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Effect of Side Amino Acid Groups on the Effectiveness of Nitric Oxide Binding by the Cobalt Complexes with Amino Acids

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The effect of metal environment on the effectiveness of nitric oxide binding by the cobalt complexes with amino acids and with amino acids and imidazole was investigated. The results of spectroscopic studies and magnetic moment measurements (NMR) indicate an equilibrium in solution between the active cobalt(II) complex and the nitrosyl cobalt(III) complex.

Introduction

As early as in 1961 the binding process of nitric oxide by the cobalt(II) system with histidine was investigated [1, 2]. This complex whose molar ratio of cobalt(II) to histidine is 1:2 was found to bind reversibly nitric oxide. Earlier it was found that this system is also an excellent inorganic oxygen carrier [3–6].

In our previous paper [7] we have presented the results obtained for reversible nitric oxide binding at 0 °C by the cobalt(II) system with various amino acids and imidazole. On the basis of electron spectroscopy and volumetric studies a Co(III)(Amac)$_2$(Himid)NO species was suggested to exist in solution. In the present paper further results of NMR studies are presented as this method appears to be more suitable for investigations of these systems. The method enabled us to follow an equilibrium existing in solution between the active cobalt(II) complex and cobalt(III) complex with bound nitric oxide (1, 1′) and to determine the effect of metal environment on the effectiveness of NO binding.

$$(1)\quad [Co(II)(Amac)_2(H_2O)_n] + NO \rightleftharpoons [Co(III)(Amac)_2NO](H_2O)_{n-1}] + H_2O$$

$$n = 1, 2$$

$$(1')\quad [Co(II)(Amac)_2(Himid)](H_2O)] + NO \rightleftharpoons [Co(III)(Amac)_2(Himid)NO] + H_2O$$

Experimental

The binding process of nitric oxide by the cobalt(II) complexes with amino acids (Co(Amac)$_2$·nH$_2$O, n = 1, 2) and the cobalt(II) complexes with amino acids and imidazole (Co(Amac)$_2$(Himid)·H$_2$O) were investigated. The following amino acids were used: glycine (Gly), alanine (Ala), sarcosine (Sar), ornithine (Orn), threonine (Thr), arginine (Arg), lysine (Lys), which were obtained from E. Merck. For synthesis of the cobalt system with imidazole and amino acids the Co(imid)$_2$ complex was used whose preparation technique was provided earlier [8]. The 'active' and nitrosyl forms of the complexes free of imidazole were prepared by dissolving Co(NO$_3$)$_2$·6H$_2$O in aqueous amino acid solution and passing argon through the solution – 'active' form, and NO – nitrosyl form. The systems under examination were prepared and stored under oxygen-free atmosphere, at room temperature. Nitric oxide was bubbled through the aqueous solution of the active form for about 15–20 minutes until the solution became saturated with nitric oxide. Then a stable dark brown colour of nitrosyl compounds was established.

The electronic spectra in the visible and near infrared regions were measured on a Cary-14 spectrophotometer, using cells of d = 0.5, 0.1 and 0.01 cm. The cells were filled with the solutions to be investigated under strictly oxygen-free conditions. The total cobalt concentrations in the complexes free of imidazole were from 5.5×10^{-2} to 7.5×10^{-2} *M* and in the complexes with imidazole 3.6×10^{-2} *M*. In order to achieve high pH values, a large excess of amino acid with respect to cobalt was applied.

Magnetic susceptibilities of solutions were measured using 2% t-butanol in D$_2$O at 22 ± 1 °C by the method described by Evans [9]. The pH was measured on a Mera-Elmat N-5122 pH-meter. The IR spectra in D$_2$O were measured on a Perkin-Elmer Model 621 spectrophotometer from 1200 to 1900 cm^{-1}.

All spectroscopic measurements were performed at room temperature.

