# Complex Formation of Nickel Ion with Aliphatic Dipeptides

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*Formation constants for complexes between nickel and a number of aliphatic dipeptides consisting of glycine, alanine, leucine and proline have been determined by potentiometric titrations. The structures of $NiLH^+$, $NiL_2H_2$ and $NiL_3H_3^-$ are discussed ($LH_2 = {}^+H_3N{-}CHR{-}CO{-}NH{-}CHR'{-}CO_2^-$). The dipeptides are found to act as bidentate ligands. Complex distribution depending on pH and metal/peptide ratio is given for the whole series of dipeptides and influences of the side chains on this distribution are discussed.*

## Introduction

Interactions between proteins or peptides and transition metals have been studied extensively [1–6] during the last two decades. As proteins show a rather complicated chemical behaviour, model substances were used to mimic the metal binding site. Dipeptides [7, 8] proved to be such model substances having three important binding abilities: N-terminal amino group, C-terminal carboxyl group and one peptide bond. Such studies have been performed by such techniques as spectroscopy [9–13], temperature jump [14], calorimetry [15, 16] and—as is being used in this work—potentiometric titrations. With this method glycylglycine [8, 14, 16, 17] and some other dipeptides [18, 19] have been studied extensively. There exists, however, still some uncertainty regarding the higher pH-range. Our investigations were carried out with most of the dipeptides consisting of glycine, alanine, leucine and proline. Such a series should give information about the influence of the side chains on complex formation between the dipeptide and the nickel ion, as well as about the dependence of formed complex species on the structure of the dipeptides. In connection with our previous work [20] on the complex formation of copper ion with the same dipeptides a comparison of the complexation behaviour of nickel and copper was also attempted.

## Experimental

### *Materials*

Nickel chloride solution was prepared by dissolving $NiCl_2 \cdot 6H_2O$ in $CO_2$-free water. The concentration of the nickel stock solution was examined by complexometric titration. The dipeptides gly-gly, gly-d,l-ala*, d,l-ala-d,l-ala*, gly-d,l-leu*, gly-l-pro, d,l-ala-gly*, d,l-ala-d,l-leu, l-ala-l-pro, d,l-leu-gly*, d,l-leu-d,l-leu*, l-pro-gly, l-pro-l-ala and l-pro-l-leu were obtained from Sigma Chemical Co. (Sigma analytical grade).

### *Measurements*

Nickel complex formation constants were calculated from potentiometric titration curves of the dipeptides in the absence and presence of nickel. Changes in pH were measured using a combined glass electrode and a Schott pH-meter CG 803. Titrations were carried out at various metal/ligand ratios from 2:5 to 2:10. The systems need considerable time to reach equilibrium between pH 7 and pH 9. For a 2:5 metal/ligand ratio equilibrium is established after ten minutes. This process can be accelerated by increasing the peptide concentration. The concentration of nickel chloride was $2.03 \times 10^{-3}$ *M* in all titrations, the titration was performed with 0.100 *M* NaOH solution. All investigations were carried out under nitrogen atmosphere at 20 °C (± 0.2°) and at an ionic strength of 0.20 *M* KCl. For the calculation of the formation constants a FORTRAN computer program [20] was used, using at least 150 titration data per system. All computations were carried out on the CDC computer of the University of Innsbruck.

*These dipeptides were used in the d,l-form. The calculated formation constants are therefore mean values for all present stereoisomers.

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## Results and Discussion

*Model used for the Simulation of the Titration Curves*

Several authors [8, 14, 16–19] report that $Ni^{2+}$ forms three simple types of complexes with aliphatic dipeptides: $NiLH^+$, $NiL_2H_2$ and $NiL_3H_3^-$. All peptide protons are retained in these complexes. Kaneda and Martel [16], as well as Brookes and Pettit [18], describe further species in which one or two peptide protons are dissociated Brookes and Pettit tried to find a comprehensive model, which would hold for a large pH range. They suggested three models, among which no further selection seemed to be possible. In this work nine models were investigated. If $LH_2$ denotes the zwitterionic dipeptide, $^+H_3NCHR–CO–NH–CHR'–CO_2^-$, the following possible reactions can be defined:

$K_1$: $LH_2 + H^+ \longrightarrow LH_3^+$

$K_2$: $LH_2 \longrightarrow LH^- + H^+$

$K_3$: $Ni^{2+} + LH_2 \longrightarrow NiLH_2^{2+}$

$K_4$: $Ni^{2+} + LH^- \longrightarrow NiLH^+$

$K_5$: $Ni^{2+} + LH^- \longrightarrow NiL + H^+$

$K_6$: $Ni^{2+} + LH^- \longrightarrow NiLOH^- + 2H^+$

$K_7$: $Ni^{2+} + 2LH^- \longrightarrow NiL_2H_2$

$K_8$: $Ni^{2+} + 2LH^- \longrightarrow NiL_2H^- + H^+$

$K_9$: $Ni^{2+} + 2LH^- \longrightarrow NiL_2^{2-} + 2H^+$

$K_{10}$: $Ni^{2+} + 3LH^- \longrightarrow NiL_3H_3^-$

As the formation of $NiLH_2^{2+}$ does not generate any proton, it is very difficult to detect such a species by means of pH-titration. Kaneda and Martell [16] involved this complex in their model. Our work showed that the pH-range from 5 to 7 could be simulated very well without considering the species $NiLH_2^{2+}$. Therefore, all further calculations were carried out neglecting this species. Thus the considered models include the following complexes:

