ORIGINAL PAPER

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Molecular modeling analysis of the ease in oxidation of planar nickel–peptide complexes

Abstract Molecular mechanics and semiempirical calculations using HyperChem 5 were carried out to investigate the ease in oxidation of 1:1 nickel(II)–peptide complexes as compared to that of hydrated Ni^{2+}. The analysis shows that the amounts of energy required to cause oxidation of nickel–peptide complexes from Ni^{2+} to the Ni^{3+} state are much smaller than that required for the oxidation of the hydrated Ni^{2+} ion, giving support to the idea that planar complexes of Ni(II) with peptides and proteins may mediate nickel-induced carcinogenesis.

Keywords Cancer · DNA damage · Free radicals · Molecular modeling · Nickel–peptide complexes · Oxidation

Introduction

Nickel compounds are known to be carcinogenic to humans and animals. Although the exact mechanism of nickel-induced carcinogenicity remains unclear, it is believed that this may involve oxidation of nickel from +2 to a higher oxidation state, namely +3, by intracellular oxidants. This may occur subsequent to the binding of nickel ions with biological ligands such as peptides and proteins. This oxidation and subsequent redox cycling of nickel, presumably through Fenton-type reactions, results in the formation of reactive oxygen species that can cause oxidative damage to DNA. [1, 2] Although oxidation of nickel from +2 to a higher oxidation state is greatly disfavored, the oxidation potential of Ni^{2+} decreases markedly when it forms planar complexes with peptides and proteins (in which Ni^{2+} is bonded to deprotonated amide nitrogens) so that it may occur in the presence of biological oxidants such as hydrogen peroxide.

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The aim of the present study was to see whether results obtained from computer modeling support the observed relative ease in oxidation of planar nickel–peptide complexes as compared to that of hydrated Ni^{2+}. To this end, the structures of 1:1 planar complexes of nickel with glycyglycylglycine (glyglygly) and glycylglycylglycylglycine (glyglyglygly) in the +2 and +3 oxidation states for nickel were optimized and their heats of formation calculated. Similarly, the heats of formation of the optimized structures of hydrated nickel ion in both the +2 and +3 oxidation states were also calculated. The calculated results show that the amounts of energy required to cause the oxidation of planar nickel–peptide complexes are much smaller than that required for the oxidation of the hydrated nickel(II) ion. The stoichiometry of the main adduct formed between nickel ion and the ligand glyglygly in basic solution was established based on UV–visible spectrophotometry using the technique of continuous variation.

Experimental

Materials

Analytical grade nickel chloride 6-water ($NiCl_2$, $6H_2O$) was purchased from Ajax Chemicals, NSW, Australia. Amino acids and the peptides glyglygly and glyglyglygly were purchased from Sigma-Aldrich Pty Ltd, NSW, Australia.

Method

From 0 to 2 ml of 33.3 mM solutions of both $NiCl_2$ and glyglygly, made in milliQ (mQ) water, were mixed in varying proportions and the total volume made up to 2 ml (Table 1). The solutions were placed in sealed plastic tubes, to which were added varying amounts of 0.50 M NaOH and mQ water (as described in Table 1) so that the total was made up to 4.00 ml in each case. The

Table 1 Interaction between 33.3 mmol l⁻¹ Ni²⁺ and 33.3 mmol l⁻¹ L glyglygly: absorbance values at λ_{max} against mQ water as blank

Solution	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
ml NiCl₂	2.00	1.88	1.75	1.63	1.50	1.38	1.25	1.13	1.00	0.88	0.75	0.63	0.56	0.50	0.44	0.38	0.25	0.13	0.00
ml L	0.00	0.13	0.25	0.38	0.50	0.63	0.75	0.88	1.00	1.13	1.25	1.38	1.44	1.50	1.56	1.63	1.75	1.88	2.00
ml 0.5 M NaOH	0.00	0.04	0.08	0.13	0.17	0.21	0.25	0.29	0.33	0.38	0.41	0.46	0.48	0.50	0.52	0.54	0.58	0.63	0.67
ml mQ water	2.00	1.86	1.92	1.87	1.83	1.79	1.75	1.71	1.67	1.62	1.59	1.54	1.52	1.50	1.48	1.46	1.42	1.37	1.33
[Ni²⁺]$_{tot}$, mM	16.65	15.65	14.57	13.57	12.49	11.49	10.41	9.41	8.33	7.33	6.24	5.24	4.66	4.16	3.66	3.16	2.08	1.08	0
[L]$_{tot}$, mM	0	1.08	2.08	3.16	3.66	4.16	6.24	7.32	8.33	8.33	10.41	11.49	11.99	12.49	12.99	13.57	14.57	15.65	16.65
Abs at 458 nm[a]	0.041	0.164	0.291	0.415	0.488	0.692	0.808	0.949	1.127	0.998	0.896	0.765	0.708	0.578	0.451	0.379	0.253	0.163	0.019

