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METAL COMPLEXES OF TETRACYCLINES

III—Formation of metal complexes and biological activity of tetracyclines

Stability constants of metal complexes of several tetracycline antibiotics were correlated with the biological activity of these compounds, measured by the inhibitory rate constants of the reactions of cell division and protein and nucleic acids synthesis. The results support the idea that the bacteriostatic action of tetracyclines is associated to the formation of mixed complexes, involving the antibiotic, the ribosomes and a metal ion such as Mn^{2+} .

In the previous papers of this series (1, 2) we presented results obtained in a study of the reactions of formation of alkaline-earth and transition metals with several tetracycline antibiotics, and raised some questions of the existence of some kind of relation between the biological activity of these compounds and their tendency to complex certain metals.

A problem to be considered beforehand is, however, that of measuring the so called biological activity, which varies even for different strains of the same microorganism. The well succeeded structure-activity correlations reported in a recent work by PARADEJORDI, MARTIN and CAMMARATA (3), in which the inhibitory rate constant of the reaction of cell division and protein and nucleic acid synthesis, studied with strains of «*Escherichia Coli*» (4, 5) in the presence of tetracyclines, was taken as a quantitative measure of the biological activity of these compounds, suggested that it might be appropriate to correlate these constants with the stability constants of the metal complexes formed with the several antibiotics studied.

The above mentioned constants k_i^T relate to the concentration of the tetracyclines $[T_i]$ by

$$k_i = k_o - k_i^T [T_i]$$

where k_o is the kinetic constant for the growth of the species in the absence of the antibiotics and k_i is the kinetic constant when variable concentrations of tetracyclines are present.

It is clear that k_i^T measures the susceptibility of the kinetic constant to the presence of a certain antibiotic T_i and this may be taken as the activity of the antibiotic.

In table I values for k_i^T are presented, both for active and inactive members of the tetracycline antibiotics (4, 5).

It is possible to devise models which explain the inhibiting action of the tetracyclines on the reactions of cell division and protein and nucleic acid synthesis, supported by the results of the study of the kinetics.

In these models it is assumed that the antibiotics bind to one or more ribosomal sites, therefore inhibiting the fixation of aminoacyl-t-RNA, and that equilibrium is established between the con-

Table I

Experimental values of «Inhibitory rate constants» of various tetracyclines (4, 5)

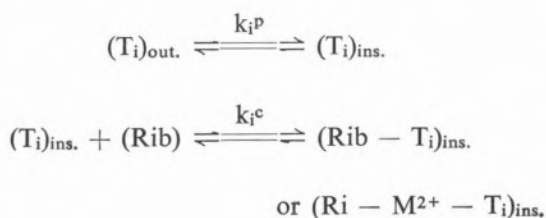
ANTIBIOTIC — Ti	k_i^T	$\log k_i^T$
7-NO ₂ -6-demethyl-6-deoxytetracycline	748 ± 53	2.87 ± 0.03
7-Cl-6-demethyltetracycline	517 ± 34	2.71 ± 0.03
7-Cl-tetracycline	401 ± 15.9	2.60 ± 0.02
Tetracycline	271 ± 11.3	2.43 ± 0.02
5-oxytetracycline	257 ± 24.5	2.41 ± 0.04
7-NH ₂ -6-demethyl-6-deoxytetracycline	181 ± 7.82	2.26 ± 0.02
9-NH ₂ -6-demethyl-6-deoxytetracycline	145 ± 8.79	2.16 ± 0.03
6-demethyl-6-deoxytetracycline	94 ± 3.06	1.98 ± 0.02
7-Br-6-demethyl-6-deoxytetracycline	51.8 ± 3.14	1.71 ± 0.03
9-NO ₂ -6-demethyl-6-deoxytetracycline	44.3 ± 1.51	1.65 ± 0.02
9-N(CH ₃) ₂ -6-demethyldeoxytetracycline	23.6 ± 1.52	1.37 ± 0.03
5a(6)-anhydrotetracycline	15.5 ± 1.23	1.19 ± 0.03
12a-deoxytetracycline	2.54	0.40
7-Cl-5a(11a)-dehydrotetracycline	0.34	— 0.47
4-dedimethylaminotetracycline	24.1 ± 1.19	1.38 ± 0.02
4-CH ₃ I-tetracycline	2.18 ± 0.22	0.34 ± 0.05
2-CN-tetracycline	0.00	— ∞
7-Cl-isotetracycline	0.00	— ∞
7-Cl-6-demethyl-6-deoxytetracycline	(84.52) (a)	
7-dimethylamino-6-demethyldeoxytetracycline	(154.49) (a)	

