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ORIGINAL PAPER

The possible production of natural flavours by amino acid degradation

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Abstract This work describes the degradation of phenylalanine and tryptophane catalysed by their complexes with Fe(II), Co(II), and Cu(II). The influences of the central atom and of the reaction conditions on the degradation of the amino acids were observed. The necessary condition of the degradation is the possibility of a redox reaction on the central atom between M(II) and M(III). Moreover, the coordination sphere of the central cation of the transition metal must not be sterically shielded. The necessary conditions are fulfilled only in the Fe(II) complexes. The degradation is strictly anaerobic because due to the influence of oxygen, an irreversible oxidation of Fe(II) to Fe(III) proceeds. This reaction results in 5-hydroxy-1*H*-indol instead of the mixture of the degradation products, such as benzaldehyde, phenylacetaldehyde, and phenylacetic acid. The influence of the temperature on the catabolism is very important because the reaction accelerates with temperature increase. The phenylalanine anion acts as a reducing agent, and Fe(II) is spontaneously reduced to Fe(0).

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Introduction

The degradation of amino acids is an important process by which various components of natural flavour complexes in foodstuffs are produced. The most important amino acid degradation catalysts are nonheme metalloproteins. They are oxidative catalysts in which the centre of the enzyme contains the coordinated cation of a transition metal. Metalloproteins are able to exist in more than one oxidation degree and because of this they are useful catalysts for the biological processes that demand electron transport [1].

From the preparative point of view of the possible production of many interesting products, the simulation of the metalloprotein reaction centers, where water is used as a reaction medium, is extremely important. In the case of the implementation of these reaction types in industrial production, the problems with energetic demands of production and problems with the waste disposal of the reaction solvents will be eliminated. Because of this, many experiments on the degradation of amino acids using various reactants were realised. Ameta et al. [2] realised the oxidative decarboxylation of amino acids by potassium permanganate.

Itoh et al. [3] reported the catalytic oxidative decarboxylation reaction of amino acids with coenzyme PQQ (Methoxantin; Fig. 1). The reaction is followed by the pH change from 7.21 to 8.58. The products of degradation are shown in Table 1. The yields and also the individual products are very interesting. Consequently, it is possible to prepare natural phenylacetaldehyde from natural phenylalanine. Phenylacetaldehyde is indispensable for the food

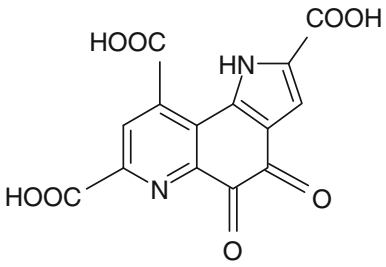


Fig. 1 Coenzyme PQQ (methoxantin) [3]

industry as a flavour compound, and it is largely deficient on the market. Another product of this degradation is phenylacetic acid, the esters of which are necessary for the production of honey flavours.

Amino acids are also interesting ligands for the preparation of transition metal complexes. These are common objects for the study of the structure and especially redox attributes.

For the production of flavour compounds, the biotransformation of amino acids, mainly phenylalanine, by the cultures of microorganisms or moulds, and by the crude extracts of enzymes is important as well. The biotransformation has previously been shown to produce variable amounts of benzaldehyde, which is the next most important flavour compound after vanillin and is used in cherry and other fruit aromas [4–6].

Researchers have found a "natural" benzaldehyde production to be possible through these biotransformations as an alternative to the extraction from vegetable sources. In these studies, benzaldehyde was a product of complex metabolic pathways that were more or less elucidated. Moreover, different enzymes were involved in these biotransformations, which led to different intermediates of reactions [5].

Okrasa et al. [5] studied a novel bi-enzymatic transformation of D-phenylalanine into benzaldehyde using D-amino acid oxidase from *T. variabilis* and peroxidase from *C. cinereus*. Although they used D-amino acid as a starting material, they supposed that their results most probably could be transferred to the systems based on L-phenylalanine and the less available enzymes L-amino acid oxidase or L-aminotransferase [5, 7]. They established several mechanisms of D-phenylalanine transformation into benzaldehyde. On the basis of their results, they proposed the mechanistic pathway: a non-oxidative decarboxylation of phenylpyruvate (or its enol form) into phenylacetate, and then an oxidative deformylation of the enol form of phenylacetaldehyde leading to benzaldehyde and formic acid, most probably through a dioxetane ring [5].

