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FORMATION OF A STABLE CHLORAMINE COMPLEX DURING INTERACTION OF CARNOSINE WITH THE HYPOCHLORITE ANION

V. E. Formazyuk, E. I. Dudina, and V. I. Sergienko

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KEY WORDS: carnosine; sodium hypochlorite; taurine; chloramine.

The dipeptide β-histidyl-L-alanine (carnosine) is nowadays regarded as a new therapeutic agent [10-12] with the rediscovery of its therapeutic properties, which were demonstrated by Soviet scientists as long ago as in the 1930s or 1940s [1, 3-5]. The wound healing, anticataract, and other therapeutic effects of the dipeptide may perhaps be due to its ability to interact with active forms of oxygen. The antioxidative properties of carnosine have been established in a number of studies [6, 10, 13, 14]. For instance, the present writers showed that carnosine can suppress chemiluminescence in the NaClO + H_2O_2 system [7], which can be only partly explained by its ability to quench singlet oxygen [8, 13].

Some free amino acids undergo oxidation by the hypochlorite anion with the formation of unstable chloramines [15]. No information of this kind is available for carnosine. The aim of the present investigation was therefore to study interaction between carnosine and the ClO^- ion.

EXPERIMENTAL METHOD

Solutions of carnosine preparations obtained by organic synthesis (from "Serva" and "Sigma," USA) and by extraction from beef (Leningrad Medical Preparations Factory) were used. Other test objects included solutions of L-alanine and L-histidine ("Serva," USA). A solution of glutathione ("Reanal," Hungary) was interesting as a sulfur-containing compound interacting actively with oxidizing agents, and taurine ("Serva," USA) as a substance forming a stable complex on interaction

Research Institute of Physicochemical Medicine, Ministry of Health of the RSFSR, Moscow.

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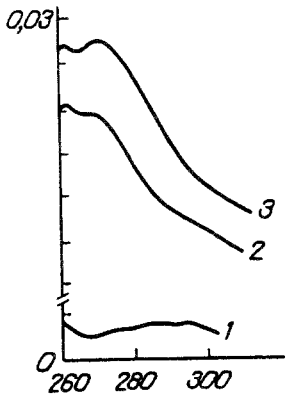

Fig. 1. Absorption of preparations of 5 mM solutions of carnosine. Abscissa, wavelength (in nm); ordinate, optical density (in relative units). 1) Natural carnosine, obtained from beef; 2 and 3) synthetic carnosines obtained from "Sigma" and "Serva" respectively.

with hypochlorite. The solutions were made up in physiological saline buffered with potassium salts of phosphoric acids (pH 7.0).

Interaction of the above-mentioned solutions with sodium hypochlorite was studied on a DU-7 photometer ("Beckman," Austria). Sodium hypochlorite was obtained by electrochemical oxidation of NaCl solution on the EDO-3 apparatus [7]. Its concentration was determined spectrophotometrically relative to the characteristic adsorption peak at 292 nm. The initial concentration of sodium hypochlorite in the reaction mixture was 4 mM and the concentration of amino acids 5 mM. This ratio between the concentrations of oxidizing agents and amino acids was chosen empirically for convenience of long-term monitoring of the kinetics of interaction between these substances. The reaction was monitored for 2 h, on the grounds that this time interval is sufficient to establish the stability of the resulting chloramine complex [15].

EXPERIMENTAL RESULTS

A study of the absorption spectra of preparations of synthetic and preparative carnosine revealed clear differences, namely additional absorption bands at 260-280 nm (Fig. 1). This fact is evidence that the natural preparation of the dipeptide has a higher degree of purity. At least it can be said that it does not contain phenolic impurities present in the reagents of chemical synthesis.

The study of interaction of natural and synthetic preparations of carnosine with sodium hypochlorite revealed no significant differences in their spectral characteristics; accordingly, in the subsequent description of the results the term "carnosine" will be taken to mean preparations of both synthetic and natural origin.

Within a minute after mixing the carnosine and sodium hypochlorite solutions, the formation of a new peak was recorded at 254 nm, evidence of the formation of a chloramine complex. Absorption of the mixture in the 292 nm region, characteristic of sodium hypochlorite, accounts for only 14.6% of the density of the control sample, in which the oxidizing agent was not used up. No significant change in the pattern could be observed 1 h after the beginning of the reaction (Fig. 2).

A dynamic study of interaction of both carnosine and taurine with sodium hypochlorite shows the processes to be identical (Fig. 2). In both cases the peak in the 292 nm region, belonging to the solution of the oxidizing agent, had disappeared, but a stable chloramine complex, recorded at 254 nm, was formed (Table 1). For instance, the optical density of the mixture of carnosine and taurine solutions with the solution of the oxidizing agent was about 84% 2 h after the beginning of incubation.

During interaction of L-alanine and L-histidine with sodium hypochlorite, the peak at 292 nm also disappeared after a minute and a new peak appeared at 254 nm. Nevertheless, the chloramine complex formed was not stable. The optical density of the peak at 254 nm decreased particularly rapidly in the case of oxidation of L-histidine (Table 1, Fig. 2).

