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Complexation behaviour of azathioprine with metal ions

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Summary

The behaviour of complex formation of azathioprine, an immunosuppressant drug, with various metal ions has been investigated by the potentiometric titration method. Azathioprine is found to form 2:1 complexes with Co(II), Cu(II) and Ni(II) in the order Cu(II) > Ni(II) > Co(II). No complex is formed with Ba(II), Ca(II), Fe(III), Mn(II) or Zn(II). The stability constants for the complexes were calculated. These differ from those of 6-mercaptopurine, of which azathioprine is a prodrug but shows an independent activity.

Introduction

Studies on degradation and related aspects of azathioprine, a potent immunosuppressive agent and an essential drug, are continuing in our laboratory. Already, the pathways of hydrolysis of azathioprine (Gupta et al., 1987; Singh and Gupta, 1988a), mechanisms (Singh and Gupta, 1988a,b) and thermodynamics (Singh et al., 1989) of its degradation reactions and a method for its specific determination in solution (Singh et al., 1988) have been reported. In this paper we report the behaviour of complex formation of azathioprine with various metal ions.

The objective in carrying out the present study has been two-fold. One, to explore the mechanism by which Na_2EDTA brings about an inhibitory influence on hydrolysis of azathioprine to 6-mercaptopurine (6-MP) when incorporated in borate buffers (Singh and Gupta, unpublished results). The conversion of azathioprine to 6-MP is of particular pharmaceutical interest, since stringent pharmacopoeial controls have been laid down which limit the presence of 6-MP in azathioprine to less than 1% w/w (BP, 1980; USP, 1985). Secondly, azathioprine is at present under intense investigation for its anti-rheumatic property (Morrill and Carmichael, 1988). Rather, it is now included in the category of slow-acting anti-rheumatic drugs. While anti-rheumatic studies on azathioprine have also involved its copper complexes as the possible active form (Roberts and Robinson, 1985), the stoichiometry and stability constants of azathioprine-metal complexes, even with that of copper, have not yet been determined.

In this paper, the behaviour of metal complex formation of azathioprine is also compared with that of 6-MP. Although azathioprine is a prodrug

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of 6-MP, it still possesses a superior immunosuppressant activity and is commercially available as an independent drug.

Materials and Methods

Materials

Azathioprine was received as a gift from The Wellcome Foundation Ltd, London, and was used as received. The metallic ions were used in the form of their chloride salts of analytical grade purity. Fresh triple-distilled water from an all-glass still (Scientronic, New Delhi) was employed in the studies. All other reagents were of analytical grade.

Titration procedure

The following procedure was employed in each run, the metal being omitted for the determination of the acid dissociation constant. A 50 ml solution of drug (4×10^{-4} M) in water at ionic strength 0.005 containing appropriate metal salt was introduced into the titration cell held in a water bath (D8, Haake, Germany) set at $25 \pm 0.1°$ C. Stirring was carried out with the help of nitrogen freed from impurities by passing through three traps of Fieser's solution (Fieser, 1955) followed by a trap of saturated solution of lead acetate. When thermal equilibrium was reached, a total of 5 ml of 0.005 N sodium hydroxide solution (carbonate free) was added in small increments of 0.25 ml each. For cases where precipitation occurred before addition of total 5 ml of titrant, the studies were repeated with a titrant increment of 0.1 ml. The pH was recorded after every addition on a Control Dynamics APX 175 digital pH meter equipped with Ingold 455 combination electrode and standardised at the temperature of study using NBS buffer solutions (Bates, 1962).

Calculation of acid dissociation constant

Although the pK_a value of azathioprine at 25° C could have been derived from the literature, it was thought preferable to determine it under the conditions of the metal chelation studies. The value of the acid dissociation constant we determined from titration results in accordance with the procedure described by Albert and Serjeant (1962) is 8.15 ± 0.02. This value is in close agreement with that of 8.2 reported at the same temperature by Wilson and Benezra (1981). Two other reported pK_a values of azathioprine, determined at 25° C by solubility and spectrophotometric methods, are 7.87 ± 0.04 and 7.99 ± 0.03, respectively (Newton et al., 1982).

