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Reversible Uptake of Nitric Oxide in Co(II)–Dipeptide–NO–OH^- Systems

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Uptake of nitric oxide by cobalt(II) chelates with selected dipeptides was investigated by volumetric and spectrophotometric methods (UV, VIS). The temperature was reduced to ~0°C in order to inhibit the autooxidation of Co(II) to Co(III). The stoichiometry of NO addition and the reversibility of the reaction (by re-acidification and re-argonation) were investigated as well. The reaction was reversible in a varying degree depending on the kind of amino acid side groups used. The kinetic stability of the formed NO adducts was followed spectrophotometrically. Present and previous results, concerning fixation of dioxygen in similar systems, are compared.

Key words: dipeptides, cobalt(II) chelates, NO coordinative addition

The significance of complexes which take up nitric oxide in a reversible manner have increased remarkably in the last years, owing to the finding of physiologic role of NO in the living systems (controlling the blood pressure, inhibiting the formation of vascular thrombi *etc*.) and also regarding the therapeutic effects, *e.g*. activity of vasodilators liberating NO to the system directly or *via* metabolic cellular processes and thus saving life (hypertonia crisis) [1–5].

The removal of excessive NO, evolved into the living system as a result of metabolic processes and vasodilator drug activity, is often necessary [6]. It is expedient, therefore, to search new complex compounds, able to take up NO, *e.g*. by coordinative addition or substitution [7,8]. Due to similarity in the electronic structure of the O_2 and NO molecules [9,10], we intend to start with the 3*d* transition metals, particularly with cobalt(II). As auxiliary ligands in the initial "active" complexes we use ligands existing in the living cells and applied previously in the dioxygen adducts [11,12], that is to say dipeptide ligands – fragments of the proteins.

EXPERIMENTAL

Reagents: The NO gas 99%, purchased by Linde Gas Poland, was washed with sodium hydroxide, sulphuric acid and passed through a cold trap. A Praxair reducing valve HSS-280-10 was used. Both in the spectrophotometric and volumetric experiments the systems were deaerated with pure argon (0.9995). The dipeptides were obtained from Sigma (AlaAla, AlaPhe, GlyAla, GlyPhe), Serva (AlaGly, PheAla and PheGly), Fluka AG (GlyGly) and Chem-Impex International (HisGly and GlyHis). The purity of the

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dipeptides was tested potentiometrically. A stock solution of cobalt(II) nitrate (POCh Gliwice) was standardized with sodium salt of ethylenediaminetetraacetic acid in the presence of murexide. The remaining reagents, *i.e*. nitric acid, sodium hydroxide and potassium nitrate, were also of the highest analytical purity.

Physical measurements: pH was measured by N5170 MERATRONIC precision pH meter with a combined OSH-10-10 semi-micro electrode. The indicating glass electrode was standardized with buffer solutions: phthalate (pH_{20} = 4.00 ± 0.02) and phosphate (pH_{20} = 7.00 ± 0.02) obtained from Russell pH Limited (Scotland). Volumetric measurements were made in a conventional isobaric laboratory apparatus. Electronic absorption spectra in the UV/VIS were recorded on a Specord M40 (Zeiss) spectrophotometer equipped with thermostated cell holders. The pH range necessary for coordination of NO was attained by adding appropriate amounts of 2.985 M NaOH with a microsyringe Hamilton Bonaduz AG (Switzerland).

RESULTS AND DISCUSSION

Volumetric and pehametric data under NO atmosphere: Aqueous solutions of total volume 8 cm^3, containing 0.1 mmole of the dipeptide, 0.02 mmole of nitric acid, 0.05 mmole of cobalt(II) nitrate and an appropriate amount of potassium nitrate (ionic strength I = 0.1 mole dm^{-3}) were totally deaerated with pure argon. Then nitric oxide was passed within 15–20 minutes and the solutions were alkalized by 40–50 μl of deareated 2.985 M NaOH. In the case of HisGly and GlyHis the solutions were acidified with higher amounts of nitric acid prior to deaeration, in order to avoid undesirable dioxygen uptake.

