial Ala to Ser replacement is exchanged by a spatially more demanding Leu residue. This residue would also be unable to promote a water-mediated contact, and so, as with the Ala mutation, this residue will be detrimental for the potent binding of saccharin. CAVI and CAXIV are also inhibited to a remarkable extent by saccharin. Interestingly, they possess a Thr residue at the site of Ala65 in CAII. This residue should be equally well suited to pick up a water-mediated contact from the ligand.

The question arises as to whether the observed potent in vitro inhibition of some of the CA isoforms has any pharmacological consequences, for example, in terms of desired or undesired cross-reactivities or side effects. Saccharin is expected to activate the sweetness-sensing receptors on

the tongue. Apart from this function, an ideal sweetener should pass through the intestinal system without any unwanted penetration into the blood stream or even passing through the blood–brain barrier. CAVII, the most strongly inhibited isoform, is located in the brain. It is unlikely, considering the acidic character of saccharin, that the compound will arrive in the brain at significant concentration by passive transportation. Only under the acidic conditions of the stomach will a considerable amount of saccharin be present in the uncharged more hydrophobic state. In this state some passive penetration might occur; however, absorption at pH 6–8 is rather unlikely. Nevertheless, it has been shown that saccharin is nearly completely absorbed from the gut and rapid elimination in the urine occurs as unchanged compound.[10] A distribution of saccharin across most organs in rats has been described; thus, a possible active transport has been suggested.[10] Clearly, the compound is chemically rather inert and not easily metabolized in vivo. Nevertheless, the decrease in plasma levels after oral dosing has been reported as being slow and limited by the rate of absorption from the gut.[11]

It remains, however, speculative as to whether this latter observation can be attributed to putative inhibition of different CAs present in various compartments of the organism. Recently, CAVI, the only secreted form of CAs, has been discussed in regard to its putative involvement in olfaction, taste, and pH regulation in the oral cavity.[12] As such, it contributes to the acidification of the enamel pellicle of teeth, and speculations about its inhibition with respect to protection against caries have been put forward.[13,14] Saccharin possesses an unpleasant bitter or metallic aftertaste, a property shared with clinically approved systemic carbonic anhydrase inhibitors.[15] This property may in fact be due to the inhibition of the salivary CAVI, for which saccharin also shows submicromolar inhibition. Nevertheless, as the various CAs are repeatedly discussed as putative targets for drug therapy with respect to diuresis, glaucoma, tumor suppression, and obesity, saccharin might teach medicinal chemists how to equip their molecules with additional features to achieve impressive selectivity discrimination.

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