TABLE I. Absorption Spectra for Cobalt Nitrosyl Complexes with Different Amino Acids.

	pH	ν_1		ν_2		ν_3		ν_4 [Co(II)]	
		$^1T_{2g} \leftarrow {}^1A_{1g}$		$^1T_{1g} \leftarrow {}^1A_{1g}$		$^3T_{1g} \leftarrow {}^1A_{1g}$		$^4T_{2g} \leftarrow {}^4T_{1g}$	
Complex		nm	(ϵ)	nm	(ϵ)	nm	(ϵ)	nm	(ϵ)
$Co(Ala)_2NO$	6.2	340	(39)	480 510	(11) (12)	600	(4)	1100	(0.3)
$Co(Ala)_2NO$	10.1	340	(119)	475 520	(50) (52)	730	(3)		
$Co(Orn)_2NO$	6.3	355	(46)	480 510	(9) (10)	680	(0.3)	1100	(2)
$Co(Orn)_2NO$	9.1	355	(334)	470 520	(123) (123)	720	(35)		
$Co(Sar)_2NO$	6.3	340	(11)	480 510	(6) (8)	720	(7)	1100	(2)
$Co(Sar)_2NO$	10.4	340	(213)	500 525	(46) (28)	720	(3)		

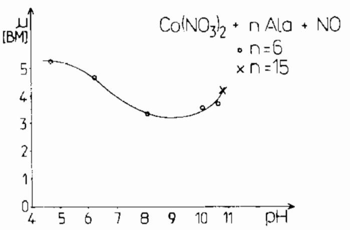

Fig. 1. The pH-dependence of magnetic moment values for $[Co(III)(Ala)_2NO{\cdot}H_2O]$ system.

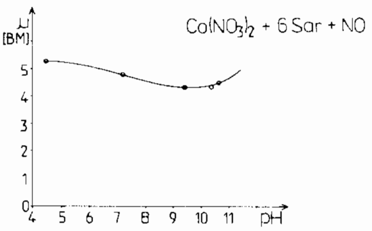

Fig. 2. The pH-dependence of magnetic moment values for $[Co(III)(Sar)_2NO{\cdot}H_2O]$ system.

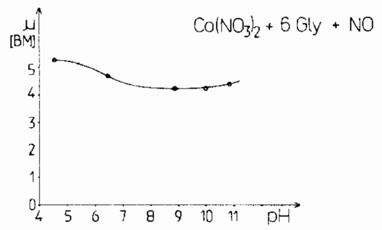

Fig. 3. The pH-dependence of magnetic moment values for $[Co(III)(Gly)_2NO{\cdot}H_2O]$ system.

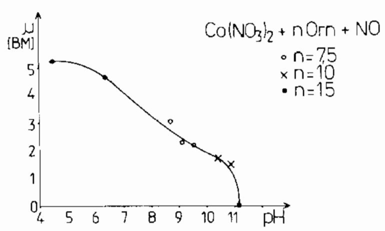

Fig. 4. The pH-dependence of magnetic moment values for $[Co(III)(Orn)_2NO]$ system.

Results

Electron spectroscopy studies for nitrosyl cobalt complexes with imidazole and amino acids ($Co(Amac)_2(Himid)NO$) performed at 0 °C are described in [7]. The electronic spectra of similar systems free of imidazole measured at room temperature are summarized in Table I. These spectra do not differ considerably from those of identical systems measured at 0 °C. Electron spectroscopy shows that as a result of the NO molecule being bound by the 'active' form, a cobalt(III) complex is formed. A comparison of the transition energies for complexes with various amino acids (Table I) shows that the effect of the ligand side chain on the symmetry of the complex formed is rather insignificant. In some cases a weak band was found at about 1100 nm which is characteristic of high-spin hexa-coordinated cobalt(II) complexes. This means that even after the solution is fully saturated with nitric oxide, there is still a cobalt(II) complex in solution in equilibrium with the nitrosyl cobalt(III) complex. Apart from the bands summarized in Table I, the electron spectrum exhibits a strong band at 260 (ϵ = 8800) which may be interpreted as a charge-transfer band in the $[Co(NO)]^{+2}$ system.