The metabolism of L-phenylalanine has been studied in several white rot fungi [8, 9]. Among the potential aroma producers, white rot basidiomycetes were probably the most versatile microorganisms. These fungi were able to produce a wide variety of volatile aryl metabolites of commercial interest, such as vanillin, benzaldehyde (bitter almond aroma), and cinnamaldehyde [10].

Lapadatescu et al. [11] studied aryl metabolite biosynthesis in the white rot fungus *Bjerkandera adusta* by using ^{14}C- and ^{13}C-labelled L-phenylalanine as precursors. The metabolite formation required de novo protein biosynthesis. The results have shown that L-phenylalanine was deaminated to *trans*-cinnamic acid by a phenylalanine ammonia lyase, and *trans*-cinnamic acid was in turn converted to aromatic acids (phenylpyruvic, phenylacetic, mandelic, and benzoylformic acids); benzaldehyde was a metabolic intermediate. These acids were transformed into benzaldehyde, benzyl alcohol, and benzoic acid [11].

The metabolism of phenylalanine is extremely complicated and can have an atypic course in many cases. One of the reasons for starting this investigation was the possibility that the defect in the metabolic hydroxylation of phenylalanine to tyrosine, which is known to be the primary fault in phenylketonuria, might be associated with a similarly abnormal hydroxylation of tryptophane.

Table 1 Oxidative decarboxylation of the α-amino acids with coenzyme PQQ [3]

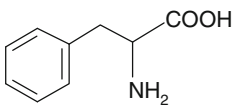
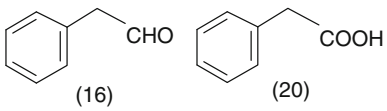
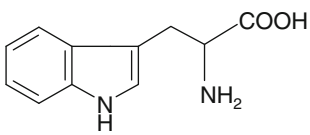
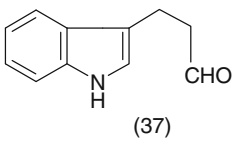
Amino acids	Initial pH	Final pH	Product [yields (%)]
	7.21	8.28	
	7.20	8.58	

Table 2 Products of the reaction of phenylalanine in the coordination sphere of Fe(II) in the complex with phenylalanine at room temperature under 24 h stirring in an inert atmosphere isolated from the reaction mixture before and after acidification

	Compound	Retention time (min)	Area (%)
Before acidification	Benzaldehyde	3.3	6.9
	Phenylacetaldehyde	4.4	38.9
After acidification	Benzaldehyde	3.2	8.3
	Phenylacetaldehyde	4.4	25.1
	Phenylacetic acid	7.5	40.8

Table 3 Products of the reaction of tryptophane in the coordination sphere of Fe(II) in the complex with tryptophane at room temperature under 24 h stirring in an inert atmosphere isolated from the reaction mixture before and after acidification

	Compound	Retention time (min)	Area (%)
Before acidification	1*H*-Indole-3-carboxaldehyde	14.0	31.5
After acidification	Indole-3-acetaldehyde	12.8	11.3
	1*H*-Indole-3-carboxaldehyde	14.0	7.7

5-Hydroxyindol is an atypic metabolite in this case [12]. Although it has been suggested by Harley-Mason that phenylalanine could serve as a precursor of 5-hydroxyindole compounds, the results of the experiments with ^{14}C-labelled amino acids make this unlikely [13].

Results and discussion

Tables 2, 3, 4, and 5 contain the results of the GC/MS analysis of the amino acid degradation products that were catalysed by transition metal complexes.

