The Binding of Metal Ions by Enalaprilat (MK422)

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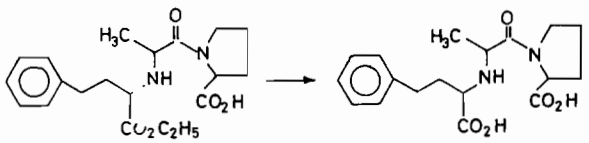
The inhibition of Angiotensin I – Converting Enzyme has become an established method for the treatment of hypertension and congestive heart failure. The first oral inhibitor, captopril 1-[2(S)-3-mercapto-2-methylpropionyl]-L-proline was introduced in 1977 and effectively treated hypertension in many patients whose blood pressure had not been controlled by beta-blockers or diuretics. However, a number of side-effects, such as loss of taste, skin rash, and neutropenia, occurred [1], and it was thought that these might be associated with the presence of a sulphydryl group and/or the complexing of metal ions, particularly zinc.

Attempts were made in several laboratories to synthesise inhibitors which had no sulphydryl group and which would, it was hoped, produce fewer side-effects. One such drug is enalaprilat (Merck, Sharp and Dohme, MK422), 1-[1-(S)-carboxy-3-phenylpropyl-L-alanyl]-L-proline, administered in the form of the pro-drug, enalapril maleate (MK421) to facilitate absorption from the gastro-intestinal tract. The maleate is hydrolysed, probably in the liver, to the active diacid form [2].



Enalapril (MK 421) Enalaprilat (MK 422)

Scheme 1.

There are not believed to be any other significant metabolites of MK421 as 94% of the administered dose of MK421 is recovered in urine and faeces in the form of MK421 and MK422 [3]. Although MK422 has no -SH moiety and is a more potent inhibitor than captopril, lower doses being required, (typically 10 mg up to 40 mg per day), there have been some reports of side effects such as the odd case of leucopenia, rash and disease of renal function [4, 5]. In addition, some cases of headache, dizziness and milder effects have been reported [6]. Some side effects such as rash and taste disorder and neutropenia can be related to zinc [7, 8] or copper [9] deficiency and MK422 contains functional groups that could complex these metals, possibly causing depletion.

The aims of this study were therefore:

(i) To determine the metal-binding properties of MK 422 *in vitro* by using potentiometry to measure the formation constants.

(ii) To insert the formation constants into computer models of blood plasma and so predict MK422 complexation of trace metals *in vivo*.

Experimental

Enalaprilat dihydrate was kindly provided by Merck, Sharp and Dohme Ltd. and used without further purification. (C, H, N analysis: Found: C, 56.5; H, 7.3; N, 6.7. Calcd. for $C_{18}H_{24}N_2O_5$: C, 56.2; H, 7.3; N, 6.7).

Solutions of enalaprilat were freshly prepared by direct weighing each day using distilled, doubly deionised degassed water. Metal chloride (Analar) solutions were analysed for metal ion concentration by EDTA titration and for hydrogen ion concentration by potentiometry and MAGEC [10] or ESTA [11] programs. Potentiometric titrations were performed as described in ref. [12] except that sodium chloride, 150 mmol dm^{-3}, was used as the background electrolyte instead of sodium perchlorate, and that the average value of pK_w determined in this laboratory (–13.31) was used. Formation constants were calculated for copper-enalaprilat and zinc-enalaprilat interactions by analysis of the potentiometric data using both MINIQUAD [13], and ESTA* programs. The species distributions and plasma mobilisation of copper and zinc by enalaprilat in plasma were computed using the ECCLES** program [14, 15].

Results

The protonation constants for enalaprilat and the formation constants for its interactions with zinc and copper are shown in Table I†, and the speciation graphs for percentage distribution of complexes existing at various pH values are shown for these metals (Figs. 1 and 2). Titrations with calcium ions

*Equilibrium Simulation for Titration Analysis.

**Evaluation of Constituent Concentrations in Large Equilibrium Systems.

†Abbreviations used in tables and figures: MK, enalaprilat; His, histidine; Gln, glutamine; Cys, cysteine.

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TABLE I. Thermodynamic Formation Constants for MK422 (Enalaprilic Acid) 37.0 °C and 150 mmol dm^{-3} Sodium Chloride ($\beta_{pqr} = [L_pM_qH_r]/[L]^p[M]^q[H]^r$).

