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Carbonic anhydrase inhibitors. Inhibition of isozymes I, II, IV, V and IX with complex fluorides, chlorides and cyanides

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Abstract—The inhibition of five human carbonic anhydrase (hCA, EC 4.2.1.1) isozymes, the cytosolic hCA I and II, the membrane-bound hCA IV, the mitochondrial hCA V and the tumour associated, transmembrane hCA IX, with complex anions incorporating fluoride, chloride and cyanide, as well as B(III), Si(IV), P(V), As(V), Al(III), Fe(II), Fe(III), Pd(II), Pt(II), Pt(IV), Cu(I), Ag(I), Au(I) and Nb(V) species has been investigated. Apparently, the most important factors influencing activity of these complexes are the nature of the central metal ion/element, and its charge. Geometry of these compounds appears to be less important, since both linear, tetrahedral, octahedral as well as pentagonal bipyramidal derivatives led to effective inhibitors. However, the five isozymes showed very different affinities for these anion inhibitors. The best hCA I inhibitors were cyanide, dicyanocuprate and dicyanoaurate (K_Is in the range of 0.5–7.7 μM), whereas the least effective were fluoride and hexafluoroarsenate. The best hCA II inhibitors were cyanide, hexafluoroferrate and tetrachloroplatinate (K_Is in the range of 0.02–0.51 mM), whereas the most ineffective ones were fluoride, hexafluoroaluminate and chloride. The best hCA IV inhibitors were dicyanocuprate (K_I of 9.8 μM) and hexacyanoferrate(II) (K_I of 10.0 μM), whereas the worst ones were tetrafluoroborate and hexafluoroaluminate (K_Is in the range of 124–126 mM). The most effective hCA V inhibitors were cyanide, heptafluoroniobate and dicyanocuprate (K_Is in the range of 0.015–0.79 mM), whereas the most ineffective ones were fluoride, chloride and tetrafluoroborate (K_Is in the range of 143–241 mM). The best hCA IX inhibitors were on the other hand cyanide, heptafluoroniobate and dicyanoargentate (K_Is in the range of 4 μM–0.33 mM), whereas the worst ones were hexacyanoferrate(III) and hexacyanoferrate(II).

1. Introduction

Anions, such as halides, pseudohalides, sulfide, act as inhibitors of many types of metalloenzymes due to their capacity of binding metal ions within the active site of such enzymes.[1,2] A particularly well-studied case is represented by the carbonic anhydrases (CAs, EC 4.2.1.1), zinc enzymes that catalyze the interconversion between carbon dioxide and bicarbonate, widely distributed all over the phylogenetic tree.[2–5] These enzymes are inhibited by inorganic, simple metal-complexing anions (such as those mentioned above) as well as by anions, which show a lower tendency to bind metal ions in solution, such as sulfate, nitrate or perchlorate, among others.[6–10]

In several previous contributions from these laboratories,[7–10] we have investigated the interaction between various α-, β- and γ-CA isozymes, such as the human isozymes hCA IV, V, IX and XIII, or the archeal isozymes Cab and Cam with a series of simple anions such as halides, pseudohalides, nitrate, sulfate, carbonate, bicarbonate, etc., or with phosphates/phosphonates.[11] Several interesting facts emerged from such studies, which allowed us, for example, to hypothesize the involvement of the newly isolated cytosolic isozyme XIII in a metabolon with anion exchangers involved in the bicarbonate/chloride transport,[10a] or to establish that the renal side effects of the antiviral drug foscarnet are probably due to its strong inhibition of the membrane-associated isozyme CA IV, highly abundant in this organ where it plays a critical role in urine formation and excretion of anions.[11] Thus, further investigations of anion inhibitors of CAs may allow a better understanding of the physiological roles of these widely

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distributed enzymes, or may lead to the design of inhibitors with pharmacological applications.[2–5]

Since anions belonging to coordination compounds have not been investigated in detail up to now (only some qualitative data of CA II inhibition with ferrocyanide are mentioned by Maren,[12] whereas Scozzafava's group[1] investigated the interaction of Co(II)-substituted CA II with dicyanocuprate and dicyanoaurate, by means of electronic and NMR spectroscopy, but no precise inhibition constant measurements were provided) we report here the first inhibition study of five α-CA isozymes (i.e., the cytosolic isozymes I and II, the membrane-bound isozyme IV, the mitochondrial isozyme V as well as the transmembrane, tumour-associated isozyme IX) with complex anions containing fluoride, chloride or cyanide. A series of such complex halides/pseudohalides, among which tetrafluoroborate; hexafluorosilicate; hexafluorophosphate(V)/arsenate(V); hexafluoroaluminate; hexafluoroferrate(III); heptafluoroniobate(V); tetrachloroplatinate(II); hexachloroplatinate(IV); dicyanocuprate(I); dicyanoargentate(I); dicyanoaurate(I); tetracyanopalladate(II); hexacyanoferrate(II) and hexacyanoferrate(III) have been included in our study.