Calculation of chelate stability constants

The formation constants of the chelates of azathioprine with various metal ions were calculated by the algebraic method described by Albert and Serjeant (1962). The calculations as such were performed only on those portions of the titration curves where the solutions were homogeneous and the precipitates were not seen. The results were validated through determinations by the graphical method of Bjerrum (1941). In addition, the stoichiometry of azathioprine-metal complexes was also verified by following Job's method of continuous variation (Job, 1928; Vosburgh and Cooper, 1941). In this case, equimolar solutions of azathioprine and metal salt were prepared in a mixture of dioxane : water (75 : 25), the pH of these two solutions was adjusted to 6.7 and they were mixed in varying proportions ranging between 9 : 1 and 1 : 9. Subsequently, absorbances of these mixtures were noted at the corresponding wavelength maximum of the complex.

Results and Discussion

Eight metals commonly supposed to form chelates with drug substances were involved in the metal complexation studies on azathioprine. The metals are copper(II), nickel(II), cobalt(II), zinc (II), barium(II), manganese(II), calcium(II) and iron(III). In preliminary investigations, these were included in the ratio of 1 : 2 in the aqueous solution of the drug. On addition of alkali, significant downward shifts of titration curves from those obtained with drug alone were observed only in the case of three metals, viz. cobalt, copper and nickel. The pattern of curves for these three is shown in Fig. 1. For all other metals, the curves obtained on titration of drug solution in the presence of the metallic ion traversed a path similar to

one of the two component curves. While for zinc, calcium, manganese and barium, the curve in presence of metal ion overlapped that obtained for drug alone, it overlapped the metal hydrolysis curve in the case of iron. These results infer that the avidity of azathioprine is very low for Zn(II), Ca(II), Mn(II) and Ba(II) and that no complex is formed between drug and Fe(III).

Table 1 summarizes the chelate formation constants of azathioprine with Co(II), Cu(II) and Ni(II). The value of the first chelate formation constant K_1 is evidently largest for copper, followed by nickel, which in turn is closely followed by cobalt. The order of the stability of the complexes of azathioprine with the three metals is thus established as Cu(II) > Ni(II) > Co(II).

TABLE 1

Values of formation constants for azathioprine and 6-mercaptopurine

Drug	Metal ion	log K_1	log K_2	log β
Azathioprine				
	Cu(II)	5.14	5.08	10.22
		(5.48) [a]	(4.50)	(10.01)
	Ni(II)	3.84	4.65	8.47
		(4.56)	(4.33)	(8.82)
	Co(II)	3.70	4.73	8.42
		(4.50)	(4.35)	(8.73)
6-Mercaptopurine [b]				
	Cu(II)	12.80	5.40	18.20
	Ni(II)	6.10	5.78	11.88

[a] The values in parentheses were interpolated from the data corresponding to the formation curves shown in Fig. 3.
[b] Values reported by Scoran and Cefola (1962).