Readings of pH and NO volume bound were taken after every two minutes (as in Figure 1).

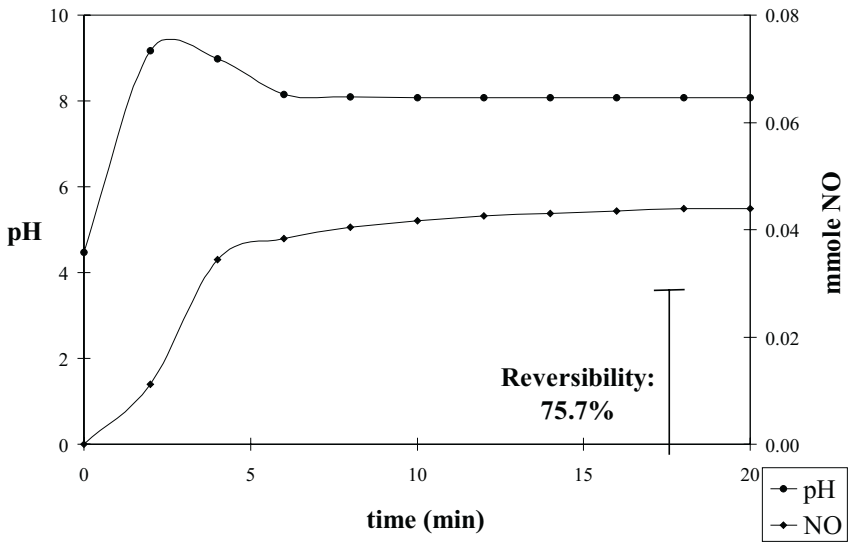


Figure 1. The pH and mmole number of NO bound after alkalization of a deaerated sample containing: 0.1 mmole of AlaPhe, 0.05 mmole of $Co(NO_3)_2$ and KNO_3 (I = 0.1 mol dm^{-3}), temp. ~0°C. The vertical segment indicates the reversibility of nitrosylation.

As it follows of Figure 1 the rise in pH after alkalization was followed by a rapid uptake of NO and drop in pH. The main uptake of NO occurred during the most rapid decrease of pH. Then, for particular ligands, the pH began to stabilize at 8–10. Simultaneously the stabilization of NO uptake was visible at *ca*. 0.05 mmole per 0.05 mmole of cobalt (*i.e*. NO:Co = 1:1) – Table 1, thus confirming a monomeric structure (not dimeric as in dioxygen systems [11]). On the other hand, the ligand to metal ratio did not influence, for particular dipeptides, the amount of NO at L:M ≥ 2:1. The mean number of mmoles of base used up to bind 1 mmole of NO ($\Delta OH^-/\Delta NO$) amounted to ~2.

Table 1. Number of mmoles of base used per mmole of bound NO and consecutive decrease in pH due to amide group deprotonation. The number of mmole of Co = 0.05.

Dipeptide	ΔNO (mmole)	$\Delta OH^-/\Delta NO$ *)	ΔpH
AlaAla	0.0495	2.02	–1.26
AlaGly	0.0481	2.08	–1.34
GlyAla	0.0505	1.98	–1.06
GlyGly	0.0498	2.01	–1.34
GlyPhe	0.0532	1.88	–1.48
PheGly	0.0460	2.17	–1.03
AlaPhe	0.0439	2.27	–1.09
PheAla	0.0537	1.86	–1.15
GlyHis	0.0516	1.94	–1.58
HisGly	0.0547	1.83	–1.96

*) $\Delta OH^- = 0.1$ (number of mmoles of base added to 0.1 mmole of the ligand, needed to neutralize the $-NH_3^+$ group).

