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CHLORAMINES AS INTERMEDIATES OF OXIDATION REACTION OF AMINO ACIDS BY MYELOPEROXIDASE

J. M. ZGLICZYŃSKI, T. STELMASZYŃSKA, J. DOMAŃSKI AND W. OSTROWSKI

Interfaculty Department of Physiological Chemistry, Medical Academy, Kraków, Kopernika 7 (Poland)

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SUMMARY

The mechanism of the deamination–decarboxylation of amino acids by myeloperoxidase is proposed. In the presence of hydrogen peroxide and chloride, myeloperoxidase catalyses the formation of HOCl which reacts with amino acids giving chloramines. The chloramines of amino acids as unstable compounds are spontaneously decomposed to NH_3, CO_2, Cl^- and corresponding aldehydes.

In previous papers[1,2] it has been reported that amino acids were deaminated and decarboxylated with a mixture of H_2O_2 and Cl^- in the presence of myeloperoxidase. Taurine was found to be a competitive inhibitor of this process.

In this report the mechanism of the above-mentioned reaction is proposed: Myeloperoxidase catalyses the formation of HOCl from Cl^- and H_2O_2; the HOCl reacts with the amino acids forming chloramines. The amino acids chloramines are unstable and decompose to NH_3, CO_2, Cl^- and corresponding aldehyde, whereas taurine chloramine is stable under reaction conditions.

The mechanism of the reaction of deamination and decarboxylation of amino acids has been studied by use of myeloperoxidase which was isolated from human leukemic granulocytes[1]. The coefficient $R_Z = A_{430}/A_{280}$ of the enzyme was 0.7. The substrates used were L-amino acids (Hoffmann – La Roche, Switzerland) and hydrogen peroxide (Electrochemical Establishments, Ząbkowice, Poland).

During incubation of alanine with myeloperoxidase at pH 4.5 in the presence of H_2O_2 and NaCl, a product with absorption at 254 nm was observed, as illustrated in Fig. 1. In the first minutes of the reaction, absorption increased and during the course of further incubation gradually decreased (Fig. 2). The increase of absorption at 254 nm was not connected with formation of the enzyme–substrate complex because substances with this spectrum easily dialyzed through a cellophane bag. Simultaneously with changes of the spectrum, content of Cl^- in the reaction medium changed. When absorbance at 254 nm and Cl^- by the argentometric method were determined during incubation of the reaction mixture, it was found (see Fig. 2) that the rise in absorption was accompanied by a drop in Cl^- content, which increased

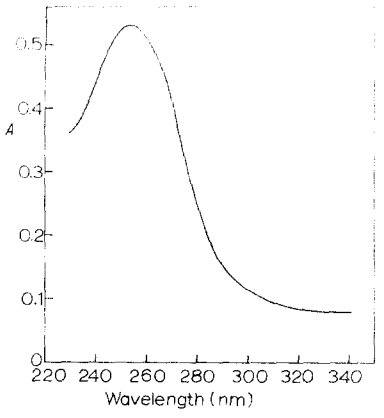

Fig. 1. Absorption spectrum of intermediate product formed during the peroxidatic deamination–decarboxylation of alanine. Sample composition: 20 μmoles of alanine, 10 μmoles of H_2O_2, 90 μmoles of NaCl, 45 μg of myeloperoxidase in 50 mM sodium acetate buffer (pH 4.5). Total volume, 3 ml; temp., 20°. Time of incubation, 6 min.

again when absorption decreased. This process was accompanied by regular increase in the content of acetaldehyde measured on the basis of absorbance of its 2,4-dinitrophenylhydrazone at 510 nm. As shown in a previous study[1], acetaldehyde is produced as a result of peroxidatic deamination and decarboxylation of alanine.

Since the reaction of peroxidatic deamination and decarboxylation fails to take place in the absence of Cl^- (see ref. 1), and Cl^- is used up in the course of the reaction for production of substances with maximum absorption at 254 nm, it has