model 1: $NiLH^+$, $NiL_2H_2$, $NiL_3H_3^-$, NiL, $NiLOH^-$

model 2: $NiLH^+$, $NiL_2H_2$, $NiL_3H_3^-$, $NiL_2H^-$, $NiL_2^{2-}$

model 3: $NiLH^+$, $NiL_2H_2$, $NiL_3H_3^-$, NiL, $NiL_2^{2-}$

model 4: $NiLH^+$, $NiL_2H_2$, $NiL_3H_3^-$, $NiL_2H^-$, $NiLOH^-$

model 5: $NiLH^+$, $NiL_2H_2$, $NiL_3H_3^-$, NiL, $NiL_2H^-$, $NiL_2^{2-}$

model 6: $NiLH^+$, $NiL_2H_2$, $NiL_3H_3^-$, NiL, $NiL_2H^-$, $NiLOH^-$, $NiL_2^{2-}$

model 7: $NiLH^+$, $NiL_2H_2$, $NiL_3H_3^-$,

model 8: NiLH1, $NiL_2H_2$, $NiL_3H_3^-$, $NiL_2^{2-}$

model 9: $NiLH^+$, $NiL_2H_2$

In order to decide whether any of these models is to be preferred to the others, it is necessary to compare the estimated experimental error with the standard deviation resulting from the calculations. No system could be simulated using model 9, indicating thus the significance of $NiL_3H_3^-$. The dipepties gly-pro and ala-pro do not have any peptide proton. Only for these two systems does the error using model 7 remain negligible. Fig. 1 shows that complex formation with $Ni^{2+}$ ion and all dipeptides having a peptide proton cannot be described satisfactorily neglecting the dissociation of the peptide proton. The models 1 and 4 on the one hand and 2 and 3 on the other are equivalent to each other. The models 2 and 3, not containing the species $NiLOH^-$, however, lead to considerably better results than the models 1 and 4. This seems to prove clearly that $NiL_2^{2-}$ represents an essential species and cannot be replaced in any approach by $NiLOH^-$. A further indication for this result was obtained by using model 6, containing all possible species. In this approach no significant contribution of $NiLOH^-$ is obtained, whereas $NiL_2^{2-}$ proves to be necessary. It is impossible, however, to distinguish between the species NiL and $NiL_2H^-$. They can simply replace each other in the simulation. Therefore, models 2, 3 and 5 yield almost the same error. The dipeptides l-pro - gly, l-pro - l-ala and l-pro - l-leu do not form the species NiL. Thus, model 3 is not able to simulate the titration curves of these systems. Neglect of these two species leads to a rather poor simulation of the titration curves (Fig. 1). Model 5 also contains the species NiL. For this reason we prefer model 2 because of its more general applicability. Other species, like polynuclear complexes, do not exist or cannot be detected with this method, because model 2 leads to a very good simulation of all titration curves, the error of simulations remaining negligible through all variations of metal/peptide concen-

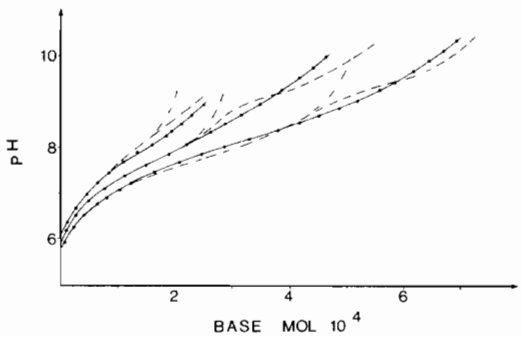



Fig. 1. Titration curves of the system nickel–d,l-ala - gly at constant nickel concentration ($2.03 \times 10^{-3}$ *M*) a: [d,l-ala - gly] = $10.08 \times 10^{-3}$ *M*. b: [d,l-ala - gly] = $6.40 \times 10^{-3}$ *M*. c: [d,l-ala - gly] = $4.24 \times 10^{-3}$ *M* ——— experimental titration curves; · · · · calculated curves using model 2 or 3 or 5; – · – · – calculated curves using model 7; – – – – calculated curves using model 8

tration ratios under investigation. It is not likely that inclusion of higher nickel/peptide ratios would lead to a different picture, since eventual new species should have appeared to some extent already at the investigated ratios and since precipitation of $Ni(OH)_2$ occurs at increasingly lower pH values with increasing $Ni^{2+}$ concentration. Furthermore, all biologically relevant systems will contain peptide or protein ligands at larger concentrations than the $Ni^{2+}$ metal ion. This means that on the basis of potentiometric titrations the following complex species are predicted to exist: $NiLH^+$, $NiL_2H_2$, $NiL_3H_3^-$, $NiL_2H^-$ and $NiL_2^{2-}$. The formation constants of these species are listed in Table I.