(a) Calculated by Paradejordi, Martin and Cammarata (3).

centration of the antibiotic inside and outside the cells.

The process according to which the tetracyclines bind to ribosomes in the inside of the cells to give a species which is inactive for the protein synthesis may involve the formation of a mixed metal complex.

The fundamental reactions will then be



where k_i^p and k_i^c are equilibrium constants. It may be admitted as a working hypothesis, that

$$k_i^T = k^o \cdot k_i^p \cdot k^c$$

or

$$\log k_i^T = \log k^o + \log k_i^p + \log k_i^c$$

One would then expect a correlation between $\log k_i^T$, which measures the «biological activity» of the tetracycline and $\log k_i^c$, which is related to the tendency for complex formation between the antibiotics, the ribosomes and, eventually, metal ions, provided that k_i^p is kept constant for that group of compounds.

It must be stressed that any conclusions which might be derived from the existence of such correlations are limited by the simplifying assumptions implicit in the working hypothesis adopted, namely:

- a) It is admitted that all the compounds investigated act in a similar way and are bonded, directly or through the formation of mixed metal complexes, to the ribosome sites to which the aminoacyl-t-RNA normally bonds.

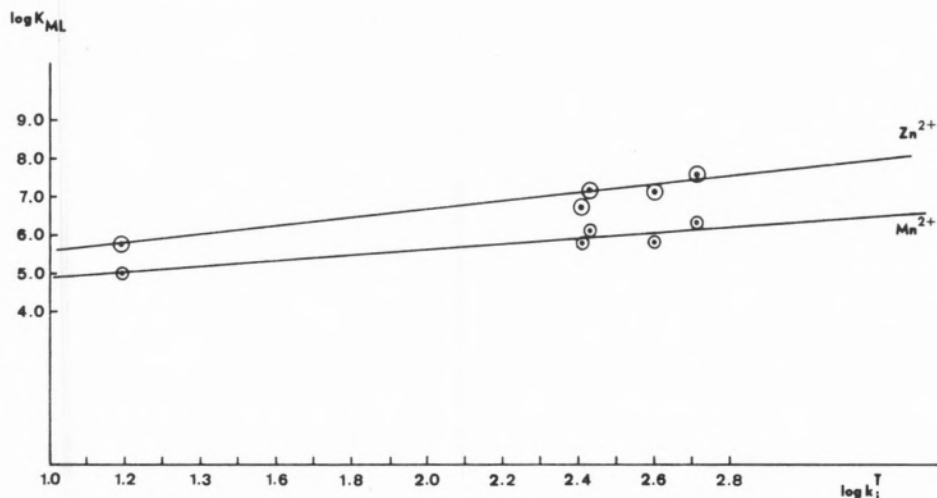


Fig. 1

Correlation of the stability constants ($\log K_{ML}$) of tetracycline, oxytetracycline, chlorotetracycline, demethylchlorotetracycline and anhydrotetracycline complexes of Zn^{2+} and Mn^{2+} with the inhibitory rate constant of the reaction of cell division and protein and nucleic acid synthesis ($\log k_i^T$).

b) It is assumed that the cell wall permeability is analogous for all compounds studied since the functional groups which are responsible for the values of the proton ionization constants are the same.

Although this two conditions are not exactly met, they may be accepted within certain limits, and the excellent correlation obtained by the above mentioned authors (3) between experimental $\log k_i^T$ values and $\log k_i^c$ values calculated from the

principles of the perturbation theory gives some support to those assumptions.