This work describes a procedure of the possible flavour compound preparation on the basis of natural precursors. It is based on the reaction simulation, which uses a catalyst as the active center of the metalloprotein. Most metalloproteins contain the coordinated cation of a transition metal. We have chosen Cu(II), Co(II), and Fe(II) because these cations belong to the most active ones in metalloproteins. For the case of simplicity, we used amino acid anions as ligands which were further transformed. We used the tryptophane and phenylalanine amino acids because of the eventual creation of interesting degradation products, such as benzaldehyde, phenylacetaldehyde, phenylacetic acid, and indol or its derivatives. We used the GC/MS method for product verification. Because of the supposition that some products of the amino acid degradation should be organic acids and because the reaction runs in alkaline medium, we used a two-stage method for isolation of the reaction products. Alkali indifferent substances were isolated by direct extraction into diethylether in the first stage. In the second stage, the rest of the reaction products was isolated after acidification to pH 2–3 by extraction into diethylether. The results of all product isolations are presented in Tables 2, 3, 4, and 5.

Table 4 Products of the reaction of phenylalanine in the coordination sphere of Co(II) in the complex with phenylalanine at room temperature under 24 h stirring in an inert atmosphere isolated from the reaction mixture

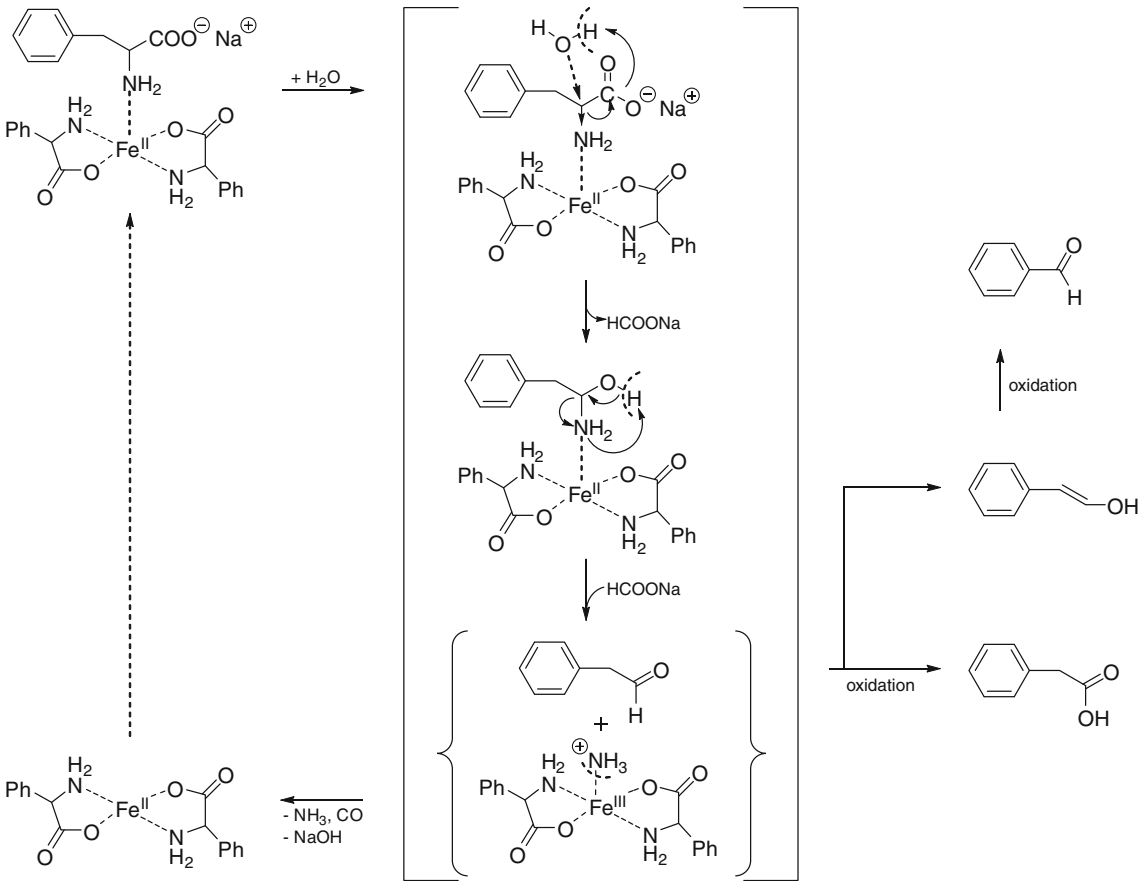
Compound	Retention time (min)	Area (%)
Benzaldehyde	3.2	4.5