*The Structures of $NiLH^+$, $NiL_2H_2$ and $NiL_3H_3^-$*

The side chain influence of the dipeptides gives some information concerning the arrangement of the ligands. Fig. 2 shows a plot of formation constants *versus* $pK_2$ [21]. For identical N-terminal amino acids the contribution of the C-terminal side chain leads only to a change of the basicity of the amino group. This holds for all three complexes, indicating that there is no steric hindrance caused by the C-terminal side chain. A strong decrease of complex stability is found, however, if the N-terminal side chain is enlarged. This result supports the proposed structures [8, 14, 16, 22] of Fig. 3 and does not agree with the assumption that the peptide ligands are tridentate [17]. If the dipeptides are only bidentate, the constants $lgK_I$, $lgK_{II}$ and $lgK_{III}$ should be similar after taking into account their different statistical probability [23].

$Ni^{2+} + LH^- \longrightarrow NiLH^+$ $\quad lg\bar{K}_I$*: 3.87[a] 3.12[b]

$NiLH^+ + LH^- \longrightarrow NiL_2H_2$ $\quad lg\bar{K}_{II}$: 3.39 2.81

$NiL_2H_2 + LH^- \longrightarrow NiL_3H_3^-$ $\quad lg\bar{K}_{III}$: 3.18 2.77

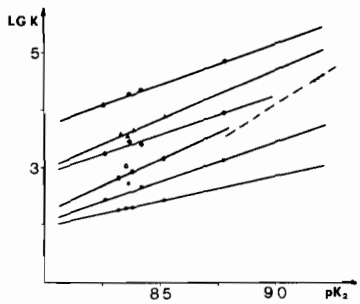



Fig. 2. Plot of formation constants *versus* $pK_2$. Notation • $lgK^{Ni^{2+}}_{NiLH^+}$, glycine - X dipeptides; * $lgK^{NiLH^+}_{NiL_2H_2}$, glycine - X dipeptides, ▪ $lgK^{NiL_2H_2}_{NiL_3H_3^-}$, glycine - X dipeptides; ▲ $lgK^{Ni^{2+}}_{NiLH^+}$, alanine - X dipeptides; ○ $lgK^{NiLH^+}_{NiL_2H_2}$, alanine - X dipeptides; ♦ $lgK^{NiL_2H_2}_{NiL_3H_3^-}$, alanine - X dipeptides; – – – $lgK^{Ni^{2+}}_{NiLH^+}$, proline - X dipeptides.

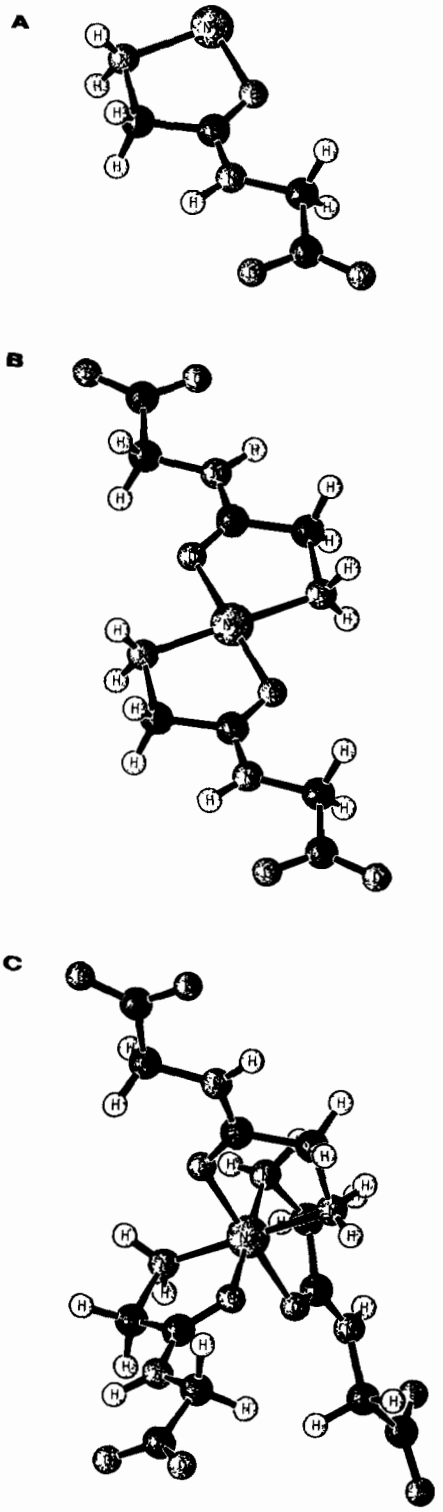



Fig. 3. Postulated structures of various nickel–glycylglycine complexes. A. $NiLH^+$, B: $NiL_2H_2$, C: $NiL_3H_3^-$.

*The constants after statistical correction. a: gly - d,l-ala, b: d,l-ala - gly.