It may be concluded that the decrease in pH (as seen in Table 1) originates from the metal promoted amide deprotonation of the ML_2 cobalt-dipeptide complexes in two steps, just as it was ascertained in numerous previous studies [13,14,15]:

$$CoL_2 \overset{k_{A1}}{\rightleftarrows} CoL(LH_{-1})^- + H^+; \qquad CoL(LH_{-1})^- \overset{k_{A2}}{\rightleftarrows} Co(LH_{-1})_2^{2-} + H^+.$$

As it was evidenced for various dipeptides [13,14], the overall deprotonation constant $pk_{A12} = pk_{A1} + pk_{A2}$ is about 18. Owing to the resulting equation, describing the approximate pH:

$$pH = \frac{1}{2}\left(pk_{A12} + \lg\frac{[CoL_2]}{[Co(LH_{-1})_2^{2-}]} \right)$$

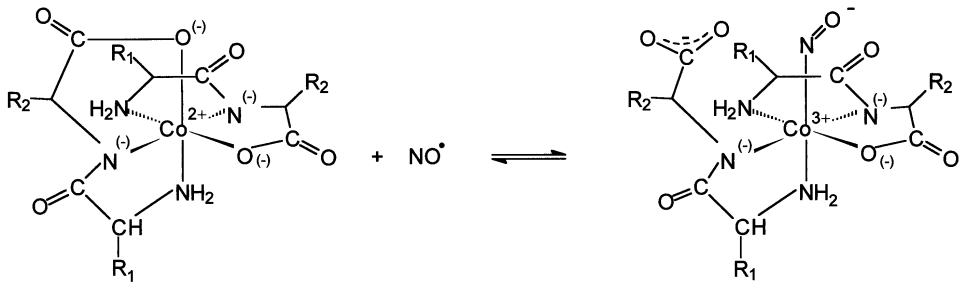
the observed decrease in pH within $\Delta pH = -1 \div -2$ corresponds to a significant, in practice almost completely, displacement of equilibrium from CoL_2 to $Co(LH_{-1})_2^{2-}$.

Thus, considering the overall reaction:

$$CoL_2 + NO + 2\ OH^- = Co(LH_{-1})_2NO^{2-} + 2\ H_2O \quad (1)$$

the uptake of NO may be presented as in Scheme 1, where $Co(LH_{-1})_2^{2-}$ is the "active" complex and the replaced function is probably the carboxyl oxygen (a relatively weak electron pair donor).

Scheme 1



$[Co(LH_{-1})_2]^{2-}$ **$[Co(LH_{-1})_2NO]^{2-}$**

The reaction in Scheme 1 might be regarded as a simple coordinative substitution, with NO functioning as a two-electron nitroso (nitrosyl) donor NO^- [2,6,17,18]. The bent end-on structure in the resulting formally Co(III) complex is the most justified one in such cases [9,10].

During the volumetric measurements an additional, although insignificant, consumption of NO was observed. After alkalization in absence of metal chelation, the uptake of NO amounted to *ca* 0.01 mmole per 10 minutes, which is a rather negligible rate as compared with the coordinative addition. The relatively slow reaction in diluted solutions of strong bases is known as disproportionation [16,17,18] of the type:

$$2\ NO + NaOH \rightarrow NaNO_2 + 1/2\ N_2O + 1/2\ H_2O \quad (2)$$

The reversibility of nitrosylation in the main experiment was tested by acidification of each sample with 3 M nitric acid – the number of mmoles added was one of sodium hydroxide used up during alkalization. From among the dipeptides tested as auxiliary ligands, the highest reversibility was indicated by PheGly and AlaPhe – Table 2. The inductive effect of the benzyl ring in phenylalanine [19] on promoting amide deprotonations in the "active" complex may be considered herein.