In Figs. 1 and 2 we present the correlations of $\log K_{ML}$ or $\log K_{MHL}$ values determined in our laboratory (1), with $\log k_i^T$ values available in the literature, where K_{ML} and K_{MHL} are the stability constants of the simple and the protonated complexes formed by the tetracycline antibiotics with a representative group of metal ions.

Although the number of data is rather small, it is apparent that there exists a correlation between

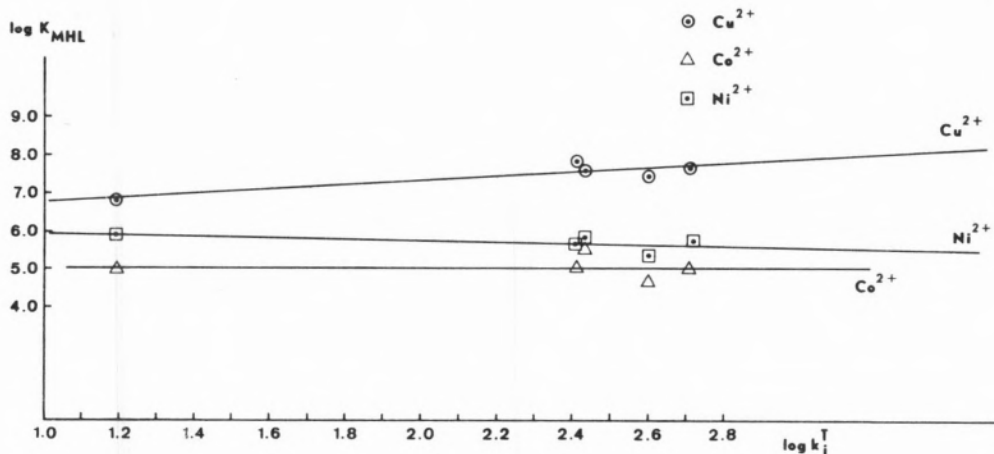


Fig. 2

Correlation of the stability constants ($\log K_{MHL}$) of tetracycline, oxytetracycline, demethylchlorotetracycline and anhydrotetracycline complexes of Cu^{2+} , Co^{2+} and Ni^{2+} with the inhibitory rate constant of the reaction of cell division and protein and nucleic acid synthesis ($\log k_i^T$).

the two pairs of corresponding constants which is such that an increase in the stability of the complex formed corresponds to an increase in biological activity.

It is interesting to note that in several previous works by other authors, the Mn^{2+} ions has been frequently mentioned as an essential element for the manifestation of the biological activity of this family of compounds (6, 7, 8); it has furthermore been found that up to a certain concentration level Mn^{2+} increases the biological activity but for higher levels the opposite effect is verified.

These observations are in agreement with the idea that the tetracyclines-ribosome bond involves the formation of a mixed metal complex, perhaps with Mn^{2+} . When this metal is not present the reaction does not occur but if a certain concentration value is exceeded some kind of competition arises thereby inhibiting again the association with the ribosomes.

This may imply the existence of Mn^{2+} already bonded to the ribosomes, and as a matter of fact, some authors have found that this ion can replace Mg^{2+} associated to the nucleic acids.

Our results give therefore some support to the idea that the mechanism of the bacteriostatic action of the tetracyclines is connected with the formation of mixed metal complexes and it is likely that it consists primarily in the inhibition of protein synthesis followed by the inhibition of the nucleic acid synthesis, in agreement with the work of MILLER, KAHLIL and MARTIN (9).

However, these authors suggest that the inhibitory action of the antibiotics is proportional to the concentration of the free «molecular» species of those substances and *not* to the concentration of the complexes, presenting as evidence a linear correlation between the kinetic constant for the reproduction reaction of the microorganisms in the presence of chlorotetracycline and the calculated concentration of the molecular form (neutral) of the antibiotic.