TABLE I Complex Formation Constants of Nickel with Aliphatic Dipeptides.

| | $NiLH^+$ | $NiL_2H_2$ | $NiL_2H^-$ | $NiL_2^{2-}$ | $NiL_3H_3^-$ |
|---|---|---|---|---|---|
| gly - gly | | | | | |
| $pK_b$ (Ref.) | −3.88[b] | −7.00 | – | – | – |
| | −3.34[c] | −7.41 | – | – | −9.91 |
| | −4.49[d] | −7.91 | – | – | – |
| | −4.17[e] | −7.32 | – | – | – |
| $pK_b$[a] | −4.08 | −7.32 | 1.80 | 11.57 | −9.68 |
| $\Delta pK_b$[h] | 0.05 | 0.06 | 0 28 | 0 18 | 0 07 |
| gly - d,l-ala | | | | | |
| $pK_b$ (Ref.) | −4.23[f] | −7.60 | – | 12.24 | −9 71 |
| $pK_b$ | −4.35 | −7.74 | 1.52 | 11.76 | −10.44 |
| $\Delta pK_b$ | 0.09 | 0.10 | 0.12 | 0.10 | 0 12 |
| gly - d,l-leu | | | | | |
| $pK_b$ (Ref.) | −4.25[d] | −7.99 | – | – | – |
| | −4.16[g] | −7.83 | – | – | – |
| $pK_b$ | −4 28 | −7.77 | 2.13 | – | −10.52 |
| $\Delta pK_b$ | 0.09 | 0.11 | 0.19 | – | 0.14 |
| gly - l-pro | | | | | |
| $pK_b$ (Ref.) | −4.76[f] | −8.65 | – | – | −11.46 |
| $pK_b$ | −4.86 | −8.83 | – | – | −11.90 |
| $\Delta pK_b$ | 0.13 | 0 15 | – | – | 0.18 |
| d,l-ala - gly | | | | | |
| $pK_b$ | −3.60 | −6.41 | 2.21 | 12.08 | −8.70 |
| $\Delta pK_b$ | 0.09 | 0.13 | 0.14 | 0.09 | 0.25 |
| d,l-ala - d,l-ala | | | | | |
| $pK_b$ (Ref.) | −3 61 | −6.77 | – | – | – |
| $pK_b$ | −3.65 | −6.57 | 2.41 | 12.31 | −8.84 |
| $\Delta pK_b$ | 0.08 | 0.10 | 0.16 | 0.09 | 0.19 |
| d,l-ala - d,l-leu | | | | | |
| $pK_b$ | −3.56 | −6.64 | 2.83 | 14.30 | −8.96 |
| $\Delta pK_b$ | 0 08 | 0.05 | 0.16 | 0.75 | 0.50 |
| l-ala - l-pro | | | | | |
| $pK_b$ | −3 93 | −7.12 | – | – | −9.57 |
| $\Delta pK_b$ | 0.10 | 0.12 | – | – | 0.15 |
| d,l- leu - gly | | | | | |
| $pK_b$ (Ref.) | −3.44[d] | −6.43 | – | – | – |
| $pK_b$ | −3 39 | −6.21 | 2 41 | 12.31 | −8.46 |
| $\Delta pK_b$ | 0.06 | 0.07 | 0.10 | 0.06 | 0 17 |
| d,l-leu - d,l-leu | | | | | |
| $pK_b$ | −3.30 | −6.18 | 3.43 | 14.15 | −8.01 |
| $\Delta pK_b$ | 0.19 | 0.13 | 0.33 | 0.86 | 0.40 |
| l-pro - gly | | | | | |
| $pK_b$ | −4.36 | −8.10 | 0.95 | 10.48 | −10.22 |
| $\Delta pK_b$ | 0.14 | 0.10 | 0.18 | 0.11 | 0.56 |
| l-pro - l-ala | | | | | |
| $pK_b$ | −4.46 | −8.21 | 0.97 | 11.09 | −10.65 |
| $\Delta pK_b$ | 0.13 | 0.11 | 0.14 | 0.14 | 0.34 |
| l-pro - l-leu | | | | | |
| $pK_b$ | −4.38 | −8.60 | 1.87 | 11.93 | −11.35 |
| $\Delta pK_b$ | 0 10 | 0.06 | 0.54 | 0.45 | 0.12 |

[a]This work; 0.20 *M* KCl, 20 °C [b]Ref. 16; 0.10 *M* $KNO_3$, 25 °C. [c]Ref 17; 0.10 *M* $KNO_3$, 25 °C. [d]Ref. 14, 0.10 *M* $NaClO_4$, 25 °C [e]Ref. 8; 0.10 *M* $KNO_3$, 25 °C. [f]Ref. 18; 0.10 *M* $KNO_3$, 25 °C. [g]Ref. 19; unknown conditions. [h]$\Delta pK_b$ serves as a measure for significance of the constants. Changing the constant by $\Delta pK_b$ leads to an increase of $\sum_i (v_i^{\text{theoret.}} - v_i^{\text{calc.}})^2$ by a factor of 2.