[a] Wavelength at which absorption is maximum

solutions were then left standing at 22.0 °C for 24 h. The UV–visible spectrum from 190 nm to 900 nm) of solution I (Table 1) was recorded using a Cary 1A UV–visible spectrophotometer, to determine the wavelengths (λ_{max}) at which the absorbance values were maximum. A scan rate of 200 nm/min and band width of 2 nm were used. The absorbance at λ_{max} was then measured for each solution A to S using mQ water as the blank. The absorbance values (given in Table 1) were plotted against the added concentrations of Ni²⁺ and glyglygly to determine the stoichiometry of the adduct formed.

HyperChem calculations

The proposed structures for the metal–amino acid complexes were optimized and their electronic spectra generated based on molecular mechanics and semiempirical calculations using the HyperChem 5 Molecular Visualization and Simulation program. [3] Geometry optimizations based on molecular mechanics (using the MM⁺ force field) and semiempirical quantum mechanical calculations using ZINDO/1 [4] were used to find the coordinates of molecular structures that represent a potential energy minimum. For geometry optimization using both molecular mechanics and semiempirical calculations, at the final stage of refinement, the Polak–Ribiere routine with RMS gradient of 0.01 as the termination condition was used. To simulate the conditions in solution, the molecule was placed in a periodic box of TIP3P water molecules [5] followed by further cycles of geometry optimization. Molecular dynamics calculations were used to obtain a lower energy minimum by enabling molecules to cross potential barriers. [6] For the optimized structures, electronic spectra were generated using the routine ZINDO/S following a singly excited configuration interaction (CI) calculation with the semiempirical method. HyperChem performs a self-consistent field (SCF) calculation to obtain the reference electronic configuration associated with the ground state. Next, it generates a series of singly excited configurations, computes Hamiltonian matrix elements between them, and then diagonalizes the matrix to get the spectrum of the electronic states. The numbers of occupied and unoccupied orbitals set in the single point CI calculations were both set equal to ten.

Results and discussion

The plot of absorbance versus concentration showed that nickel formed mainly a 1:1 complex with glyglygly (Fig. 1). In this complex, glyglygly acts as a tetradentate ligand, being bonded to nickel via the amino nitrogen, carboxyl oxygen and two deprotonated amide nitrogens (Fig. 2). Although it is known that in acidic solutions Ni²⁺ forms a chelate ring [7] with glyglygly involving amino nitrogen and the carbonyl oxygen of the first peptide bond, in basic solutions it forms a yellow planar
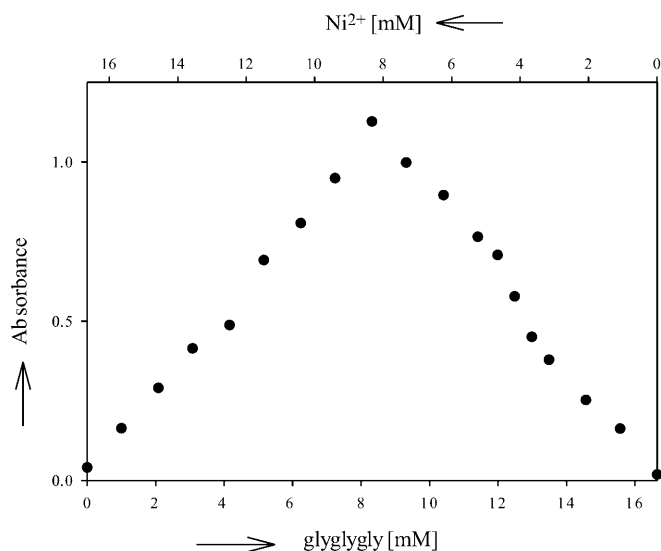
Fig. 1 Absorbance versus concentration plot applying to continuously varying mixtures of NiCl$_2$ and glyglygly
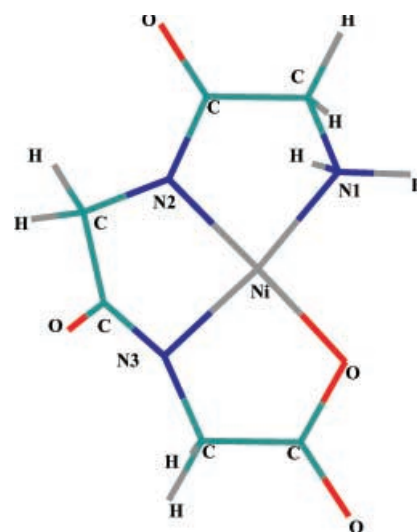
Fig. 2 Proposed structure of Ni–glyglygly in which nickel is believed to be coordinated to the amino nitrogen, carboxyl oxygen and the two amide nitrogens