ESTA						MINIQUAD				
Species			lg β (sd)	Obj.	R	lg β (sd)	Obj.	R	No.	No.
p	*q*	*r*		Func.			Func.		Pts.	Titns.
Protonation										
1	0	1	7.537 (0.002)	70	0.0023	7.538 (0.002)	3.94E-6	0.0025	342	5
1	0	2	10.799 (0.003)			10.804 (0.003)				
1	0	3	12.435 (0.009)			12.431 (0.004)				
Copper										
1	1	0	7.660 (0.006)	609	0.007	7.663 (0.004)	1.13E-7	0.0040	384	6
2	1	0	11.36 (0.03)			11.38 (0.03)				
3	1	0	14.75 (0.02)			14.69 (0.04)				
2	2	−1	11.16 (0.03)			11.19 (0.05)				
2	1	−1	2.63 (0.01)			2.56 (0.02)				
3	2	0	21.94 (0.03)			21.82 (0.07)				
Zinc										
2	1	2	18.85 (0.02)	148	0.003				499	7
2	1	1	13.84 (0.03)							
1	1	0	4.51 (0.005)							
2	1	0	7.70 (0.008)							
1	1	−1	−3.89 (0.02)							
2	1	−1	−1.25 (0.03)							

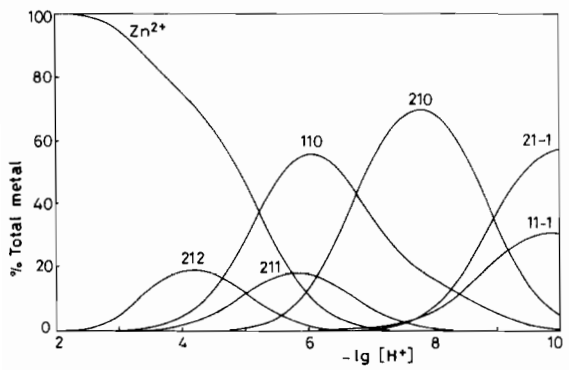

Fig. 1. Species distribution with pH for: total enalaprilat = 9.0 mmol dm^{-3}, total zinc = 3.0 mmol dm^{-3}, Species 110 – see Table I.

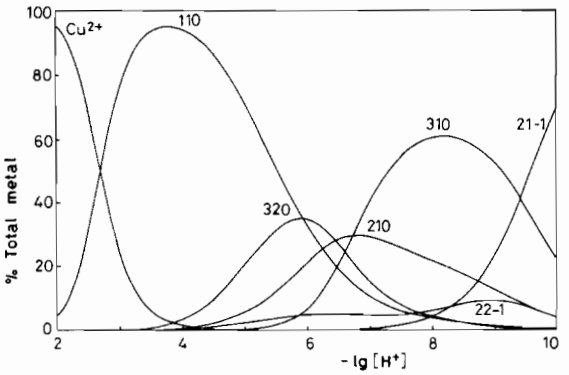

Fig. 2. Species distribution with pH for: total enalaprilat = 9.0 mmol dm^{-3}, total copper = 3.0 mmol dm^{-3}.

indicated that complexing by enalaprilat was not significant. Preliminary studies with manganese resulted in precipitation and this metal was not investigated further. The PMI curves for zinc and copper–enalaprilat are shown in Fig. 3. The zinc–penicillamine curve is shown for comparison.

Discussion

Zinc and copper were studied first because, as already indicated, they were considered to be the metals most likely to be involved in the side-effects. Only at an enalaprilat concentration of 10^{-3} mol dm^{-3} are the PMIs for these metals greater than zero with 20% of low molecular weight plasma zinc being bound in a ternary complex $MKCysZn^{2-}$, and 4% of copper in a ternary complex MKHisCu. The computed distributions of enalaprilat species are shown in Table II. However, the highest pharmacological levels of the drug reported for a single 10 mg dose are of the order of 10^{-7} mol dm^{-3} so the model suggests that mobilisation of both

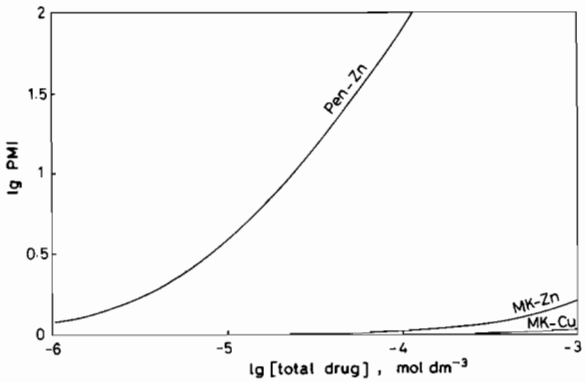

Fig. 3. PMI curve for the mobilisation of zinc and copper by enalaprilat compared with that of zinc by D-penicillamine.

TABLE II. Simulated Low Molecular Weight Zinc and Copper Complexes in Human Blood Plasma when (Enalaprilat) = 10^{-3} mol dm^{-3}.

Species	% Metal Bound
Copper(II)	
CuMKHis	4.4
CuMKGln	0.4
Zinc(II)	
ZnMKCys	20.1
ZnMK	5.2
ZnMKHis	4.3
$ZnMK_2$	3.4

metals from normal blood plasma by MK422 would be negligible. This prediction is supported in respect of zinc by a recent report on the effects of single doses of enalapril on urinary excretion of electrolytes in healthy adults which showed that it accelerated the rate of zinc excretion initially but decreased the amount of zinc excreted in 24 hours though this was not considered statistically significant [16]. The study did not measure the excretion of copper.

Further studies are being carried out in this laboratory to examine the interactions of the prodrug enalapril maleate (MK421) with metal ions.

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