Inorganica Chimica Acta, 56 (1981) 41–44

Metal Complexing Ability of Two Inducers of Reverse Transformation

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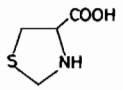
Received March 12, 1981

The metal complexing properties of sodium thiazolidine-4-carboxylate and 2-amino-2-thiazoline hydrochloride with H^+, Mg^{2+}, Ca^{2+}, Ni^{2+}, Mn^{2+}, Cu^{2+} and Zn^{2+} have been investigated. These agents have been reported to be a new type of anticancer agent which induce reverse transformation. Computer simulations of blood plasma suggest that this type of ligand drug disturbs the normal biochemistry of manganese and, to a lesser extent, zinc.

Introduction

Gosálvez *et al.* [1–3] have reported that the compounds thiazolidine-4-carboxylic acid (thiaproline) (I) and 2-amino-2-thiazoline hydrochloride (II) are both capable of inducing 'reverse transformation' in tumour cells. (This term was coined a decade



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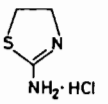

II

ago for those agents which caused tissue cells in culture to lose the characteristics of tumoral transformation and to assume a morphology approaching that of normal cells. It includes the restoration of 'contact inhibition' in tumour cells previously devoid of it) [4].

These two compounds have undergone clinical trials in advanced human cancer and therapeutic activity is reported [5–7]. Thiazolidine-4-carboxylic acid was selected as a chelating agent possibly capable of chelating a metal from a protein complex in the plasma membrane which was the origin of contractil microfilaments [1, 8]. 2-Amino-2-thiazoline hydrochloride was the only available analogous chemical of thiazolidine-4-carboxylic acid which is known to induce reverse transformation of tumour cells [9].

We have developed techniques for estimating the degree of competitive complexing of metal ions by ligands *in vivo* [10–13], and we have now investigated the efficacy of thiazolidine-4-carboxylate and 2-amino-2-thiazoline as ligands capable of complexing metals *in vivo*. This could suggest that chelation was involved in the mechanism of action as proposed by Gosálvez.

This paper reports stability constants for thiazolidine-4-carboxylic acid and 2-amino-2-thiazoline hydrochloride-H^+, -Ca^{2+}, -Mg^{2+}, -Mn^{2+}, -Ni^{2+}, -Cu^{2+} and -Zn^{2+} interactions for blood plasma conditions (37 °C and I = 150 mmol dm^{-3} NaCl) and then uses the ECCLES blood plasma model to assess their metal ion binding ability *in vivo*.

Experimental

L-Thiazolidine-4-carboxylic acid (Merck. Found: C, 36.0; H, 5.3; N, 10.2. Calc. for $C_4H_7NO_2S$: C, 36.07; H, 5.30; N, 10.52%), was used without further purification. 2-Amino-2-thiazoline hydrochloride (Trans World Chemicals, Washington, D.C.) found: C, 26.3; H, 5.10; N, 20.3. Calc. for $C_3H_7N_2SCl$: C, 26.00; H, 5.09; N, 20.21%) was also used as supplied. Metal chloride solutions were prepared from analytical grade salts and metal and mineral acid concentrations analysed by two independent methods.

Potentiometric titrations were performed under an atmosphere of oxygen-free nitrogen using Ingold thermostatted (37 °C) titration vessels, a digital voltmeter (Radiometer type pHM64), and glass and calomel electrodes (Russell pH Ltd.).

Titrations were performed following our usual 'grid' approach whereby a range of metal:ligand concentrations are used spanning the pH range 2–10.

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TABLE I. Log Formation Constants for (Metal ion)$_p$(thiazolidine-4-carboxylate)$_q$(Proton)$_r$ Complexes at 37 °C; I = 150 mmol dm^{-3} NaCl. Literature results from reference 17 are also included. s = standard deviation, n = number of experimental points.

Cation	p	q	r	logβ	s	n	logβ [17]
H^+	1	0	1	6.104	0.004	203	6.109
	1	0	2	7.829	0.005		7.616
Ni^{2+}	1	1	0	4.007	0.005	269	3.925
	2	1	0	7.259	0.005		7.207
	3	1	0	9.239	0.019		8.825
Zn^{2+}	1	1	0	3.190	0.008	266	3.103
	2	1	0	5.753	0.013		5.629
	3	1	0	7.883	0.022		
Cu^{2+}	1	1	0				6.02
	2	1	0				11.22
	1	1	1				7.85
Mg^{2+}	1	1	0	1.683	0.024	131	
Ca^{2+}	1	1	0	1.657	0.018	113	
Mn^{2+}	1	1	0	1.904	0.022	118	
Zn^{2+}, thiazolidine-4-carboxylate, Histidinate ternary 1,1,1 complex				8.87	0.03	231	

TABLE II. Log Formation Constants for (Metal ion)$_p$(2-amino-2-thiazoline)$_q$(Proton)$_r$ Complexes at 37 °C, I = 150 mmol dm^{-3} NaCl.