complex (which is a consequence of the planarity of the peptide bond) in which the peptide nitrogens are deprotonated so that the metal ion can be coordinated to the amino nitrogen, carboxyl oxygen, one or more of the peptide nitrogens and one or more of the carbonyl oxygens.

The results of semiempirical calculations using ZINDO/1 (with no constraints applied in optimization) show that the four angles around nickel in Ni(II)(glyglygly)$^-$ are N1–Ni–N2 (87.3°), N2–Ni–N3 (88.2°), N3–Ni–O (90.3°) and O–Ni–N1 (95.2°), which together add up to 361° (see Fig. 2 for the numbering of coordi-

nated nitrogens), indicating that the geometry around the metal ion is indeed close to planar. It will be seen later that for Ni(II)(glyglyglygly), the four N–Ni–N angles around the metal ion add up to 370.2° (10° more than 360°), indicating a significant deviation from planar geometry. It is difficult to decide whether this deviation from planarity is real. If it is, it may be a consequence of the geometrical constraint imposed by the coordination of the metal ion to the amino nitrogen and three deprotonated amido nitrogens.

For the solvated Ni(II)–glyglygly ion optimized using ZINDO/1, a single-point calculation using ZINDO1/S

Table 2 Optimization of Ni(II)–glyglygly and Ni(III)–glyglygly using HyperChem 5

Ni(II)–glyglygly			Ni(III)–glyglygly		
Program used	Heat of formation (kcal mol^{-1})	Predicted spectral[a] lines in nm[b]	Program used	Heat of formation (kcal mol^{-1})	Predicted spectral lines in nm
ZINDO/S	−137,29.97	215.1 (0.121); 215.7 (0.077); 221.8 (0.031); 224.7 (0.169); 228.2 (0.089); 232.6 (0.295); 306.8 (0.001); 316.6 (0.002); 318.7 (0.001); 311.4 (0.018); 434.4 (0.007); 440.5 (0.011); 473.1 (0.009)	ZINDO/S	−136,41.32	206.6 (0.011); 209.1 (0.058); 211.2 (0.014); 213.7 (0.009); 216.6 (0.005); 221.8 (0.020); 224.1 (0.053); 229.9 (0.064); 236.6 (0.005); 239.4 (0.050); 240.0 (0.012); 243.0 (0.004); 245.3 (0.007); 250.7 (0.016); 250.7 (0.016); 252.0 (0.006); 254.8 (0.031); 260.7 (0.021); 261.6 (0.001); 264.3 (0.004); 278.5 (0.001); 297.7 (0.001); 303.3 (0.003); 310.3 (0.005); 317.8 (0.003); 331.4 (0.002); 344.4 (0.018); 353.9 (0.016); 363.2 (0.004); 378.0 (0.002); 397.7 (0.019); 415.3 (0.015); 471.4 (0.006); 490.5 (0.005); 545.8 (0.005)

[a] Observed broad bands extending from 195 to 350 nm (maximum at 290 nm) and 400 to 700 nm (maximum at 458 nm)
[b] Numbers in parentheses indicate the oscillator strength

Table 3 Optimization of $Ni(H_2O)_6^{2+}$ and $Ni(H_2O)_6^{3+}$ using Hyper-Chem 5

$Ni(H_2O)_6^{2+}$		$Ni(H_2O)_6^{3+}$	
Program used	Heat of formation (kcal mol^{-1})	Program used	Heat of formation (kcal mol^{-1})
ZINDO/S	−85,956.97	ZINDO/S	−82,987.89

shows that (in the +2 oxidation for nickel) it has a heat of formation of −13,729.97 kcal mol^{-1} (given in Table 2). The corresponding value for the +3 oxidation state for nickel is −13,641.32 kcal mol^{-1}. The results show that about 88.65 kcal mol^{-1} of energy is required to cause the oxidation of the complex from the +2 to the +3 state for nickel.