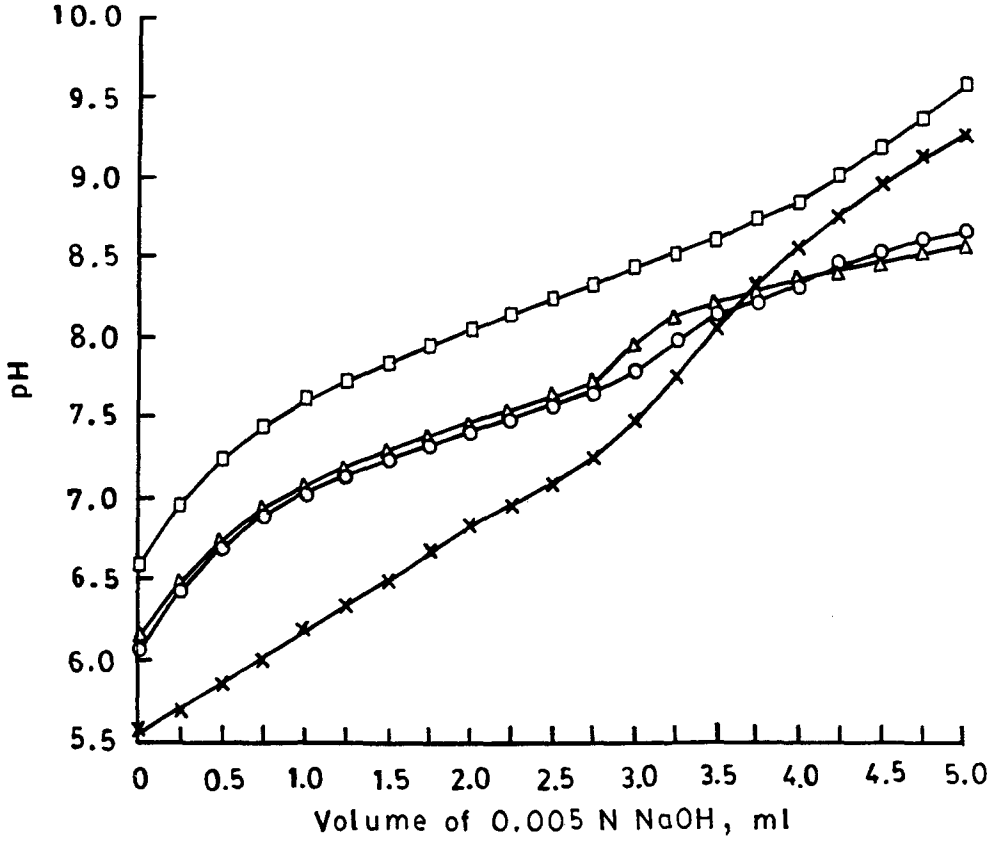


Fig. 1. Potentiometric titration curves in water of 4×10^{-4} M azathioprine with 0.005 N sodium hydroxide at 25°C, ionic strength = 0.005 in the absence of metal ions (□) and in the presence of 2×10^{-4} M each of copper (×), nickel (○) and cobalt (△).

In studies involving the pH titration method, the drug : metal stoichiometry for all the three complexes was indicated to be 2 : 1. In all cases, the number of ligand molecules attached to an ion of the metal (n) approached 2.0. The study of the stoichiometry of the azathioprine : metal complexes by Job's continuous variation method confirmed the 2 : 1 stoichiometry of azathioprine : metal complexes. The same is evident from the mole fraction ratio vs absorbance curves in the presence of copper and cobalt (Fig. 2) in which there is change of slope at mole fraction values of 0.64 and 0.67, respectively. Similar study in the case of nickel, however, was not possible as sufficient colour did not develop in its solutions. Further, studies were also conducted at drug : metal ratios of 3 : 1, 4 : 1 and 6 : 1 with each of the complex-forming metals. In no case did the value of n exceed 2.0, meaning that azathioprine does not form higher complexes with copper(II), nickel(II) or cobalt(II).

The formation functions of azathioprine complexes with Cu(II), Ni(II) and Co(II) are shown in Fig. 3. The values of formation constants read from these plots (Table 1) closely coincide with those obtained by the algebraic method. It is reasonable not to expect the exact coincidence of the two set of values as the former method is based on the titration of a heterogeneous and precipitating system which is not in instant equilibrium while the latter method takes into account only the homogeneous solution prior to the appearance of precipitation.

The metal binding properties of 6-MP, of which azathioprine is a prodrug, have already been determined and were reported by Scoran and Cefola (1962) in water at 23.56 ± 0.04 °C and by Cheney et al. (1959) in 50% v/v dioxane-water system at