The small differences between the statistically corrected constants $\lg\bar{K}_I$, $\lg\bar{K}_{II}$ and $\lg\bar{K}_{III}$ may be due to slight differences in bond strength and bond lengths for higher coordinations [24, 25]. When leucine is the C-terminal amino acid the complex stability of $NiL_2H_2$ and $NiL_3H_3^-$ is higher than expected, especially for l-pro - l-leu. The experimental results do not yet give any satisfactory explanation for this. We thus plan to involve quantum chemical calculations in a further stage of these investigations, in order to obtain more information about binding and electronic effects in such systems.

*Species Distribution depending on pH and Nickel/Ligand Ratio*

Complex formation of nickel ion and aliphatic dipeptides starts at pH 4.5. Fig. 4 demonstrates the successive formation of the species $NiLH^+$, $NiL_2H_2$ and $NiL_3H_3^-$. At pH 7, 8–8.5 and 8.5–9 these complexes attain their highest concentration. Beginning with pH = 7, dissociation of one peptide proton takes place. The thus formed species $NiL_2H^-$ reaches a maximum at pH 9.5–10.5. The second peptide proton dissociates from pH > 8. The resulting species $NiL_2^{2-}$ dominates the alkaline solution, until precipitation occurs.

Increasing ligand concentration influences both the pH distribution and the relative concentration of the various complex species (Fig. 4.).

*Influence of the Side Chains on Stability and Concentration Distribution*

*$NiL_2^{2-}$*

Both side chains show a distinct influence on the stability of the complex (Fig. 5). Increasing R′ leads to a lower stability of the complex, causing complex formation to begin at higher pH values. As nickel hydroxide precipitates at pH 10–10.5, the constants of $NiL_2^{2-}$ cannot be determined exactly, if the C-terminal amino acid is leucine. A larger R leads to a similar decrease of the stability, but changes in the species distribution are not observed.

*$NiL_2H^-$*

The influence of R and R′ is very similar to the case of $NiL_2^{2-}$, but the concentration maximum is shifted to lower pH values, if R becomes larger.

*$NiLH^+$, $NiL_2H_2$ and $NiL_3H_3^-$*

As has been demonstrated already in Fig. 2, R′ does not cause any steric hindrance. The change of stability is only due to the induced changes of basicity. The N-terminal side chain, however, leads to a strong decrease of stability within the series glycine, alanine, leucine. R seems to have a steric influence in all species. If the change of basicity is rather small, the steric influence may dominate, as can be seen in

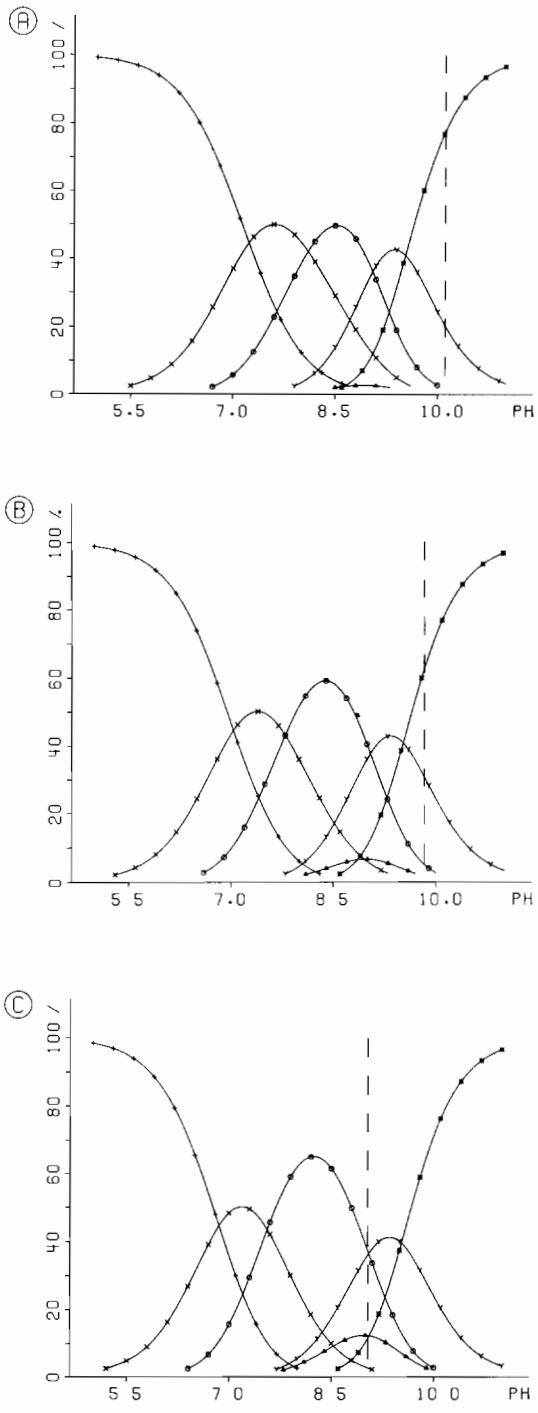



Fig. 4. Species distribution depending on nickel/ligand ratio and pH at constant nickel concentration ($2.00 \times 10^{-3}$ *M*) and varying peptide concentration (a: $5.00 \times 10^{-3}$ *M*, b: $7.00 \times 10^{-3}$ *M*, c: $1.00 \times 10^{-2}$ *M*). The dipeptide used was l-pro - gly. Concentration is given in percent of total metal concentration. Notation: +: $Ni^{2+}$, ×: $NiLH^+$, ○: $NiL_2H_2$, △: $NiL_3H_3^-$, y: $NiL_2H^-$, ∗: $NiL_2^{2-}$. – – – Precipitation of $Ni(OH)_2$ begins.