Cation	p	q	r	log β	s	n
H^+	1	0	1	8.483	0.001	256
Zn^{2+}	1	1	0	3.15	0.18	116
Zn^{2+} (2-amino-thiazoline), Alaninate ternary 1,1,1 complex				7.25	0.13	63

TABLE III. Computer Simulation of Low Molecular Weight Distribution of Thiazolidine-4-carboxylate (TC) 100 μmol dm^{-3}) in Blood Plasma.

Metal ion	% l.m.w. metal ion complexes to drug	Complexes formed	log β used
Zn^{2+}	0.2	Zn(TC)(His)°	8.87
Cu^{2+}	nil		–
Mn^{2+}	0.4	$Mn(TC)^+$	1.90
Ca^{2+}	0.3	$Ca(TC)^+$	1.66
Mg^{2+}	0.3	$Mg(TC)^+$	1.68

TABLE IV. Distribution of Thiazolidine-4-carboxylate (TC) in Blood Plasma at pH = 7.4 Expressed as Percentage of Drug Administered at a Dose of 100 μmol dm^{-3} in Plasma.

Free carboxylate	88.7%
$Ca \cdot TC^+$	4.5%
H·TC	4.5%
$Mg \cdot TC^+$	2.2%

Data were analysed using the ZPLOT, PSEUDOPLOT and MINIQUAD computer programs [14–16]. The binary stability constants obtained for thiazolidine-4-carboxylic acid are listed in Table 1.

Zinc tends to form mixed ligand ternary complexes involving histidinate *in vivo* and so the zinc-thiazolidine-4-carboxylate histidinate constant was also determined. The 2-amino-2-thiazoline metal ion systems proved to be exceedingly insoluble in aqueous solution and so only the protonation constant was readily available. However, by using two ligand competition for Zn^{2+}, an estimate of the binary zinc-2-amino-2-thiazoline formation constant was obtained. The constant for the zinc, 2-amino-2-thiazoline-alaninate complex was also measured for comparative purposes. The results are shown in Table II.

These formation constants were then included in the blood plasma model and the ECCLES program was used to calculate the effect of the ligands on the normal metal ion distribution [11–13]. The results for thiazolidine-4-carboxylic acid administration to blood plasma are summarised in Tables III and IV. Different concentrations of drug in plasma were scanned, representative ones being noted in the table. The effect of 2-amino-2-thiazoline upon metal ions in blood plasma is expected to be even less than thiazolidine-4-carboxylic acid because of the much lower solubility of the former.

Since the computer simulation indicated that manganese and zinc are complexed by the agents concerned *in vivo*, it is desirable to establish the mode of bonding because the solution potentiometry produces stoichiometry details, not bond site data. Secondly, those metal–ligand complexes which are insoluble in water may well be actively involved in the organic environment of the lipid-protein cell membranes. Thus, the solid complexes formed by thiazolidine-4-carboxylic acid and 2-amino-2-thiazoline with zinc ions were isolated from both aqueous and ethanolic solutions and subjected to

TABLE V. Elemental Analyses for Thiazolidine-4-carboxylate (TC) and 2-Amino-2-thiazoline (AT) andTheir Solid Zinc Complexes.

	%C	%H	%N	%Zn	%Cl
TC analyses	36.0	5.3	10.2	–	–
TC theoretical	36.1	5.3	10.5	–	–
AC analyses	26.3	5.1	20.3	–	–
ACtheoretical	26.0	5.1	20.2	–	–
Zn–TC analyses Theoretical for $Zn(TC)_2 \cdot 2H_2O$	26.1	3.7	7.6	17.3	–
	26.4	3.9	7.7	17.9	–
Zn–AT analyses Theoretical for $Zn(AT)_2Cl_2$	21.2	3.6	16.4	19.1	18.4
	21.2	3.6	16.4	19.2	20.8

TABLE VI. Infrared Absorption Bands for Thiazolidine-4-carboxylate (TC) and 2-Amino-2-thiazoline (AT) and Their Zinc Chelates (units = cm^{-1}).

	TC	Zn–TC
ν_{NH}	3040	3250
$\nu_{NH_3^+-COO^-}$	2600–2100	
ν_{asNH}	1625	1615
$\nu_{axC=O}$	1585	1580
ν_{sCOO^-}	1425	1425
ν_{CCN}	860, 880, 905	855, 890
$\nu_{Coord.\ H_2O}$		825, 715
ν_{CS}	622	625–630
ν_{MN}		565
	AT	Zn–AT
ν_{NH}	3350–3200	3450, 3380
$\nu_{RNH_3^+}$	3100–2500	
$\nu_{C=H}$	1680	1665
δ_{NH_2}	1585	1585
ν_{CN}	1340, 1305, 1255	1355, 1305, 1285
ν_{MN}	735	520–570

elemental and infrared analyses (Perkin Elmer 557). Metal determination was by EDTA back titration using xylenol orange indicator, chloride was analysed by the Schöniger approach and then ion selective electrode analysis, and carbon, hydrogen and nitrogen analyses were obtained from a Perkin-Elmer elemental analyser type 240 [24, 25]. The results are shown in Tables V and VI.