The heat of formation of the solvated of $Ni(H_2O)_6^{2+}$ obtained from single-point calculation with ZINDO1/S after its optimization with ZINDO/1 is found to be −85,956.97 kcal mol^{-1} (Table 3). The corresponding value for the solvated $Ni(H_2O)_6^{3+}$ is −82,987.89 kcal mol^{-1}, showing that about 2,969 kcal mol^{-1} of energy is required to oxidize the hydrated nickel ion from the +2 to +3 state, a value which is much greater (about 33 times as large) than that required for the oxidation of the triglycine complex (considered earlier). The experimentally observed values for the heats of formation of Ni^{2+} and Ni^{3+} in the gaseous state are 280.2 and 700.3 kcal mol^{-1}, respectively, [8] showing that about 420 kcal mol^{-1} of

energy is required to oxidize Ni^{2+} to Ni^{3+} in the gaseous state. The corresponding values calculated by ZINDO/S are 674.9 and 1416.0 kcal mol^{-1}, respectively, showing that as per the calculation about 741 kcal mol^{-1} of energy is required to oxidize Ni^{2+} to Ni^{3+} in the gaseous state. We can deduce from the results that there is a scale factor about 2:1 between calculated and observed values. Whether the same scale factor exactly applies to the hydrated metal ions and the complexes in solution or not, what is clear is that it would be much harder to oxidize $Ni(H_2O)_6^{2+}$ to $Ni(H_2O)_6^{3+}$ than to oxidize Ni(glyglygly) complex from Ni(II) to Ni(III) state.

The calculated Ni–O, Ni–N1, Ni–N2 and Ni–N3 bond distances in Ni(II)(glyglygly)$^-$ are found to be 186, 201, 190 and 185 pm, respectively. The corresponding values for Ni(III)(glyglygly) are 182, 200, 185 and 199 pm, respectively. The results also show that (as expected) the Ni–O bond distance is slightly shorter than the Ni–N bond distance and that for the Ni(III) state the bond distances are generally shorter than the corresponding values in the Ni(II) state. It is also found that in the Ni(III) state, unlike that in the Ni(II) state, the coordination geometry around nickel in the Ni(glyglygly) complex deviates significantly from planar (the four angles add up to about 370°).

It was assumed that nickel also formed a 1:1 complex with glyglyglygly. It was further assumed that in basic solution nickel ion was coordinated to glyglyglygly through the amino nitrogen and three deprotonated amide nitrogens similar to that found in the structure of a

Table 4 Optimization of Ni(II)–glyglyglygly and Ni(III)–glyglyglygly using HyperChem 5

Ni(II)–glyglyglygly			Ni(III)–glyglyglygly		
Program used	Heat of formation (kcal mol^{-1})	Predicted spectral[a] lines in nm[b]	Program used	Heat of formation (kcal mol^{-1})	Predicted spectral lines in nm
ZINDO/S	−5,149.78	206.9 (0.062); 207.6 (0.017); 208.9 (0.009); 214.1 (0.045); 216.2 (0.068); 216.9 (0.035); 229.9 (0.009); 232.0 (0.029); 251.0 (0.050); 260.1 (0.015); 271.6 (0.026); 290.8 (0.036); 311.0 (0.021); 315.0 (0.023); 316.2 (0.003); 319.3 (0.002); 331.0 (0.040); 332.8 (0.035); 361.6 (0.015); 484.8 (0.029); 643.7 (0.020); 692.8 (0.001)	ZINDO/S	−5,313.72	232.4 (0.007); 233.3 (0.001); 233.5 (0.001); 237.5 (0.001); 252.9 (0.007); 267.6 (0.001); 268.5 (0.001); 268.8 (0.001); 292.0 (0.001); 297.4 (0.001); 300.6 (0.001); 330.7 (0.001); 341.1 (0.005); 344.0 (0.001); 345.5 (0.001); 352.6 (0.001); 386.2 (0.010); 402.7 (0.001); 404.5 (0.001); 412.8 (0.001); 413.7 (0.019); 419.5 (0.001); 432.3 (0.001); 442.0 (0.005); 443.5 (0.003); 446.4 (0.001); 461.3 (0.001); 469.5 (0.001); 476.5 (0.001); 481.5 (0.003); 496.3 (0.003); 509.8 (0.014); 509.8 (0.014); 519.3 (0.006); 547.2 (0.005); 551.4 (0.001); 580.2 (0.001); 601.6 (0.006); 611.9 (0.001); 637.5 (0.001); 649.5 (0.001); 675.2 (0.001); 699.4 (0.002); 730.0 (0.003); 755.2 (0.042); 759.5 (0.001)