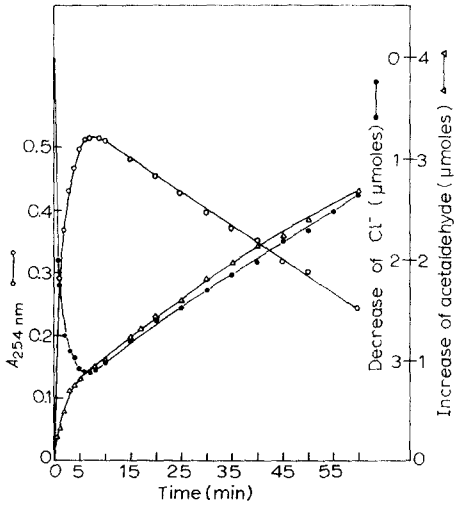

Fig. 2. Variations of absorbance at 254 nm, Cl^- and acetaldehyde amount during the peroxidatic deamination–decarboxylation of alanine. Composition of sample as in Fig. 1. In order to determine acetaldehyde, 0.2 ml 40 mM KI was added to 0.5 ml of the sample. Then liberated I_2 was titrated with 8 mM $Na_2S_2O_3$, and 0.5 ml of 0.2% 2,4-dinitrophenylhydrazine in 2 M HCl was added. After 10 min the sample was extracted with 2 ml of CCl_4. 4.5 ml of 3% KOH in methyl alcohol were added to 0.5 ml of CCl_4 solution of acetaldehyde 2,4-dinitrophenylhydrazone, and exactly after 2 min, the absorbance at 510 nm was measured.

been postulated that Cl^- is a substrate for myeloperoxidase, and is oxidized in the presence of H_2O_2 to the most probable form, *i.e.* HOCl.

The reaction of HOCl with amino compounds leading to the respective chloramines has long been known[3,4]. Some authors have shown[3,5] that chloramines produced during the reaction of HOCl with various amines are characterized by a spectrum with maximum absorption in the range 240–270 nm. It has been suggested therefore that the intermediate product formed during peroxidatic deamination decarboxylation of amino acids is a chloramine of the respective amino acid. In order to prove this concept, alanine chloramine was synthesized by treating alanine with NaOCl. At 20° temperature, 20 μmoles of alanine were mixed with 10 μmoles of NaOCl in a final volume of 2 ml and adjusted to pH 4.5 by addition of 1 ml of 150 mM sodium acetate buffer solution. Immediately after mixing the solutions, chloramine characterized by a spectrum with maximum at 254 nm was produced, as shown spectrophotometrically. In accordance with the known properties of this chloramine[4], it was unstable, showing a decrease in absorption at 254 nm with time. As can be seen in Fig. 3, the spectrophotometrically observed decomposition of alanine chloramine was accompanied by accumulation of acetaldehyde and Cl^-.

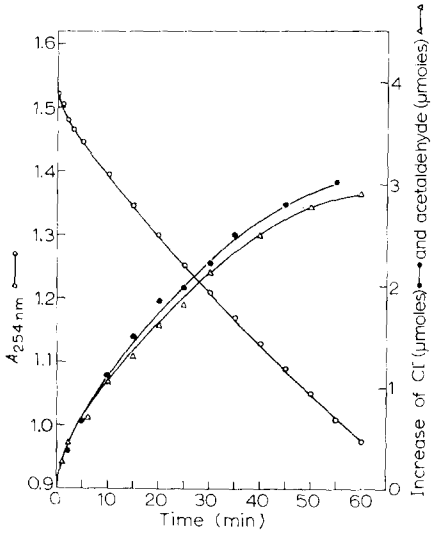

Fig. 3. Decomposition of alanine chloramine obtained by the reaction of alanine with NaOCl. Reaction mixture: 11 μmoles of NaOCl + 20 μmoles of alanine adjusted to pH 4.5 with sodium acetate buffer, 50 mM in final concentration. Total volume, 3 ml; temp., 20°. (Stock solution of NaOCl was obtained by titration of Cl_2 solution in water with 0.4 M NaOH. The concentration of NaOCl was determined from the 295 nm mmolar extinction coefficient ε_{mM} 0.35.)

The velocities of decomposition of synthetic and enzymatically produced chloramine, as measured on the basis of quantity of aldehyde formed and chloride liberated, were similar (see Figs. 2 and 3). Both chloramines were characterized by identical absorption in ultra violet light, capacity for decomposition, and identical decomposition products. In addition, synthetic chloramine as well as chloramine produced in the enzymatic reaction oxidized iodide with liberation of free iodine and recovery of alanine. In the presence of cysteine or reduced glutathione both chlor-

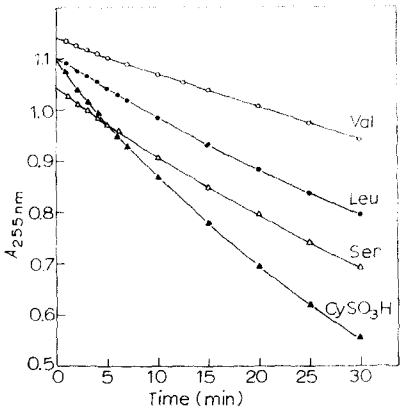

Fig. 4. Decomposition of leucine, valine, serine and cysteic acid chloramines. Reaction mixture: 9 μmoles of NaOCl + 20 μmoles of amino acid adjusted to pH 4.5 with sodium acetate buffer: 50 mM in final concentration. Total volume, 3 ml; temp., 20°.

amines decomposed to alanine. The chloramines did not oxidize bromide at pH's above 6; this made it possible to distinguish them from hypochlorite.