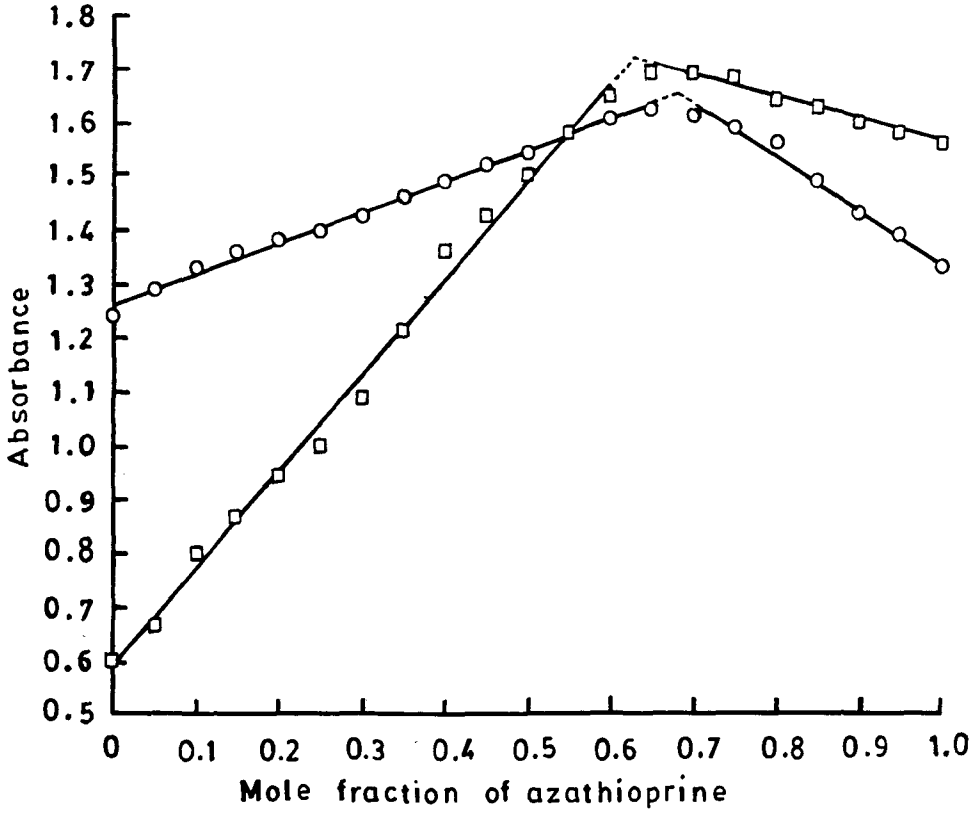


Fig. 2. Absorbance vs mole fraction curves representing Job's method of continuous variation. The spectrophotometric absorbances of azathioprine solutions containing copper (□) were determined at 390 nm and of those containing cobalt (○) at 350 nm.

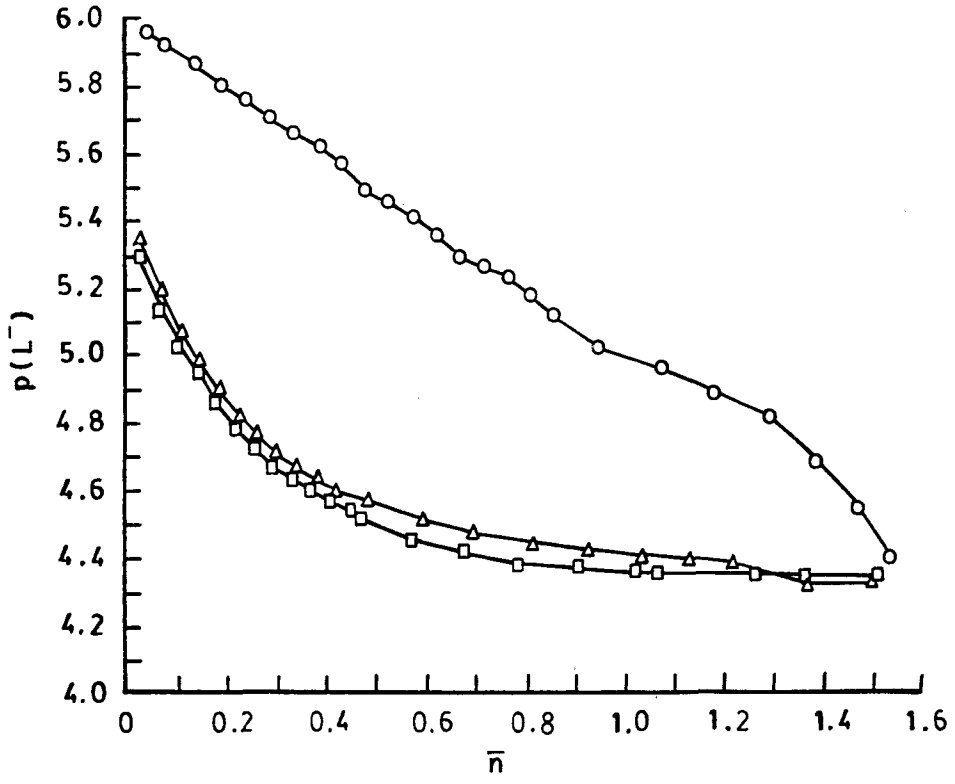
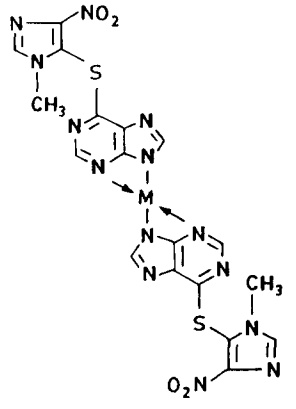


