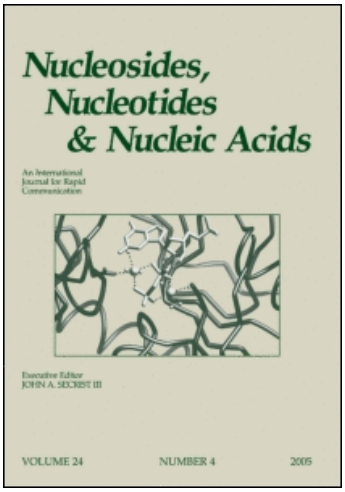


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Transition Metal Complexes of 5-Amino-1-β-D-Ribofuranosylimidazole-4-Carboxylic Acid 5'-Phosphate, an Intermediate in the *de novo* Biosynthesis of Purine Nucleotides: Synthesis and Effects on Enzyme Activity

J. R. Chipperfield[a]; R. W. Humble[b]; G. Iveson[b]; K. Kadir[b]; G. Mackenzie[b]; G. Shaw[c]
[a] University of Hull, Hull, U.K. [b] Humberside College of Higher Education, Hull, U.K. [c] University of Bradford, Bradford, U.K.

To cite this Article Chipperfield, J. R. , Humble, R. W. , Iveson, G. , Kadir, K. , Mackenzie, G. and Shaw, G.(1987) 'Transition Metal Complexes of 5-Amino-1-β-D-Ribofuranosylimidazole-4-Carboxylic Acid 5'-Phosphate, an Intermediate in the *de novo* Biosynthesis of Purine Nucleotides: Synthesis and Effects on Enzyme Activity', Nucleosides, Nucleotides and Nucleic Acids, 6: 1, 353 — 358
To link to this Article: DOI: 10.1080/07328318708056220
URL: http://dx.doi.org/10.1080/07328318708056220

NUCLEOSIDES & NUCLEOTIDES, 6(1&2), 353-358 (1987)

TRANSITION METAL COMPLEXES OF 5-AMINO-1-β-D-RIBOFURANOSYLIMIDAZOLE-4-CARBOXYLIC ACID 5'-PHOSPHATE, AN INTERMEDIATE IN THE de novo BIOSYNTHESIS OF PURINE NUCLEOTIDES: SYNTHESIS AND EFFECTS ON ENZYME ACTIVITY

J.R. Chipperfield[a], R.W. Humble[b], G. Iveson[b], K. Kadir[c], G. Mackenzie[b] and G. Shaw[c].
University of Hull, Hull, HU6 7RX (U.K.)[a]; Humberside College of Higher Education, Hull, HU6 7RT (U.K.)[b]; University of Bradford, Bradford BD7 1DP (U.K.)[c].

Abstract: Transition metal complexes [Cu(II), Co(II) and Ni(II)] of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxylic acid have been prepared and shown to form a series of stoichiometry ML_2, $nM(OH_2)$ (n = 0,1,2) and structures have been assigned. Analogous complexes of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxylic acid 5'-phosphate (CAIR), a central intermediate in the de novo pathway to purine nucleotides, produced in aqueous solution have been found to affect the activity of the enzyme AIR- carboxylase (E.C.4.1.1.21).

In earlier publications[1,2], we have described the effects of several close structural analogues of the aminoimidazole ribotide (1a) (CAIR) as competitive inhibitors of the enzyme phosphoribosylaminoimidazole carboxylase (E.C.4.1.1.21, AIR - carboxylase)[3] which is found in the biosynthetic pathway to purine nucleotides. A maximum of about 50% inhibition was found[1,2] to occur with the analogues examined which would suggest that the uninhibited section of the enzyme is highly specific and interacts with natural substrates alone. With this in mind our aim was to synthesise metal complexes of CAIR as analogues which possess very minimal changes in structure and which might interact with such a specific active site.