Table 5 Products of the reaction of phenylalanine in the coordination sphere of Co(II) in the complex with phenylalanine at 52 and 64 °C under stirring in air isolated from the reaction mixture

Compound	Retention time (min)	Area (%)
5-Hydroxy-1*H*-indol	30.1	100.0

Comparison of yields of the reactions catalysed by the complexes containing various central atoms is very interesting. It was observed that the reaction catalysed by the Fe(II) complex has the most rapid progress, whereas the reaction catalysed by the Cu(II) complex has the slowest progress (practically zero), which is suggested also by the reaction kinetics. In the case of the Fe(II) complex, the products of the reaction have been isolated in excellent yield unlike the Cu(II) complex, where no product of the reactant transformation has been isolated. After the evaluation of the reaction products and yields, we proposed the mechanism of the destructive amino acid transformation catalysed by transition metal complexes (Scheme 1).

The mechanism is based on the redox reaction between water and the amino acid anion. It is compatible with the



Scheme 1

mechanism suggested by Okrasa et al. [5]. The original supposition of Lapadatescu et al. [11] that the reaction in the in vivo experiments is connected with the eliminated amino acid deamination leading to the formation of the substituted propenoic acid was not confirmed.

The presented facts are the consequence of kinetically controlled reactions. The reaction was dramatically changed if the reaction was thermodynamically controlled. The influence of increased temperature in the case of the Fe(II) complex led to the spontaneous reduction of Fe(II) to metallic iron, which precipitated in the form of a metallic mirror.

DSC analysis indicates that there is a substance with an atypic degradation in two stages. The degradation is more penetrative in the presence of oxygen than in an inert atmosphere. The first stage of degradation has the maximum under inert atmosphere at a temperature of about 225 °C, and the second stage has the maximum at about 380 °C (Fig. 2). The analogous temperatures in the presence of oxygen are moved to lower values (Fig. 3). From the loss of weight, which is comparable in both cases, it is possible to deduce that a release of a fixed CO_2 might be observed.

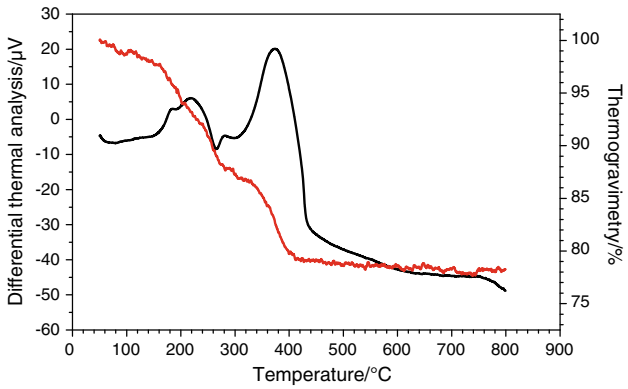


Fig. 2 Thermogravimetry (*TG*) and differential thermal analysis (*DTA*) of the reaction mixture containing reduced metal under inert atmosphere

On the basis of the experimental results, the negative influence of oxygen on the reaction was detected. We have observed the oxidation of Fe(II) to Fe(III) accompanied by a colour change of the reaction mixture and with the inactivation of the oxidative catalyst. This reaction resulted in 5-hydroxy-1*H*-indol instead of the mixture of degradation products such as benzaldehyde, phenylacetaldehyde, and phenylacetic acid. It is very interesting that the

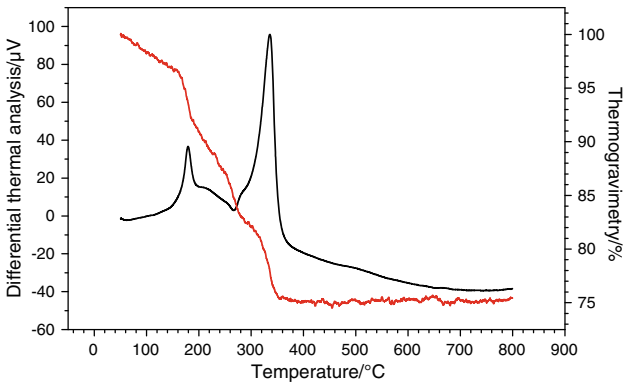


Fig. 3 Thermogravimetry (*TG*) and differential thermal analysis (*DTA*) of the reaction mixture containing reduced metal in the presence of oxygen

phenylalanine metabolism in the case of some genetic defects (such as phenylketonuria and Down syndrome) is comparable with the process of metabolism in our suggested mechanism [12–15].