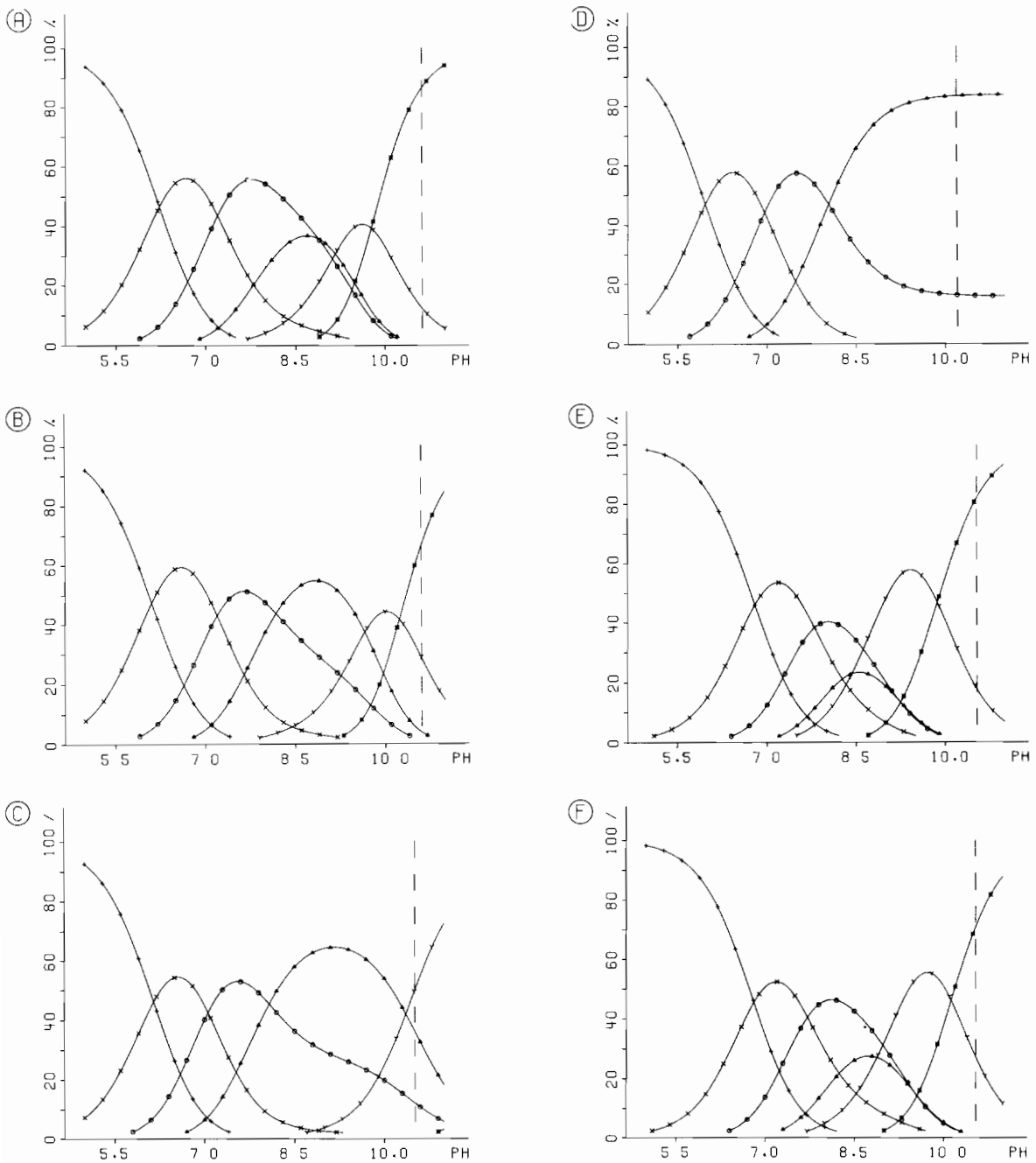
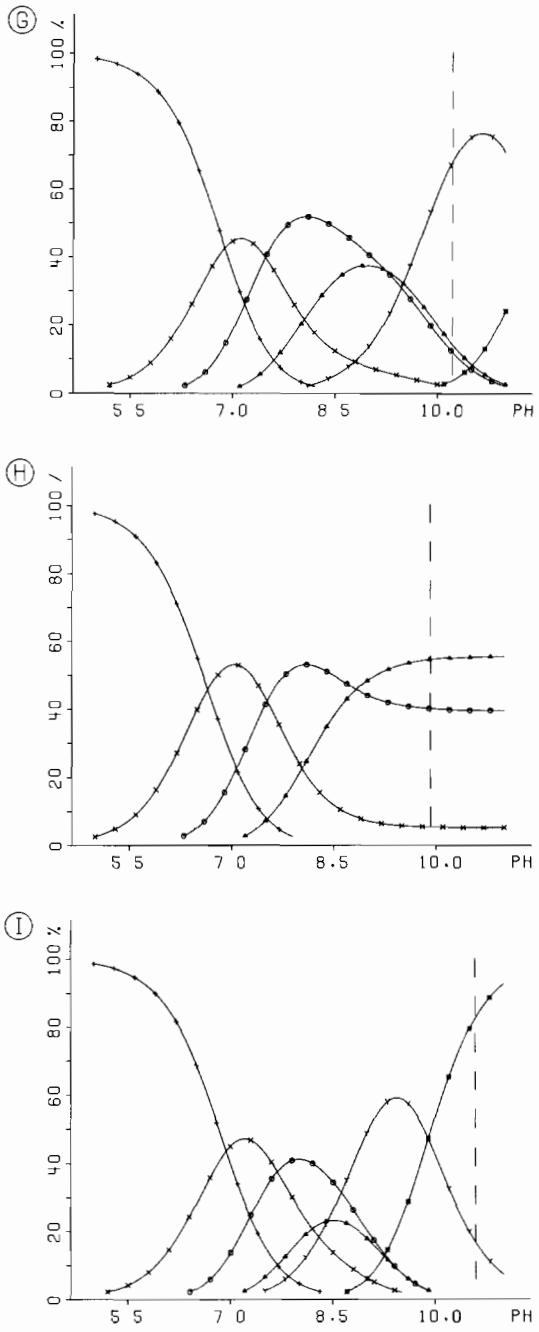