[a] Observed broad bands extending from 195 to 300 nm (maximum at 280 nm) and 300 to 550 nm (maximum at 415 nm)
[b] Numbers in parentheses indicate the oscillator strength
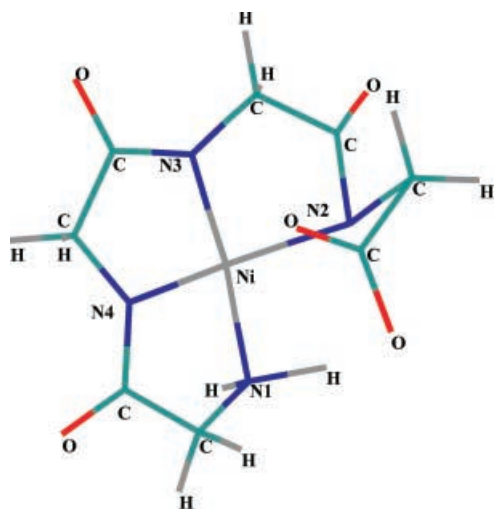
Fig. 3 Proposed structure of Ni–glyglyglygly in nickel is believed to be coordinated to the amino nitrogen and the three amide nitrogens

crystal of the complex of Ni^{2+} with glyglyglygly. [9] When this structure was optimized using ZINDO/1, a single-point calculation using ZINDO1/S showed that the solvated complex ion had a heat of formation of –5149.68 kcal mol^{-1} (Table 4) in the +2 oxidation state of nickel. As before, the proposed structure was also optimized for the +3 oxidation state of nickel. The heat of formation for the Ni(III)–glyglyglygly was –5,313.72 kcal mol^{-1}, showing that the Ni–glyglyglygly complex is more stable in the Ni(III) state than in the Ni(II) state. It is clear from the results that nickel ion in the complex can easily be oxidized from the +2 to +3 state. The calculated Ni–N1, Ni–N2, Ni–N3 and Ni–N4 bond distances for Ni(glyglyglygly) are found to be 209, 216, 201 and 198 pm, respectively. Once again, the Ni–ligand bond distances are found to be shorter in the Ni(III) state than in the Ni(II) state. The four N–Ni–N angles, N1–Ni–N2 (88.6°), N2–Ni–N3 (87.1°), N3–Ni–N4 (83.5°) and N4–Ni–N1 (111.0°) (see Fig. 3 for the numbering of coordinated nitrogens), together add up to 370.2° indicating (as stated earlier) a significant deviation from planar geometry around nickel in the Ni(II)(glyglyglygly) complex ion.

The predicted UV–visible spectral lines for Ni–glyglygly and Ni–glyglyglygly in +2 and +3 oxidation states of nickel are given in Tables 2 and 4, respectively. It is found that, for the Ni–glyglyglygly complex, the predicted spectral lines in the Ni(III) state agree more closely with the observed λ_{max} values than do those in the Ni(II) state, which can be explained in terms of oxidation of the complexes from Ni(II) to Ni(III). More importantly, it can be deduced that, when the complexes are prepared in the presence of air, these may exist in the higher oxidation state for nickel. Indeed Burrows et al. [2] have found that planar nickel–peptide complexes are oxidized by O_2 alone or by O_2 in the presence of other oxidants.

The relative ease in oxidation explains why Ni(II)–glyglyglygly is able to disproportionate hydrogen peroxide at pH≥8 and to catalyze the oxidation of guanine residues in 2'-deoxyguanosine, calf thymus DNA and calf thymus nucleohistone by H_2O_2 at physiological pH, [2, 10] and thus supports the contention that planar complexes of nickel with peptides and proteins may mediate nickel-induced carcinogenesis by causing oxidative damage to DNA.

Conclusion

Molecular modeling calculations support the observation that planar nickel–peptide complexes are more easily oxidized than the $Ni(H_2O)_6^{2+}$ ion, giving credence to the idea that planar complexes of nickel with peptides and proteins may be involved in the molecular mechanism of nickel-induced carcinogenesis.

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