From all our experiments, we can deduce that the most active catalyst of the amino acid degradation is the Fe(II) complex. The reason is that the Fe(II) complexes have a square-planar configuration of the coordination sphere unlike the Co(II) complexes with a tetrahedral coordination sphere. It implies that in the case of the Fe(II) complexes, the axial positions are not shielded and so a charge transfer starts between the central atom and other donor group. In the case of the Co(II) complex, mainly in the coordination with large ligands, other positions around the central atom are strongly sterically shielded. For successful degradation in the coordination sphere of the complex, the ability of a redox reaction between M(II) and M(III) of the central atom is necessary. Strongly electron-deficient groups originate in another incoming ligand on the entering of a donor group into the coordination sphere of the metal cation. These groups have a tendency to attract electrons from the metal cation while the metal oxidation degree increases (see Scheme 1). After the reactions between ligand and water, the reverse reduction of the metal cation to the original oxidation degree proceeds. This idea is supported by the finding that in the case of the Cu(II) complex, the reaction in the coordination sphere of the complex under any conditions does not run because under any described conditions, the change from Cu(II) to Cu(III) is not possible.

Experimental

Materials

Both amino acids, phenylalanine, and tryptophane, were purchased from Merck. Iron(II) sulfate heptahydrate, cobalt(II) sulfate heptahydrate, and copper(II) sulfate pentahydrate were purchased from Lachema. Sodium hydroxide, diethylether, hydrochloric acid, and anhydrous sodium sulfate were purchased from Microchem.

Apparatus

Products were analysed by GC/MS and TG/DTA methods. Gas chromatography (GC) was carried out using an Agilent Technologies 6890 gas chromatograph equipped with an Agilent Technologies 5973 inert mass selective spectrometer and with chromatographic column model no. J&W 122-503 E DB-5, 30 m × 0.25 mm × 0.5 μm. The records of thermogravimetry (TG) and differential thermal analysis (DTA) were performed on the DTG-60 (Shimadzu, Japan) with a mass range TG ± 500 mg, apparatus sensitivity 0.001 mg, and range of DTA measurement ±1,000 μV.

Synthesis

Transition metal complexes with phenylalanine and tryptophan were synthesised by a modification of the reaction described in Ref. [14]. Accordingly, 1 mmol aqueous solution of the metal sulfate hydrate at pH 5.0 was added to an aqueous solution of the sodium salt of amino acids containing 2 mmol of the ligand (molar proportion metal:ligand of 1:2) at pH 9.0.

These reactions were carried out:

1. at room temperature under stirring for 24 h in an inert atmosphere;
2. at 52 and 64 °C under stirring in air;
3. at 70 °C under heating in an inert atmosphere.

In all experiments, a mixture of 10 mmol amino acid with 10.5 cm^3 1 M solution of NaOH and 10 cm^3 distilled water was added to the prepared complexes. After adding the mixture, greyish-green Fe(II) solution was spontaneously oxidised to Fe(III) (brick red solution) in the second experiment, whereas in the third experiment it was spontaneously reduced to Fe(0) (black precipitate). After finishing the reaction, the mixture was extracted by 1 × 100 cm^3 diethylether and dried by anhydrous sodium sulfate. In the first experiment, the water part of the first extraction was acidified by 10% HCl to pH 2–3, extracted by 1 × 100 cm^3 diethylether, and dried by anhydrous sodium sulfate. The ethereal solutions of the extracts in all experiments were concentrated to 5 cm^3 and analysed by GC/MS.

In the third experiment the reduced iron was filtered at usual laboratory conditions. Consequently, the spontaneous oxidation of iron to the mixture of oxides and carbonates was observed. The filtrate was extracted by 2 × 50 cm^3

toluene and methanol at the reflux after drying. The product was dried at the normal temperature and analysed by TG/DTA methods.

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