We have earlier recorded[4] the synthesis of model aminoimidazole complexes of type (2), which were isolated in crystalline form and elemental analysis suggested the stoichiometry $CuL_2 . 2H_2O$. We now report the synthesis of transition metal complexes [Cu(II), Co(II) and Ni(II)] of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxylic acid (1b). The sodium salt of the nucleoside ligand was obtained by

hydrolysis of ethyl 5-amino-1-β-D-ribofuranosylimidazole-4-carboxylate (1c)[5] with 0.5 M aqueous ethanolic hydroxide for 2h. In making the Cu(II) complexes, for example, the foregoing solution was adjusted to pH 7 and treated with one equivalent of Cu(II) nitrate. Thin layer chromatographic examination of the resulting green solution revealed at least three green coloured absorbing spots which, additionally, gave positive colours with the Bratton Marshall[6] spray for a diazotisable primary amine. Column chromatography of the solution on silica gel and elution with water-ethanol (1:1) resulted in the isolation of two complex salts, each homogeneous on t.l.c. Analysis of the salts indicated the structures $CuL_2 . n\ Cu(OH)_2$, n = 1 and 2. The complex n = 0 was probably present in solution but was not isolated under these conditions. In a similar manner entirely analogous metal complexes of the form $ML_2 . nM(OH)_2$, n = 1 and n = 2, were obtained from the sodium salt with Co(II) and Ni(II) nitrates with evidence for the presence of the complexes where n = 0.

In order to examine the effect of related transition metal complexes of the nucleotide (1a) on AIR-carboxylase we have used a solution of CAIR at pH 7.45 to which was added solutions of each of the metal Cu(II), Co(II) and Ni(II) salts (either chloride or nitrate). On addition of excess CAIR over cation concentration (over 2:1 respectively - TABLE 1), colour changes in the resulting solutions were found to be identical with those produced by the nucleoside, where loss of the visible absorption maxima characteristics of each free cation is complete. This evidence suggests that the complexes are essentially undissociated in aqueous solution.

Structure assignments given to the complexes (2) and (3) are based on pK measurements: it was found that the carboxylate anion has a pK 3.2[7] and that the doubly bonded nitrogen has a pK 2.2[8]. The nucleotide complexes were considered to have the same type of structure since, when they are in aqueous solution at pH 7.45, neither the carboxylate anion nor the doubly bonded nitrogen will be protonated, thus permitting their involvment in complex formation.

Previous work[9] has shown that transition metal ions inhibit acid catalysed decarboxylation of CAIR to at least pH 4.5 which includes the region of the pH rate constant profile maximum at pH 5.8 in the absence of metal ions. From this evidence it would appear, therefore, that the

complexes formed with metal ions are far more stable than either the free acid or zwitterion. This evidence is supported by results obtained from calculated stability constants[10,11] of metal complexes of CAIR in phosphate buffered (pH 7.45, 0.1 moldm^{-3}) solution which have been used to further calculate relative concentrations (data for Cu(II) complexes are given in TABLE 1) of free M(II) ions and the complexes M(II)(CAIR) and M(II)$(CAIR)_2$ in solutions used for enzyme activity determinations. Calculations took into account the following equilibria:

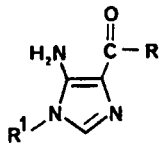
$$M(II) + CAIR \rightleftharpoons M(II)(CAIR)$$
$$M(II)(CAIR) + CAIR \rightleftharpoons M(II)(CAIR)_2$$
$$M(II) + HPO_4^{2-} \rightleftharpoons M(II).HPO_4$$
$$M(II) + OH^- \rightleftharpoons Cu\ OH^-$$
$$M(II) + 2OH^- \rightleftharpoons Cu(OH)_2$$

Although stability constants have not been measured for CAIR as a ligand, values have been estimated by comparison with other values obtained[10,11] for analogous ligands such as carboxyimidazole and glycine.