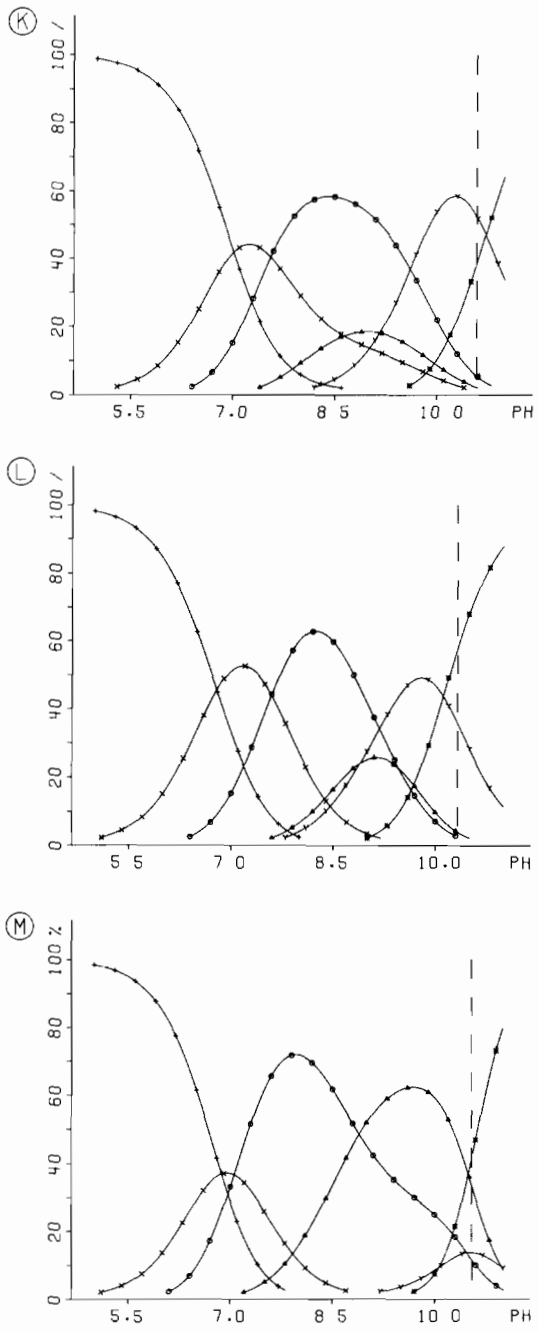