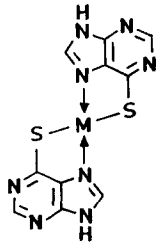
Fig. 3. Formation curves of the azathioprine-copper (○), azathioprine-nickel (△) and azathioprine-cobalt (□) complexes. The average number of molecules ($\bar{n}$) of azathioprine united to one atom of metal has been plotted against negative logarithm of the chelating species of azathioprine (L^-).

25 °C. Of the metals Cu(II), Ni(II), Ca(II), Ba(II), Fe(II), Fe(III), Co(II) and Hg(II), Scoran and Cefola (1962) have presented quantitative data for 6-MP-copper and 6-MP-nickel drug-metal complexes only. Qualitative tests with other metals gave either no evidence of complex formation or formation of complex only when the solution was rendered basic. The latter was a specific case with cobaltous ions. The values of chelate formation constants given by Scoran and Cefola (1962) for Cu(II) and Ni(II) complexes with 6-MP, being in water, are included in Table 1 for comparison. Evidently, the values of chelate formation constants of 6-MP with the two metals are larger than those of azathioprine, the difference being much more than what can be explained by the small difference of 1.5 °C in the temperatures of the two studies. Azathioprine hence is shown here to form weaker complexes with metals than those formed by 6-MP.

This difference in stability of complexes between azathioprine and 6-MP may be explained upon consideration of the probable sites for the metal binding on the two drugs. Cheney et al. (1959) have shown that in 6-MP where the thiol hydrogen of the thiol group is available, the interaction with metal ions takes place at the 6-mercapto group. However, in 6-methylmercaptopurine (6-MMP), where the free thiol hydrogen is substituted by the methyl group, the metal reaction occurs at the imino group in the imidazole moiety of purine. Similar to the case of 6-MMP, the thiol hydrogen in azathioprine is substituted by the 1-methyl-4-nitroimidazole moiety and, therefore, its imino group is also the likely group to be involved in the formation of the metal complex. In our study, evidence of this effect was provided when the IR peak at 3191 cm^{-1} owing to the N-H stretching characteristic of a purine function in azathioprine (Wilson and Benezra, 1981)



(1)



(2)

was found to be lost in the IR spectra of azathioprine-metal complexes. The calculated lone pK_a value of 8.15 of azathioprine also corresponds to the dissociation of the imine hydrogen (Newton et al., 1982). 6-Mercaptopurine, on the other hand, shows two ionisation steps in the neutral to alkaline pH regions, the two pK_a values being 7.80 and 9.94 at 23.5 °C (Scoran and Cefola, 1962). The pK_{a2} and pK_{a3} values of 6-MP correspond to the dissociation of mercapto and imino groups, respectively (Cheney et al., 1959). Going from azathioprine to 6-MP, there is a great increase in basicity of the imino hydrogen, which is rationalised on the premise that further dissociation from an anion is involved in 6-MP. In azathioprine, an electronic interaction is expected between the electron-withdrawing 1-methyl-4-nitroimidazole group and the imino group of the purine moiety resulting in a decrease in the basicity of the nitrogen atom, i.e. an increase in the acidity of the imino hydrogen. The possibility of such an interaction occurring through the sulfide bridge was discussed earlier (Singh and Gupta, 1988b).

Based on the foregoing discussion, formulae 1 and 2 are proposed as the probable structures representing azathioprine-metal and 6-MP-metal complexes, respectively in water.

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