It seems also interesting that while comparing GlyHis and HisGly (dipeptides with coordinating side groups) the observed reversibility of nitrosylation was quite similar as in dioxygen fixation. Namely, in the case of GlyHis it was high for O_2 [15]

but also above 50% for NO. Likewise, in the case of HisGly the reversibility was low for O_2 (coordination of histidine type with participation of imidazole nitrogen) and also low for NO (< 20%). Interactions for this group of dipeptides will be discussed in details in a separate paper.

Table 2. Reversibility (in lowering order) of NO uptake in the Co(II)–dipeptide–OH^-–NO systems. Temperature ~0°C

Dipeptide	Reversibility
PheGly	85.8%
AlaPhe	75.7%
AlaAla	63.9%
PheAla	61.6%
GlyHis	52.8%
GlyPhe	49.6%
GlyAla	48.5%
GlyGly	40.0%
AlaGly	25.5%
HisGly	18.6%

Spectrophotometric UV/VIS data: A UV-grade cell of 1 cm path length was tightly covered with a silicone stopper. The initial content of the sample consisted of 0.2 mmole of cobalt(II) nitrate, 0.6 mmole of the dipeptide and potassium nitrate to attain the ionic strength $I = 0.1$ mol dm^{-3}. The total initial volume amounted to 2.6 cm^3.

The first step of the spectrophotometric experiments was carried out in a UV/Vis range (33–11) × 10^3 cm^{-1}. After 15–20 minutes of argonation, the "active" complex was prepared by adding through the silicone stopper 60 μl (or 100 μl in the case of HisGly and GlyHis) of deaerated 1.04 M NaOH. The spectral curve of the pink "active" form is presented in Figure 2 (curve 10). Afterwards, the sample was cooled at temperature ~0°C and rinsed with purified NO (the color turned to almost black) and then the sample was re-argonated – in some cases the color turned back near to the initial pink and the spectral curve came close to the one of the "active" complex, as well (Figure 2, curve 11). Another sample with the NO complex was prepared in the same way but in this case the sample was spectrophotometrically followed in time. The results confirmed the high stability of the nitrosylated form at ~0°C, since during the first hours no absorbance was observed. A rise of optical density, however, like in Figure 2 (curves: 1 to 7), was due to the slow dissolution of gaseous NO from above the sample, moderated by a concomitant process – the irreversible autooxidation of Co(II) to Co(III), known already in the dioxygen uptake [12].

At last, after quite a long time (> 10 hours), a decrease in absorbance at the main bands was observed (curve 8), now exclusively as a result of the proceeding cobalt(II) autooxidation. Then curve 10, after two days, shows a characteristic rise in the UV, in-

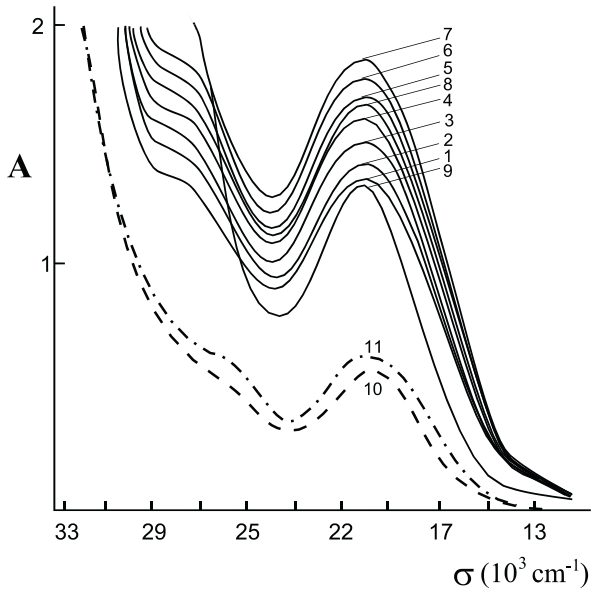


Figure 2. UV/VIS absorption spectra of the Co(II)-AlaGly-OH^--NO system. Nitrosylated sample after: 1 – 1 min, 2 – 20 min, 3 – 45 min, 4 – 75 min, 5 – 2 h, 6 – 3 h, 7 – 4.5 h, 8 – 1 d, 9 – 2 d; Active complex – curve 10; Sample nitrosylated and then argonated – curve 11.

dicating a decided displacement of equilibrium towards irreversible Co(III) species – the color of the sample turned to dark violet.