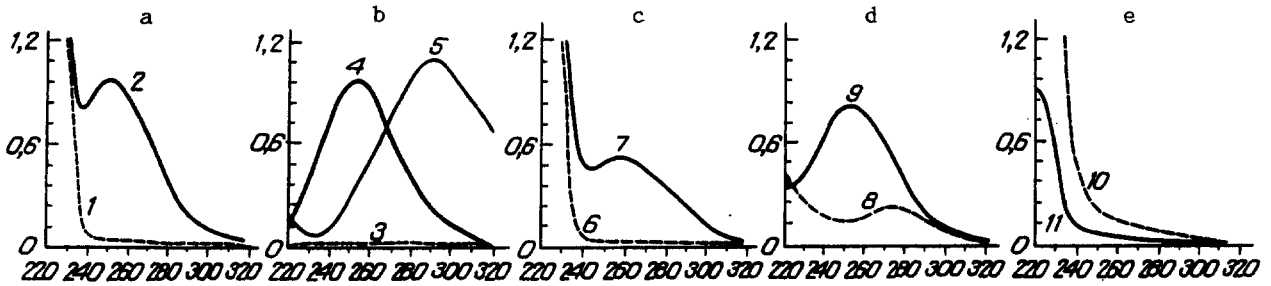

Fig. 2. Absorption of 5 mM solutions of carnosine and amino acids before and 1 h after mixing with a 4 mM solution of sodium hypochlorite: 1) carnosine, 2) carnosine + NaClO, 3) taurine, 4) taurine + NaClO, 5) sodium hypochlorite (NaClO), 6) histidine, 7) histidine + NaClO, 8) alanine. Remainder of legend as to Fig. 1.

TABLE 1. Changes in Optical Density of Reaction Mixture at 254 nm, %

Reaction mixture	Time, min						
	1	20	40	60	80	100	120
	Optical density, %						
Carnosine + sodium hypochlorite	100	98	96	93,1	92,1	91,1	84,2
Taurine + sodium hypochlorite	100	99	97,1	96	92,1	85,3	84,4
L-alanine + sodium hypochlorite	100	82,2	78,5	71	67,5	52,3	41,4
L-histidine + sodium hypochlorite	100	70,6	56,9	45,6	40,4	33	27,5

Legend. Concentration of amino acids and dipeptide 5 mM, of sodium hypochlorite 5 mM; temperature 20°C.

The rate of disintegration of the complex formed by interaction of sodium hypochlorite with L-alanine and with L-histidine after 2 h was 2 and 3.4 times higher respectively than that formed with carnosine.

Investigation of interaction of glutathione solution with sodium hypochlorite solution showed that the reaction between these substances proceeds extremely rapidly, just as in the case described previously. Only 1 min after mixing of the oxidizing agent and the amino acid, the sodium hypochlorite peak disappeared. However, no chloramine complex was formed under these conditions. A kinetic study of the spectra of the reaction mixture showed that optical density in the 230-260 nm region in this case was reduced about by half 20 min after the beginning of the reaction; later, evidently, the reaction was slowed. Thus after 40 min of incubation there was a further small decrease in optical density in this region, after which the spectra of the test samples did not change significantly (Fig. 2).

In a recent study it was stated that carnosine does not interact clearly with the hypochlorite anion [9]. However, the authors cited used an indirect method of assessing interaction between these compounds: based on the de-activating action of HClO on antiperoxidase in the presence of this dipeptide.

As our investigations showed, carnosine, like taurine, interacts with the hypochlorite anion with the formation of a stable chloramine complex. Conversely, free amino acids which are components of the dipeptide are oxidized without the formation of stable chloramines.

The writers previously found myeloperoxidase (MPO) in the lens and retina of the eye with the aid of enzyme immunoassay [2]. Before our investigations all that was known was that this enzyme is present in considerable quantities only in granulocytes. The function performed by MPO in the lens and retina, however, have not yet been studied.

The amino acid taurine (like the dipeptide carnosine) has the property of neutralizing the toxic action of the hypochlorite anion and also of delaying the development of age-related opacities of the mammalian lens [10]. The identity of action of these two related compounds, which we have found, may perhaps lie at the basis of their anticataract effect.

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EFFECT OF MODERATE PRENATAL EXPOSURE TO ALCOHOL ON CORTICAL CAPILLARY ULTRASTRUCTURE IN THE OFFSPRING

É. N. Popova

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KEY WORDS: capillaries; sensomotor cortex; offspring; moderate exposure to alcohol in pregnancy.

After exposure of the pregnant animal to alcohol, a wide spectrum of changes is observed in the offspring, ranging from the development of a full alcohol syndrome to its partial manifestations or finer disturbances of behavioral reactions [10]. In the offspring of animals exposed to prenatal intoxication with high doses of alcohol a decrease in size of the brain has been found, especially of the frontal region, together with polymicrogyria and thinning of the cortex [11], and a decrease in area of the corpus callosum and anterior commissure of the brain [13]. If there is moderate prenatal exposure to alcohol, leptomeningeal neuroglial heterotopia is found in the frontal pole, with a decrease in the density of the neurons and gliosis in the surface layers of the frontal cortex, and reduction and dysplasia of the lateral geniculate body [11]. Delay of development takes place, with changes in the neurons and interneuronal connections in the sensomotor cortex [4, 6, 8].

Laboratory of Brain Ultrastructure, Brain Research Institute, Academy of Medical Sciences of the USSR, Moscow.

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