Fig. 5. Influence of the side chains on the concentration distribution of all species at metal/peptide ratio of 1:5. a) gly - gly, b) gly - d,l-ala, c) gly - d,l-leu, d) gly - l-pro, e) d,l-ala - gly, f) d,l-ala - d,l-ala, g) d,l-ala - d,l-leu, h) l-ala - l-pro, i) d,l-leu - gly, k) d,l-leu - d,l-leu, l) l-pro - l-ala, m) l-pro - l-leu. Notation is the same as used in Fig 4. The formation constants are taken from model 2.

the case of gly - X, ala - X and leu - X dipeptides. In the case of pro- X dipeptides, however, steric hindrance plays a minor role, because of high basicity. Thus, it is impossible to discuss steric influence taking into account gly-, ala-, leu- and pro- dipeptides as well. The significant differences in the plots of Fig. 2 for both types of peptide complexes indicate, however, that there exists a steric influence of the N-terminal proline group as well.

The concentration of different species at physiological pH may be interesting in relation to biological systems. A summary of these data is given in Table III. At pH 7.4 and at a metal/peptide ratio of 2:5, only three complexes, all of them containing their peptide protons, reach a concentration higher than




one percent of the total metal concentration. $NiLH^+$ dominates such a system and the concentration of $NiL_3H_3^-$ is rather low. About 10 to 30 percent of the nickel ion does not form any complex. Increase of the ligand concentration leads to an increase of $NiL_2H_2$ and $NiL_3H_3^-$. At a metal/peptide ratio of 2:10, the concentration of $Ni^{2+}$ decreases to 2 to 10 percent, according to the side chains of the peptides.

*Comparison between Copper and Nickel Complexes*

Regarding to the Irving–Williams series [26] complex formation of $Ni^{2+}$ is weaker than that of $Cu^{2+}$ (Table II). All species formed with aliphatic dipeptides, except $NiL_3H_3^-$, obey this rule. $NiLH^+$ and $NiL_2H_2$ are about thirty times less stable than $CuLH^+$ and $CuL_2H_2$. $Ni^{2+}$ forms a 1:3 complex, however, which does not exist in the system of copper. This is to be explained by the Jahn–Teller effect appearing in the case of $Cu^{2+}$. The formation constants of the species $ML_2^{2-}$ are smaller by seven orders of magnitude.

That species retaining their peptide protons dominate the nickel systems, whereas species having lost their peptide protons are the most important ones

for the copper systems. The peptide proton, which does not dissociate in a strong alkaline solution, becomes rather acidic if copper ion is added. The pK value is about 4, indicating a stronger acid than acetic acid. In the case of nickel ion one observes a much weaker dissociation of the peptide proton with a pK of 10. If the charge of the species becomes negative, such as $ML_2H^-$, the dissociation tendency of the peptide proton decreases for the copper system and remains almost stable for the nickel systems.

## Acknowledgement

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TABLE II Complex Formation Constants of Copper and Nickel with Aliphatic Dipeptides.

| | Copper | Nickel | R* |
|---|---|---|---|
| $lgK^{M^{2+}}_{MLH^+}$ | −5.1 – −6.5 | 3.6 – 4.9 | 30 |
| $lgK^{MLH^+}_{ML_2H_2}$ | 4.0 – 6.0 | 2.8 – 4.2 | 30 |
| $lgK^{ML_2H_2}_{ML_3H_3^-}$ | – | 2.2 – 3.1 | – |
| $pK^{M^{2+}}_{ML_2H^-}$ | −4.5 – −6.3 | 3.4 – 1.0 | $3 \times 10^7$ |
| $pK^{M^{2+}}_{ML_2^{2-}}$ | 7.3 – 4.6 | 14.5 – 10.5 | $3 \times 10^6$ |
| $pK^{ML_2H_2}_{ML_2H^-}$ | 6.0 – 5.0 | 10.5 – 8.8 | $1 \times 10^4$ |
| $pK^{ML_2-H}_{ML_2}$ | 11.5 – 10.0 | 11.0 – 9.5 | 0.3 |

*Ratio of the equilibrium constants of copper and nickel.

TABLE III. Concentrations of all Nickel Complexes at pH 7.4 for Metal/Peptide Ratios of 2.5 and 2.10 in percent of Total Metal Concentration. Species below 1% were neglected.

| Peptide | $NiLH^+$ | $NiL_2H_2$ | $NiL_3H_3^-$ | $NiL_2H^-$ | $NiLH^+$ | $NiL_2H_2$ | $NiL_3H_3^-$ | $NiL_2H^-$ |
|---|---|---|---|---|---|---|---|---|
| | | (2:5)[a] | | | | (2:10) | | |
| gly - gly | 54 | 30 | 2 | 0 | 35 | 51 | 10 | 1 |
| gly - d,l-ala | 55 | 30 | 3 | 0 | 34 | 49 | 14 | 1 |
| gly - d,l-leu | 49 | 35 | 4 | 0 | 28 | 52 | 18 | 0 |
| gly - l-pro | 48 | 40 | 4 | 0 | 24 | 57 | 18 | 0 |
| d,l-ala - gly | 51 | 12 | 1 | 1 | 51 | 27 | 4 | 2 |
| d,l-ala - d,l-ala | 50 | 13 | 1 | 0 | 50 | 29 | 4 | 1 |
| d,l-ala - d,l-leu | 44 | 18 | 1 | 0 | 42 | 37 | 5 | 0 |
| l-ala - l-pro | 53 | 18 | 1 | 0 | 47 | 37 | 5 | 0 |
| d,l-leu - gly | 44 | 13 | 1 | 1 | 45 | 29 | 5 | 2 |
| d,l-leu - d,l-leu | 41 | 15 | 0 | 0 | 43 | 33 | 2 | 0 |
| l-pro - gly | 48 | 16 | 0 | 0 | 48 | 35 | 1 | 1 |
| l-pro - l-ala | 51 | 15 | 0 | 0 | 50 | 34 | 1 | 1 |
| l-pro - l-leu | 37 | 31 | 1 | 0 | 29 | 57 | 4 | 0 |

[a]Metal/peptide ratio.

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