Ultraviolet absorption spectra for 2-amino-2-thiazoline in aqueous solutions which were acidic or neutral showed that there was no hydrolysis of the thiazoline ring but, in the alkaline pH range and in the presence of a biological catalyst, hydrolysis is likely to occur *in vivo* [23].

Discussions and Conclusions

The slight general increase in formation constants reported in Table I compared with those of Fazakerley *et al.* [17] at 25 °C, I = 150 mmol dm^{-3} $NaClO_4$ probably arises from the changes in temperature and ionic background salt. The pK values of 6.1 and 8.4 for thiazolidine-4-carboxylic acid and 2-amino-2-thiazoline respectively corresponds to the protonation of the secondary and primary nitrogens, whereas the value of 1.62 for the former evidently relates to the carboxylate group. Metal ion binding in solution is assumed to be mainly through the nitrogen donors for zinc, manganese, nickel and copper but having some carboxylate involvement as appropriate whereas for calcium and magnesium the carboxylate group will be prime complexing group.

The zinc precipitates formed from both aqueous and alcoholic solutions had identical infrared spectra and elemental analyses. The latter corresponds to $Zn(thiazolidine\text{-}4\text{-}carboxylate)_2 \cdot 2H_2O$ and $Zn(2\text{-}amino\text{-}2\text{-}thiazoline)_2Cl_2$ (see Table V) and this suggests that nitrogen alone bonds to the metal ion in 2-amino-2-thiazoline complexes whereas the carboxylate takes up an additional coordinating position in thiazolidine-4-carboxylic complexes. This is in agreement with other workers [18–23].

Our studies do not produce evidence concerning processes within membranes because the computer simulations refer to the aqueous blood plasma rather than the lipid-protein membrane. Nevertheless, the lack of hydrophilicity for these metal complexes suggests a definite lipophilicity and grounds for predicting that they can complex within cell membranes. In addition, the agents may be involved in

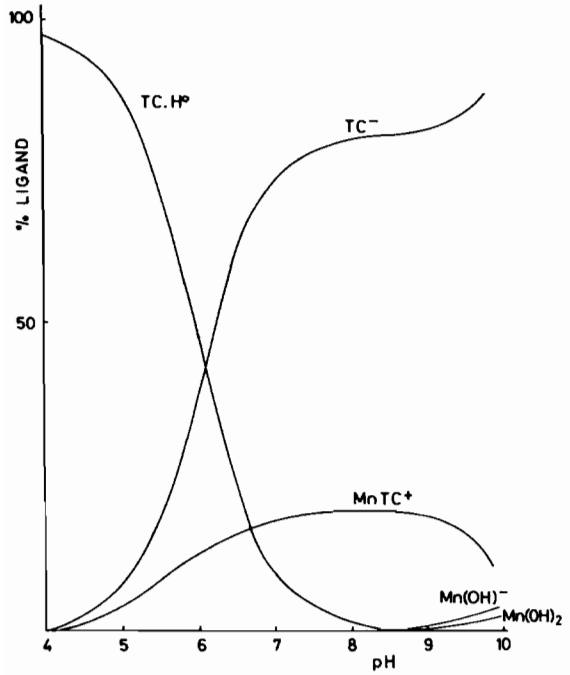

Fig. 1. Species distribution plot for Mn^{2+} and thiazolidine-4-carboxylate (TC) (6.7 and 20.0 μmol dm^{-3} respectively).

the biological mechanism that suppresses contact inhibition.

Table IV reflects the fact that calcium and magnesium are quite prevalent in blood plasma, whereas transition series metals exist at only low concentrations. Thus, although a fair amount of thiazolidine-4-carboxylic acid is found as its calcium complex, the greater disruptive effect upon low-molecular-weight equilibria manifests itself through manganese and zinc.

An apparent paradox occurs in that log β (zinc) > log β (manganese) but more low-molecular-weight manganese complex than zinc is formed *in vivo*. Relative to the other drugs being studies in this laboratory, thiazolidine is a feeble ligand and its disruption to the body's aqueous biochemistry is very weak. However, it is noteworthy that the new low-molecular-weight complex formed is positively charged and that cancer cells have an overall negative charge. Further, the role of manganese and cancer cell proliferation has been reviewed elsewhere [26, 27].

Figure 1 shows the distribution of thiazolidine-4-carboxylic acid between species present in a 3:1 solution of ligand and manganese. It is noteworthy that at stomach pH the protonated ligand species, being neutral, could well be taken up by passive diffusion through the stomach wall whereas at small intestinal pH (~6.5) very little lipophilic protonated complex exists. The manganese complex accounts for up to 19% of the ligand at these pH values. Similar plots for 2-amino-2-thiazoline suggest that it is neutral at duodenal pH and so is probably absorbed in the small intestine. Previous work from this laboratory has used metal ions to increase the bioavailability of ligand drugs by encouraging the formation of neutral complexes in the stomach or intestine [28, 29].

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