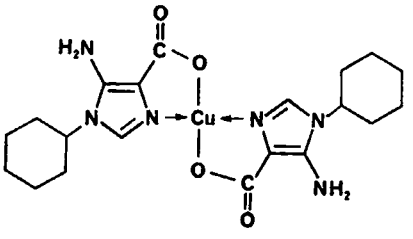
The metal complexes of CAIR examined were those of Cu(II), Ni(II), Co(II), Mg(II), Mn(II) and Zn(II). The calculations showed that the concentration of complexes varied with CAIR concentration ([CAIR]). Also, with Mg(II), Mn(II) and Zn(II) the metal phosphates ($M(II).HPO_4$) were the predominant species. However, in the formation of Cu(II) and Co(II) complexes, a constant concentration of Cu(II) $(CAIR)_2$ and Co(II) $(CAIR)_2$ was achieved on addition of excess CAIR. Thus, taking copper as our example (TABLE 1), the calculations showed that on adding excess CAIR ($[CAIR]_{FREE}$), over that required for Cu(II)$(CAIR)_2$ complex formation, a direct proportional relationship resulted with increase in non-complexed CAIR ($CAIR_{FREE}$). These results enabled enzyme kinetic studies (for Cu(II), Co(II) and Ni(II)) to be established in which the concentration of metal complex ([inhibitor]) was kept constant whilst the substrate concentration ($[CAIR]_{FREE}$) was varied, against initial velocity (Vo). The results for the enzyme kinetics involving Cu(II)$(CAIR)_2$ are given in FIG 1. The concentration of non-complexed Cu(II), Co(II) or Ni(II) (given as $Cu(II)_{FREE}$ IN TABLE 1) were considerably (some 10,000 times) less than the respective M(II)$(CAIR)_2$ complexes and were therefore considered to be insignificant with respect to enzyme inhibition.

TABLE 1: Equilibria involving Cu(II)-COMPLEX formation at given [CAIR] concentrations when Cu(II) is 0.5 x 10^{-4}moldm^{-3}

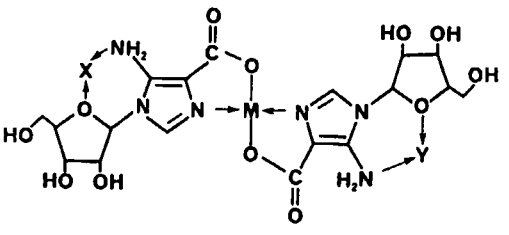
[CAIR] TOTAL /10^{-4} moldm^{-3}	[CAIR] FREE /10^{-5} moldm^{-3}	[Cu(II)] FREE /10^{-10} moldm^{-3}	[Cu(II)(CAIR)] /10^{-7} moldm^{-3}	[Cu(II)(CAIR)$_2$] /10^{-5} moldm^{-3}	[Cu(II)HPO$_4$] /10^{-8} moldm^{-3}
1.00	0.32	75.00	18.00	4.70	71.00
1.10	1.07	7.20	5.60	4.90	6.80
1.20	2.03	2.00	3.00	5.00	1.90
1.30	3.02	0.91	2.00	5.00	0.86
1.40	4.02	0.51	1.50	5.00	0.49
1.50	5.01	0.33	1.20	5.00	0.31
1.60	6.01	0.23	1.00	5.00	0.22
1.70	7.01	0.17	0.88	5.00	0.16
1.80	8.01	0.13	0.77	5.00	0.12
1.90	9.01	0.10	0.68	5.00	0.10
2.00	10.00	0.08	0.61	5.00	0.08



(1) (a) R^1 = 1-β-D-ribofuranosyl 5'-phosphate R^2 = OH(CAIR)
(b) R^1 = 1-β-D-ribofuranosyl R^2 = OH
(c) R^1 = 1-β-D-ribofuranosyl R^2 = OEt



(2)