It so happens that the experimental data of these authors as well as others was obtained in media containing metal ions and although the concentration of these ions was not accurately measured or controled it is possible to show that it was such that metal complexes with the tetracyclines were

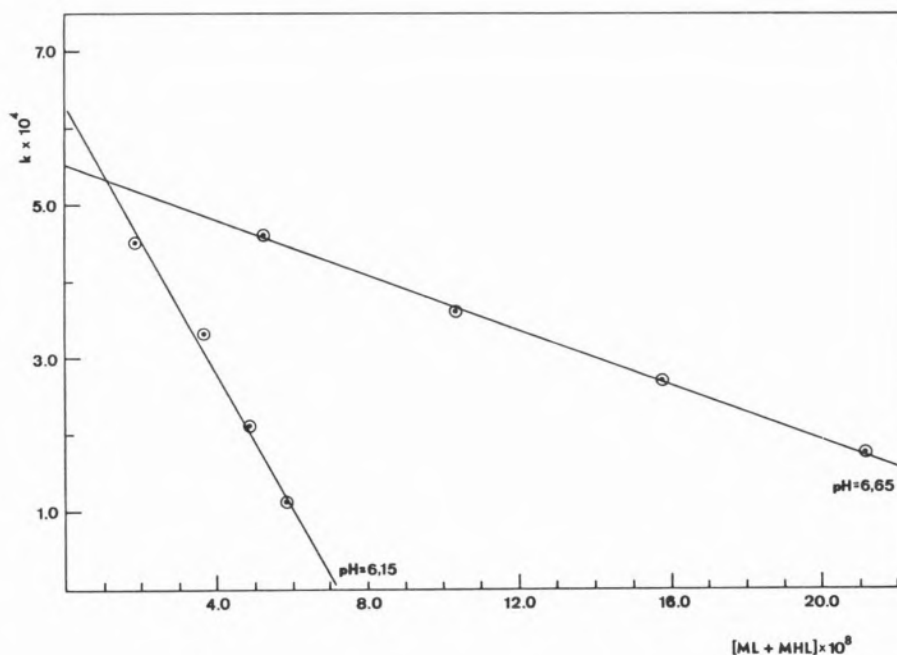


Fig. 3

Correlation of the kinetic constant for the growth of «*Escherichia Coli*» in the presence of 7-chlorotetracycline and the total concentration of Mn^{2+} complexes formed at pH 6.15 and 6.65
($C_L \times 10^6 = 0.25, 0.50, 0.75, 1.00M$; $C_M = 10^{-4}M$)

certainly formed and that the correlation presented by MILLER *et al.* is entirely equivalent to a correlation between the same kinetic constant and the degree of formation of the complexes of chlorotetracycline.

In our calculations we have taken Mn^{2+} as a representative metal ion, considered that its concentration was of the order of 10^{-4} M, as the above mentioned authors did, and calculated the total concentration of metal complexes formed $\{ [ML] + [MHL] \}$ at the two different pH values adopted by the same authors: 6.15 and 6.65.

The correlations obtained are presented in Fig. 3 and show that those previously presented constitute no real support for the suggestions of MILLER *et al.* (9), as well as for similar proposals made by JONES and MORRISON (10, 11) and BENBOUGH and MORRISON (12); indeed, the kinetic constant for the inhibited reaction of reproduction of the microorganisms is clearly shown to depend as well on the concentration of the complexed antibiotic.

Hence, the hypothesis that the Mn^{2+} ion might be associated to the mechanism of such reaction, either as promotor or supressor of the bacteriostatic action of the tetracyclines, depending on the level of concentration in the reaction media, stands without restriction and with some support from our work. The promoting effect seems however to be related to the presence of this ion in the ribosomic sites, since it is verified only for low concentrations, up to the level necessary for full occupancy of those sites, eventually by substitution of Mg^{2+} .

These findings stimulate the interest for the development of work on mixed complexes of the tetracycline antibiotics, which was initiated with some positive results to be reported at a later stage.

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RESUMO

Relacionam-se as constantes de estabilidade de complexos metálicos de várias tetraciclinas com a respectiva actividade biológica, tomando como medida desta a constante de inibição das reacções de divisão celular e síntese de proteínas e ácidos nucleicos. Os resultados são compatíveis com a hipótese de que a acção bacteriostática das tetraciclinas está relacionada com a formação de complexos mistos, envolvendo o antibiótico, os ribossomas e certos iões metálicos, como por exemplo o Mn^{2+} .