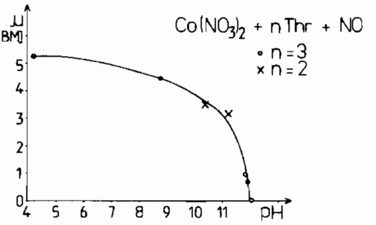

Fig. 5. The pH-dependence of magnetic moment values for [Co(III)(Thr)$_2$NO] system.

TABLE II. The Magnetic Moment Values and the Abundances of Co(II) and Co(III) Complexes for Solutions in which the Equilibrium of the NO Fixing was reached (see Reaction 1), for Different pH and for the Various Amino Acid Excesses (n = [Amac]/[Co]).

Amac	n	pH	μ	Co(II) %	Co(III) %
Thr	3	11.86	0	0	100
	6	11.64	0	0	100
	15	11.60	0	0	100
Arg	6	10.36	3.85	73	27
	15	11.02	3.77	72	28
	25	11.36	2.49	47	53
Lys	6	7.76	3.55	68	32
	6	10.36	2.71	52	48
	6	10.78	2.89	55	45
	15	11.01	2.91	55	45
	20	11.04	2.61	50	50

TABLE III. The Magnetic Moment Values and the Abundances of Co(II) and Co(III) Complexes for Solutions in which Equilibrium of Reaction (1′) was Reached (n = [Amac]/[Co]).

Amac	n	pH	μ	Co(II) %	Co(III) %
Ala	6	8.50	3.90	74	26
	6	10.14	1.94	37	63
Gly	6	8.16	3.27	62	38

No information was provided by the IR studies in D_2O since the spectrum of the nitrosyl cobalt complex with each of the amino acids under examination exihibits one band at about 1600 cm^{-1} which consists of the asymmetric frequency of a free carboxyl group, bound carboxyl group and also the frequency of the NO group bound with cobalt(III).

The most favourable method which enabled us to investigate the equilibrium in solution between the active cobalt(II) complex and nitrosyl cobalt(III) complex (1, 1′) appeared to be the NMR method. The active cobalt(II) complex (Co(II)(Amac)$_2$·nH$_2$O or Co(II)(Amac)$_2$(Himid)·H$_2$O) is a high-spin complex and its magnetic moment is 5.24 BM whereas the nitrosyl cobalt(III) complex is diamagnetic. The values of magnetic moments for the NO containing systems under examination in various pH regions are summarized in Tables II and III and in Figs. 1–5. From among the systems under investigation one can isolate a group of amino acids which, when bound with cobalt, exhibits a certain pH range (at about 7–9) where the nitric oxide binding process is most effective. This group includes amino acids which have no functional groups in side chains, such as glycine, alanine and sarcosine. In the pH region 7–9 in systems of such amino acids with cobalt the main species in solution is the complex where the ligand to metal ratio is 2:1 and in which two carboxyl groups and two amino groups are bonded with the metal [10]. For glycine and sarcosine the minimum moment is about 4.20 BM which corresponds to an equilibrium of 20% nitrosyl cobalt(III) complex and 80% cobalt(II) complex; for alanine the equilibrium is established at 37% nitrosyl cobalt(III) complex and 63% cobalt(II) complex. In order to compare these three systems, approximate values of the equilibrium constant for reaction (2) were determined:

$$K' = \frac{[\mathrm{Co(III)NO}]}{[\mathrm{Co(II)}]}$$

$$\text{Co(II) complex} \rightleftharpoons \text{Co(III)(NO) complex} \qquad (2)$$

'active' form

These constants were calculated from the molar fractions of cobalt(III) and cobalt(II) complexes determined from the magnetic moment values. The cobalt(II) concentration determined in this manner represents all complex forms of Co(II). The K′ constants are 0.59 for alanine, 0.25 for glycine and 0.22 for sarcosine, respectively. These results indicate that the system which binds most effectively nitric oxide from among the three ones mentioned above is the cobalt complex with alanine.