The autooxidation observed in the NO adducts may be interpreted as an irreversible redox rearrangement of the cobalt(II) d_{z2} electron density to the antibonding $\pi^* 2p_y$ orbital of NO^-. It is also proper to notice that the reversibility of nitrosylation, reported previously in related ternary amino acid–imidazole systems [8] and tested by the same method of re-argonation, has been confirmed herein also for the binary, "active" dipeptide systems. The equilibrium may be written as:

$$\text{"active" form} \underset{\text{Ar}}{\overset{\text{NO}}{\rightleftharpoons}} \text{nitrosylated form} \qquad (3)$$

The absorption spectra were in a good agreement with the assignments proposed in [20] for Co–NO of approximately C_{4v} symmetry, *i.e.* $\nu_1 \sim 28 \times 10^3$ cm^{-1}, an asymmetric band at $\sim 21 \times 10^3$ cm^{-1} probably composed of two transitions: ν_2 as well as ν_3, and then a very weak band ν_4 at $\sim 13 \times 10^3$ cm^{-1} (Table 3). The ν_5 transition: 3 a_1 [σ(NO), d_{z^2}] $\rightarrow$ 2 e [d_{xz}, d_{yz}, π^*(NO)] [20] was invisible in UV/VIS.

Table 3. Main absorption bands and shoulders in the UV-VIS spectra of nitrosylated Co(II)–dipeptide complexes (10^3 cm^{-1}) [a]. $\bar{\varepsilon}$ – mean molar absorption coefficient.

System	ν_1 ($\bar{\varepsilon}$) shoulder	ν_2 ($\bar{\varepsilon}$)	ν_3 ($\bar{\varepsilon}$) shoulder	ν_4 ($\bar{\varepsilon}$) shoulder
Co(II)–AlaAla–OH^-–NO	~ 28 (212)	20.7 (157)	~ 17.4 (108)	~ 13.2 (14)
Co(II)–AlaGly–OH^-–NO	~ 27.8 (115)	20.2 (116)	~ 17 (70)	——
Co(II)–GlyAla–OH^-–NO	~ 27.3 (226)	21 (212)	~ 17.2 (153)	~ 13 (37)
Co(II)–GlyGly–OH^-–NO	~ 26.9 (163)	20.7 (155)	~ 17.1 (126)	~ 13 (15)
Co(II)–GlyPhe–OH^-–NO	~ 27 (206)	22 (156)	~ 19.5 (110)	~ 13 (26)
Co(II)–PheGly–OH^-–NO	~ 28.4 (197)	21.2 (198)	——	~ 14 (17)
Co(II)–AlaPhe–OH^-–NO	~ 27.6 (250)	20.6 (211)	~ 17.2 (145)	~ 13.5 (28)
Co(II)–PheAla–OH^-–NO	~ 28.4 (230)	21 (88)	——	~ 13.4 (18)
Co(II)–HisGly–OH^-–NO	——	20.8 (140)	~ 16.5 (60)	~ 13.2 (26)

[a] assignments according to [20]:

ν_1 3 a_1 [σ(NO), d_{z^2}] $\rightarrow$ 4 a_1 [d_{z^2}, σ(NO)]

ν_2 3 a_1 [σ(NO), d_{z^2}] $\rightarrow$ 1 b_1 ($d_{x^2-y^2}$)

ν_3 3 a_1 [σ(NO), d_{z^2}] $\rightarrow$ 3 e [π^*(NO), d_{xz}, d_{yz}]

ν_4 3 a_1 [σ(NO), d_{z^2}] $\rightarrow$ 1 b_2 (d_{xy})

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