2. Chemistry

Buffers and sodium/potassium salts (tetrafluoroborate; hexafluorosilicate; hexafluorophosphate(V); hexafluoroarsenate(V); hexafluoroaluminate; hexafluoroferrate(III); heptafluoroniobate(V); tetrachloroplatinate(II); hexachloroplatinate(IV); dicyanocuprate(I); dicyanoargentate(I); dicyanoaurate(I); tetracyanopalladate(II); hexacyanoferrate(II) and hexacyanoferrate(III)) were of highest purity available, from Sigma–Aldrich (Milano, Italy) and were used without further purification. CA isozymes were prepared as previously reported by our group.[7–10]

3. CA inhibition data

Inhibition data against five CA isozymes involved in critical physiological/pathological processes, that is, hCA I, hCA II (cytosolic forms), hCA IV (membrane associated), hCA V (mitochondrial) and hCA IX (transmembrane, tumour associated),[13–17] with the above mentioned anions are shown in Table 1. Inhibition data for fluoride, chloride and cyanide (the anions from which the complexes investigated here are derived) are also provided for comparison, as they were recently reported by our groups.[7–10]

Data in Table 1 allow us to draw the following conclusions regarding CA isozyme interaction with the anions investigated here (i) against hCA I, cyanide, dicyanocuprate and dicyanoaurate act as very potent inhibitors, with inhibition constants in the range of 0.5–7.7 μM. The most potent inhibitor is just cyanide, whereas its participation in the complex anions mentioned above leads to a slightly diminished inhibitory capacity. Other complex cyanides, such as dicyanoargentate, tetracyanopalladate and the two hexacyanoferrates are on the other hand much weaker hCA I inhibitors, with K_Is in the range of 0.50–9.5 mM. Clearly, the nature of the central metal ion (and its geometry to a lower degree) seems to be the most important parameters influencing potency among these complex anions. Submillimolar hCA I inhibitors were also hexafluoroferrate(III), hepta-

Table 1. Inhibition constants of anionic inhibitors against human isozymes hCA I, II, IV, V and IX for the CO_2 hydration reaction, at 20 °C[18]

Inhibitor	K_I[#] (mM)				
	hCA I[a]	hCA II[a]	hCA IV[b]	hCA V[c]	hCA IX[d]
F^-[e]	>300	>300	0.07	241	48
Cl^-[e]	6	200	0.09	156	33
CN^-[e]	0.5×10^{-3}	0.02	0.77	0.015	0.004
BF_4^-	11.6	25.5	126	143	18.3
SiF_6^{2-}	2.9	6.4	4.7	1.8	7.5
PF_6^-	11.0	17.5	19.1	>300	8.1
AsF_6^-	>300	9.4	5.5	75.6	7.9
$[AlF_6]^{3-}$	52.4	>300	124	43.6	18.5
$[FeF_6]^{3-}$	0.20	0.44	0.34	2.71	1.62
$[NbF_7]^{2-}$	0.20	0.60	0.50	0.44	0.10
$[PtCl_4]^{2-}$	0.37	0.51	27.5	41.7	35.2
$[PtCl_6]^{2-}$	13.8	6.23	17.4	10.8	3.63
$[Cu(CN)_2]^-$	6.9×10^{-3}	0.56	9.8×10^{-3}	0.79	4.77
$[Ag(CN)_2]^-$	0.50	0.76	97	1.75	0.33
$[Au(CN)_2]^-$	7.7×10^{-3}	0.58	5.36	1.91	31.3
$[Pd(CN)_4]^{2-}$	1.27	1.22	1.69	2.00	0.89
$[Fe(CN)_6]^{4-}$	5.74	9.7	0.01	3.13	87
$[Fe(CN)_6]^{3-}$	9.5	39.0	1.00	9.8	>300

[#] Errors were in the range of 3–5% of the reported values, from three different assays.
[a] Human recombinant isozymes.
[b] Truncated human isozyme lacking the first 20 amino acid residues.
[c] Full length human isozyme.
[d] Catalytic domain of human, recombinant isozyme.
[e] From Refs. 7–10.