For the cobalt systems with glycine and alanine and with imidazole in the axial position (reaction 1′) lower values of magnetic moments were obtained (Table III) as compared with the systems free of imidazole (reaction 1) at the same pH. This means that coordination of the imidazole nitrogen, apart from the two bound amino groups of the amino acid, results in a considerable increase in the effectiveness of nitric oxide binding.

A much higher effectiveness of NO binding is exhibited by the cobalt complexes with amino acids which possess functional groups in side chains capable of metal coordination. One of the most effective is the cobalt complex with ornithine. At high pH values and at a large excess of ornithine with respect to cobalt the equilibrium in solution is shifted fully towards the formation of a nitrosyl cobalt(III) complex (μ = 0 BM). An increase in the effectiveness of NO binding as well as oxygen binding [11] occurs in strongly basic solutions. Under such conditions (pH > 10) the main species in a solution containing cobalt(II) and ornithine at the molar ratio of 1:10 is the $[Co(HL)L]^+$ complex where the following functional groups of ornithine act as donors for the metal ion: two COO^- groups, two α-NH_2 groups and one amino group of the side chain [12]. Such a complex form in which one amino acid molecule is a tridentate ligand is the nitric oxide sensitive cobalt(II) complex.

The systems examined with lysine and arginine, *i.e.* with the amino acids which contain the NH_2 groups in their side chains do not reach the zero magnetic moment. This means that the ϵ-NH_2 group of lysine is not capable of coordinating the cobalt(II) atom because of its considerable remoteness resulting from the formation of an eight-membered ring. Likewise in the system with arginine the nitrogen donors from the side chain do not coordinate with cobalt(II).

Another system which exhibits a magnetic moment of 0 BM after binding the nitric oxide is the cobalt complex with threonine. It is most probable that coordination of the OH^- group in the side chain of threonine results in the formation of a more stable and more effective NO-binding cobalt complex.

Discussion

The results of studies presented for a number of cobalt complexes with various ligands indicate different effects of these ligands on the effectiveness of nitric oxide binding.

In the cobalt systems with such ligands as glycine, alanine and sarcosine at pH > 7 when the metal is bound with two COO^- groups and with two NH_2 groups there is an equilibrium between the nitrosyl cobalt(III) complex and the initial cobalt(II) complex (reaction 1). By binding the imidazole group in the axial position the effectiveness of NO binding is increased considerably. Coordination of imidazole involves strong electron donation from the ligand to metal and produces a partial electron transfer from the metal to nitric oxide, forming a complex which, in fact, consists of cobalt(III) and the NO^- group.

A comparison of the systems with and without imidazole makes it possible to find out that the Fallab rule derived for oxygen binding by similar systems [13] is also valid for NO binding. *Viz.*, the cobalt complex with three nitrogen donors in the coordination sphere binds nitric oxide to a much higher extent than the cobalt complex with two nitrogen donors. Binding of the third nitrogen atom from the side chain in the case of ornithine increases the stability of the cobalt complex by forming an additional chelate ring. This also affects favourably the effectiveness of NO binding by this complex. An exception to the 'three nitrogen' rule is the cobalt system with threonine. A high affinity of this system to nitric oxide means that even chelation of the OH^- group in the side chain of threonine is a sufficient condition for complete NO binding by the system. The nitrosyl cobalt(III) complex which is formed in reaction (1, 1') contains a bent Co–N=O group. This fact results from electron density considerations of the system under examination [14, 15].

From the results obtained it is difficult to establish if the cobalt complex with bound nitric oxide is a monomer containing the NO group or a dimer with the N_2O_2 group [16, 17]. It is possible that in the first stage of reaction (1, 1') a monomeric complex is formed which is later transformed into a complex containing a N_2O_2 bridge (red isomer) [17].

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