Purine

It was suggested that with increasing concentration purine associates through stack formation [6]. Hewson *et al.* [7], concluded that purine associates through hydrogen bonding. In this work we noticed that there is no change in the chemical shift of proton 2, 6 and 8, but N_9H moves to higher field with increasing concentration. It seems that purine associates in a manner similar to that in pyrrol [8]. No change was observed with all acceptors except with acetyl acetone and acridon where N_9H shifted to low field upon the mixing of purine with those two acceptors, this is strong evidence for the formation of hydrogen bonding between purine and each of acetyl acetone and acridon.

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Evidence for Different Types of Interaction between Anions and the Copper(II) Site of Superoxide Dismutase

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It seems now well established that the two equivalent copper(II) ions in dimeric superoxide dismutase are exposed to solvent as well as to solute molecules, while the two zinc(II) ions are not capable of such interactions [1–4]. Water proton relaxation studies [5] have shown that anions bind the copper atom by displacing the coordinated water molecule; the obtained affinity constants roughly compare with those calculated through inhibition measurements [5], suggesting the identity between the water and the inhibitor binding sites.

The present report aims to show evidence for a second copper binding site for anions, not involving displacement of water; this site may also be of importance for the catalytic process.

Previous investigations have shown that the thiocyanate ion is able to affect the ESR spectra of copper(II) in superoxide dismutase [6], whereas it has been subsequently shown not to affect the catalytic activity. Therefore, a detailed study of the interaction between the above ion and superoxide dismutase has been undertaken, by means of electronic, ESR, and ^1H and ^{13}C NMR spectroscopy.

While the electronic spectra of the copper chromophore as well as the water proton relaxation of enzyme solutions remain substantially unchanged up to 0.5 *M* KNCS concentrations, the ESR parameters and the ^{13}C relaxation times of the anion are significantly affected. In particular, the ^{13}C signal of NCS^- dramatically broadens, consistently with a direct binding to the paramagnetic metal ion. From the dependence of the paramagnetic effect on the thiocyanate concentration an affinity constant of 50 ± 10 M^{-1} can be estimated. The ESR data on frozen solutions qualitatively agree with the above value, as judged from the progressive variation of A from 145 to 160 $cm^{-1} \times 10^4$ upon anion addition.

By increasing the thiocyanate concentration above 1 *M* a further range is observed in the ESR spectra, which show a *decrease* in the A value; a slight blue shift is observed in the electronic spectra and the water proton relaxation is reduced. Limit values of the above parameters could not be obtained; the affinity constant can be estimated to be in the range 0.1–1 M^{-1}.

All of the above results strongly suggest the existence of a second, non inhibitory anion binding site on copper in superoxide dismutase which, in the case of the thiocyanate anion, is preferred to the site of water. The electronic and ESR spectra of the adducts indicate that there are not major variations in the coordination geometry of the copper chromophore, which seems to be more consistent with the detachment of one of the four inplane imidazole donors that with the addition of the thiocyanate ion to a sixth coordination position.

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The *In Vitro* Adsorption study of Isoniazide and Pyrazinamide on Indigenous Bentonite

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In the research series on the interactions of some drugs with bentonites, having a special interest for the pharmaceutical science we studied the adsorbing capacity of revived and HCl activated bentonites from Valea Chioarului. The adsorbed drugs were isoniazide and pyrazinamide. The adsorbing capacity was expressed in the amount of drug adsorbed per unit mass of adsorbent.

The practical application of the experimental data was made using the liquid phase adsorption isotherm of Freundlich and of Langmuir.

The characteristic parameters of the bentonite–drug system were obtained processing the experimental data by regression analysis. The adsorption coefficient (a) and the adsorption capacity at the equilibrium concentration equivalent to unity (b) were determined. The spreading of the data show obedience to the Langmuir pattern.

The derivatographic study of the bentonite drug system allowed the calculation of the activation energie (*E*) and of the reaction order (n) characteristic parameters of the dehydration kinetics. For this purpose the differential method of Freeman and Carroll was used.

The electronic spectra of bentonite–drug system in KBr pellets, shows hypsochromic shifts of the 265 nm band characteristic for isoniazide and the 269 nm band characteristic for pyrazinamide.

The IR spectra for bentonite–drug system show significant changes in the 1700–1500 cm^{-1} range characteristic for $\nu_{C=O}$ and δ_{H_2O} modes.

TABLE

System		Freundlich Method	Langmuir Method	n	*E* (kcal/mol)
Bentonite–Isoniazide	a	0.69	0.298		
	b	0.0075	0.0019	0.7	10.67
	s^2	4.84×10^{-5}	0.87×10^{-5}		
Bentonite–Pyrazinamide	a	0.13	0.87		
	b	2.99	3.01	0.7	6.54
	s^2	0.041	5.39×10^{-5}		

The Nature of the Type 2 Copper Binding in Blue Oxidases

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Of the three different forms of copper usually contained in the blue oxidases, the Type 2 Cu is a divalent paramagnetic form characterized by a 'normal' EPR spectrum, similar to that of tetragonal copper complexes, with $A_{\parallel}$ of 150–200 gauss [1]. The Type 2 Cu site is open to solvent access and binds dissolved monovalent anions, such as azide, cyanide and fluoride [1]. These anions have an inhibitory effect on the oxidase activity much stronger than their affinity for the resting enzyme would predict. For tree laccase the affinity of the Type 2 Cu for azide increases on reduction of the Type 3 Cu [2], a likely event during turnover. All oxidases show in the presence of azide an intense absorption band at 400 nm and a shoulder at about 500 nm. The latter is never observed in mononuclear Cu(II)–azide complexes and is assigned to a charge-transfer from the bridging azide to the Cu(II) in the Cu(II)–N_3–Cu(I) group of azide-bound met-hemocyanine [3]. This suggests that a similar type of binding might be present in the oxidases. The EPR spectra of the Type 2 Cu(II)–N_3 and –CN adducts of tree laccase, measured under reducing conditions, are very similar to those of the corresponding complexes of the Cu(II) substituted carbonic anhydrase, where the Cu(II) is believed to be pentacoordinated in a distorted square pyramidal geometry [4]. They are characterized by low $A_{\parallel}$ in the range 106–137 gauss. The metal ion is bound to three hystidyl residues and one water molecule, besides the anion, in the Cu(II) carbonic anhydrase [4], to at least one water [1] and one hystidyl residue [5] in the tree laccase.

Selective removal of the Type 2 Cu abolishes the oxidase activity. In the tree laccase both the rate of