Nickel Peroxide as a Glycine-selective Chemical Model of Peptidylglycine α-Amidating Monooxygenase

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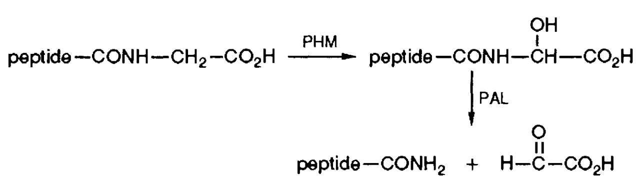
In a process, which is analogous to that catalysed by peptidylglycine α-amidating monooxygenase, nickel peroxide cleaves *N*-benzoylamino acid methyl esters to give benzamide, with a selectivity for reaction of the glycine derivative.

The bioactivation of many peptide hormones and neuropeptides involves oxidative cleavage of carboxy-terminal glycine-extended precursors (Scheme 1).[1,2] The process is catalysed by the enzyme peptidylglycine α-amidating monooxygenase (PAM), which comprises two subunits.[2] One of these, peptidylglycine α-hydroxylating monooxygenase (PHM), requires copper ions, ascorbate and molecular oxygen, and facilitates α-hydroxylation of glycine residues. The other, peptidylhydroxyglycine α-amidating lyase (PAL), cleaves the intermediate α-hydroxyglycine derivatives.

A range of chemical models of PAM has been developed[3] and used to elucidate various features of the enzyme-catalysed reactions. We now report that nickel peroxide[4] is an alternative model for PAM, with the particular feature that it shows selectivity for reaction of glycine residues akin to that displayed by the enzyme.[5] To the best of our knowledge, the basis of this substrate selectivity by PAM has not previously been examined.

When the glycine derivative **1a**[6] (0.05 mol dm^{-3} in benzene) was treated with 2.6 equiv. of nickel peroxide, at reflux under nitrogen for 1 h, filtration of the heterogeneous reaction mixture to remove nickel salts, followed by chromatography on silica, gave benzamide in 39% yield and recovered starting material in 51% yield. By comparison, the derivatives of alanine **1b**[6] and valine **1c**[6] were less reactive. The reaction of the alanine derivative **1b** under the same conditions as those used for the reaction of the glycine derivative **1a** gave benzamide in 13% yield, the dehydroalanine derivative **5**[7] in 9% yield, and 69% recovered starting material, while similar treatment of the valine derivative **1c** gave only a trace of benzamide and 93% recovered starting material. This qualitative observation of the selective reaction of the glycine derivative **1a** was confirmed in competitive experiments using mixtures of the alanine derivative **1b** with either the glycine derivative **1a** or the valine derivative **1c**, and the results are presented in Table 1.



Scheme 1

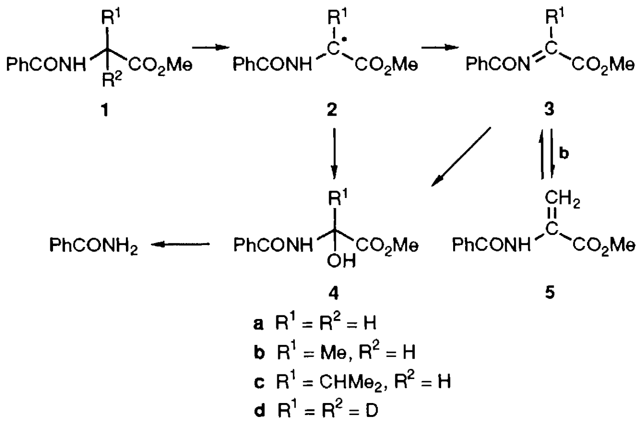
The reactions of the amino acid derivatives **1a–c** to give benzamide may be rationalized as shown in Scheme 2. Following their complexation to nickel, hydrogen-atom transfer from the substrates affords the corresponding α-carbon-centred radicals **2a–c**. Those radicals react to give the corresponding α-hydroxy amino acid derivatives **4a–c**, either directly or indirectly *via* the respective *N*-acylimines **3a–c**. Subsequent hydrolysis of the α-hydroxy amino acid derivatives **4a–c** affords benzamide. Formation of the dehydro amino acid derivative **5** in the reaction of the alanine derivative **1b** may be attributed to tautomerization of the *N*-acylimine **3b**. In a separate experiment, the dehydroalanine derivative **5** gave benzamide on treatment with nickel peroxide, consistent with the proposal that it is an intermediate in the reaction of the alanine derivative **1b**.

In a competitive experiment using 0.025 mol dm^{-3} solutions of each substrate, the glycine derivative **1a** reacted 2.9 ± 0.5

Table 1 Relative rates of reaction of the amino acid derivatives **1a–c** with nickel peroxide[a]

	Relative reaction rates		
Substrate	At 80 °C, with 0.025 mol dm^{-3} of each substrate	At 80 °C, with 0.0025 mol dm^{-3} of each substrate	At 20 °C, with 0.025 mol dm^{-3} of each substrate
Glycine **1a**	10.0 ± 2.5	4.5 ± 0.4	4.0 ± 0.5
Alanine **1b**	1.0[b]	1.0[b]	1.0[b]
Valine **1c**	0.14 ± 0.03	0.43 ± 0.03	—[c]

[a] Reaction in benzene with *N-tert*-butylbenzamide as internal standard. [b] Assigned as unity in each experiment. [c] Absolute reaction rate too slow for relative rate to be determined.



Scheme 2

times faster than the deuteriated analogue **1d**,[6] indicating that α-hydrogen transfer from the amino acid derivatives **1a–c** is a rate-determining step in their reactions with nickel peroxide. It is likely that the ease of complexation of the amino acid derivative **1a–c** with the nickel also affects the reactivity of these species, otherwise it is difficult to rationalize the effects of substrate concentration and reaction temperature on their relative rates of reaction. On this basis, the preferential reaction of the glycine derivative **1a** can be attributed to its selective binding to the nickel surface and subsequent reaction to give the glycyl radical **2a**.

The oxidative cleavage of the amino acid derivatives **1a–c** by nickel peroxide is similar to the process catalysed by PAM, and the selectivity of nickel peroxide for reaction of the glycine derivative **1a** is analogous to the substrate selectivity displayed by the enzyme. Those factors that result in the preferential reaction of glycine derivatives in the present work and earlier studies, that is the preferential complexation of glycine derivatives by metal ions[8] and the relative ease of formation of glycyl radicals,[6] may also contribute to the substrate selectivity shown by PAM. At the least it seems likely that the natural substrates of PAM are synthesized with glycine at the carboxy-terminus because that residue is so easily removed by oxidation.

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