In a previous study it was shown that the reaction of peroxidatic deamination-decarboxylation is common for amino acids[1]. Experiments with leucine, valine, serine and cysteic acid showed production of chloramines from these amino acids. Chloramines produced in the peroxidatic reaction used up Cl^- during their production and exhibited characteristic absorption in ultra violet light, (λ_{max}: leucine, 253 nm; valine, 255 nm; serine, 254 nm; cysteic acid, 255 nm). Velocity of decomposition of the studied chloramines varied, depending on the amino acid from which they were produced (see Fig. 4). The decomposition products were the respective aldehydes, NH_3, CO_2 (see ref. 1) and Cl^-. The chloramines of the aforementioned amino acids, enzymatically as well as synthetically obtained (by the method described for alanine), were identical and exhibited all the above-described properties of alanine chloramine. The results presented above demonstrated that the peroxidatic degradation of amino acids previously described[1] ran through an intermediate stage of unstable amino acid chloramines which decomposed spontaneously to NH_3, CO_2, Cl^- and the respective aldehydes. The sequence of the reactions illustrating this process is as follows:

$$H_2O_2 + Cl^- + H^+ \xrightarrow{\text{myeloperoxidase}} HOCl + H_2O$$

$$HOCl + \underset{\displaystyle COOH}{\overset{\displaystyle CH_3}{\overset{|}{\underset{|}{CH}}}}-NH_3^+ \rightarrow H^+ + H_2O + \underset{\displaystyle COOH}{\overset{\displaystyle CH_3}{\overset{|}{\underset{|}{CH}}}}-NH-Cl$$

$$\underset{\displaystyle COOH}{\overset{\displaystyle CH_3}{\overset{|}{\underset{|}{CH}}}}-NH-Cl + H_2O \rightarrow CH_3CHO + NH_4^+ + CO_2 + Cl^-$$

Experiments were performed also with taurine as competitive inhibitor of peroxidatic deamination and decarboxylation of amino acids. It was found that during the reaction with myeloperoxidase, H_2O_2 and Cl^-, taurine gave chloramine

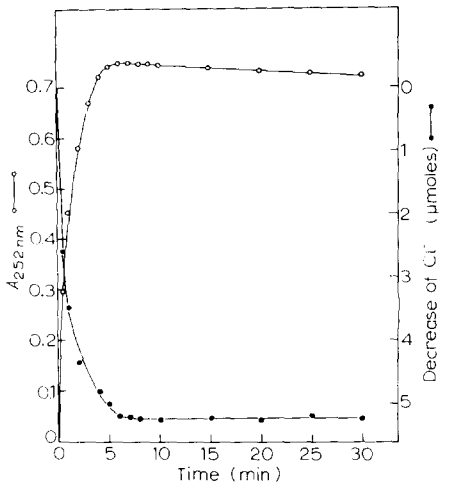

Fig. 5. Peroxidatic formation of taurine chloramine. Sample composition: 20 μmoles of taurine, 10 μmoles of H_2O_2, 90 μmoles of NaCl, 45 μg of myeloperoxidase in 50 mM sodium acetate buffer (pH 4.5). Total volume, 3 ml; temp., 20°.

with absorption maximum at 252 nm. Production of taurine chloramine was accompanied by utilization of Cl^- from the reaction medium (Fig. 5). In contrast with the amino acid chloramines, taurine chloramine was a stable compound and could be separated from taurine by electrophoresis (in 50 mM phosphate buffer, pH 7.0 at 120 V, 30 min) and by paper chromatography (in *tert.*-butanol–acetone–water, 60:20:20, by vol. + 1 g sodium acetate). Taurine chloramine produced during the enzymatic reaction was identical with synthetically produced chloramine (by the same procedure as for alanine). It exhibited the same degree of stability, identical electrophoretic and chromatographic mobility, and capacity for reacting with iodide and thiols leading to the recovery of free taurine.

It seems that the production of chloramines described above may play an important role in the physiological function of the phagocytizing granulocytes. All the conditions required for this reaction exist in these cells. Myeloperoxidase and Cl^- are present in considerable amounts[6,7], H_2O_2 synthesis is intensive[8], and the pH within the phagocyte[9] is near the optimum for the investigated reaction. Hence the formation of chloramines known as bactericidic agents[4,10], especially taurine chloramine in view of the high concentration of taurine in leucocytes[11], may play some role in the nonspecific resistance to infection.

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