(3) X = Y = O
X = M(OH)$_2$, Y = O
X = Y = M(OH)$_2$
M = Cu, Co, Ni

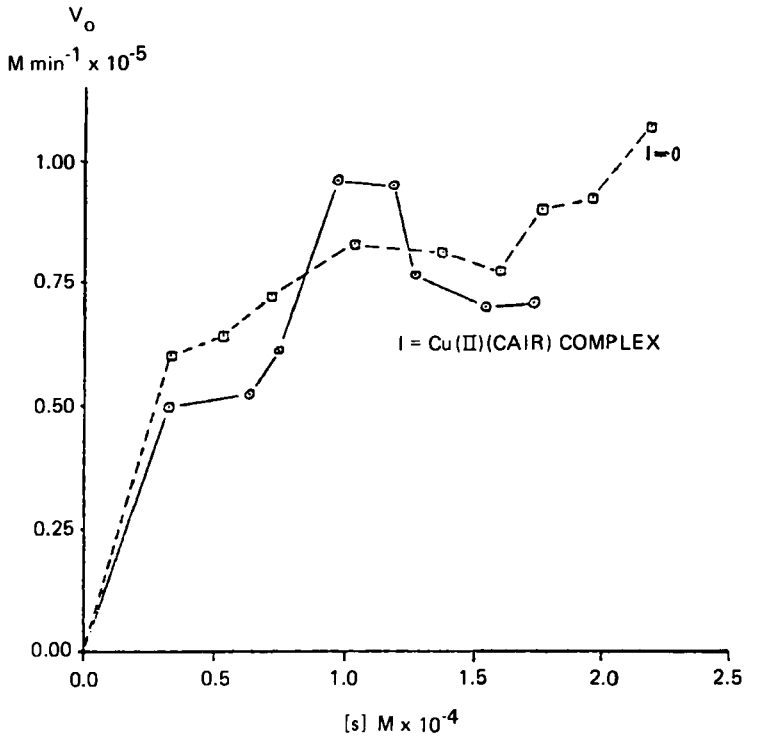


FIG 1. Effect of Cu(II)$(CAIR)_2$ on AIR-carboxylase activity.

TABLE 2: Inhibition of AIR-Carboxylase by Nucleotide (CAIR) - metal complexes

Metal Ion	Max inhibition /%	[S] /10^{-4}moldm^{-3}	Max enhancement /%	[S] /10^{-4}moldm^{-3}
Cu(II)	30	0.63	15	0.97
Co(II)	15	1.33	No significant enhancement seen	
Ni(II)	45	1.32	20	0.37
Mn(II), Mg(II), Zn(II)	Predominant species are metal phosphates			

Characteristics observed in the plots of initial velocity versus substrate concentration for each of the metal Cu(II), Co(II) and Ni(II) complexes ($M(II)(CAIR)_2$) studied were typified by that shown in FIG 1 for the effects of $Cu(II)(CAIR)_2$ on AIR-carboxylase activity: repetition of the experiments revealed consistency in the results. It was found (TABLE 2) that enzyme activity was inhibited by each of the $M(II)(CAIR)_2$ complexes but on increased substrate concentration an enhancement in activity was also observed in the experiments involving Cu(II) and Ni(II) complexes respectively.

We have previously reported[12] evidence to suggest that AIR-carboxylase is a branch point allosteric enzyme which involves both the de novo pathway to purine nucleotides and thiamine biosynthesis. We therefore suggest that the metal complexes are effecting changes in AIR-carboxylase activity as a result of interfering, in some way, with the control mechanisms inherent within the enzyme.

REFERENCES

(1) G. Mackenzie, G.Shaw and S.E. Thomas, J.C.S. Chem.Comm., 453(1976).
(2) G. Mackenzie and G. Shaw, J. Chem.Research, (S), 184(1977);(M),2145 (1977)
(3) C.A.H. Patey and G. Shaw, Biochem.J.,135. 543(1973).
(4) N.J. Cusack, G. Shaw and G.T. Litchfield, J.Chem.Soc.,(C),1501(1971).
(5) N.J. Cusack, B.J. Hildick, D.H. Robinson, P.W. Rugg and G.S.Shaw, J.C.S. Perkin I, 1720(1973).
(6) A.C. Bratton and P.K. Marshall, J.Biol.Chem., 128,647(1939).
(7) G.T. Litchfield and G.Shaw, J.Chem.Soc., (C), 817(1971).
(8) G. Mackenzie, A.E.Platt, H.A.Wilson and G.Shaw, J.C.S. Perkin II, 2055(1985).
(9) G.J.Litchfield and G.Shaw, J.Chem.Soc.,(B), 1474(1971).
G.J. Litchfield and G.Shaw, Chem.Comm., 564(1965).
(10) L.G. Sillen and A.E.Martell, "Stability Constants of Metal Ion Complexes", Chemical Society Special Publication 17(1964) and 25(1971), The Chemical Society, London.
(11) A.E. Martell and R.M. Smith, "Critical Stability Constants" 1 (1974),4(1976), 5(1982), Plenum, New York.
(12) R.W. Humble, G.Iveson, G.Mackenzie and G.Shaw, "Bio-Organic Heterocycles'86", Elsevier Sc.Publ. B.V., P.O.Box 330, 1000 AH Amsterdam, the Netherlands.