DIRECT AND INDIRECT ANTIAGGREGATING ACTION OF SODIUM HYPOCHLORITE ON PLATELET-ENRICHED BLOOD PLASMA

M. A. Murina, V. I. Sergienko,
and D. I. Roshchupkin

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Sodium hypochlorite (NaOCl) is synthesized by enzyme action in activated neutrophils; it belongs to the number of compounds which participate in inactivation of microbial cells [4, 6]. NaOCl is used in medicine as a disinfecting agent [2]. NaOCl can modify not only microbial cells, but also animal cells. The writers have shown that NaOCL weakens platelet aggregation in platelet-enriched plasma (PEP) [1]. The mechanism of this antiaggregating action of hypochlorite has not yet been explained.

In the investigation described below interaction between NaOCl and various compounds present in PEP and the possibility of inhibition of platelet aggregation in PEP by two mechanisms – by its direct action on the cells, and indirectly as a result of modification of the plasma – was studied.

EXPERIMENTAL METHOD

PEP was obtained from citrated rabbit blood [1]. To isolate the plasma and cells, a specimen of PEP was centrifuged at 20 min at 1850g. A concentrated platelet suspension was obtained by resuspending the sample in plasma containing 1% sodium citrate. The reconstituted PEP was a mixture of a concentrated suspension of platelets and plasma. Platelet aggregation was induced by the addition of 0.1 ml of ADP (final concentration 10 μM) to 1 ml of PEP. Born's turbidimetric method [3] was used to record aggregation. The maximal change in light transmission (degree of aggregation, ΔT) of PEP was used as a quantitative parameter of platelet aggregation.

NaOCL was obtained by electrolysis of 0.9% sodium chloride solution. Bilirubin, alanine, and glutathione were dissolved in buffered physiological saline. In the case of bilirubin, a concentrated solution was prepared beforehand in 0.01 M NaOH.

EXPERIMENTAL RESULTS

Judging from the results of a study of pure compounds [5, 6], NaOCl can react with many compounds (chemical groups) present in PEP. To decipher the mechanism of the functional action of hypochlorite on PEP, it is important to establish by what degree each active compound is modified. This degree must be determined by the ratio between the velocity constants of interaction of the compounds with NaOCl and the ratio between their concentrations. We studied from this point of view reactions of NaOCl with bilirubin, alanine, serine, and reduced and oxidized glutathione. In alanine and serine the amino group reacts with NaOCl [5], whereas in the case of glutathione, the amino groups and sulfur-containing group must take part in the reaction [6]. The action of NaOCl was analyzed on a mixture of two compounds: bilirubin and amino acid or peptide. Destruction of bilirubin was monitored by measuring the decrease in optical density at 450 nm. Concentrations of amino acids were chosen so that destruction of bilirubin in the mixture took place only slightly (by 5-15%). The optical density of the bilirubin solution (40 μM; pH 7.35) fell due to the action of NaOCl (80 μM) to

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TABLE 1. Antiaggregating Action of NaOCl on Platelets Under Different Conditions

Composition of specimen	Conditions of addition of NaOCl	Concentration of NaOCl, mM	$\Delta T/\Delta T_c$, %
0.9 ml plasma, 0.1 ml platelet suspension	Beforehand, to plasma	1	41±9
0.9 ml plasma, 0.1 ml platelet suspension	To mixture	1	31±5
0.9 ml plasma, 0.1 ml platelet suspension	» »	0,5	66±4
0.45 ml plasma, 0.45 ml physiological saline, 0.1 ml of platelet suspension	» »	0,5	39±5

Legend. ΔT_c and ΔT denote degree of platelet aggregation in control samples (mixture of cells with ordinary plasma or with plasma diluted by physiological saline) and after addition of NaOCl.

25 ± 3, 87 ± 1.4, 89 ± 0.7, 95 ± 0.8, and 85 ± 4% without amino acids and in the presence of alanine (150 μM), serine (340 μM), and reduced (45 μM) and oxidized (40 μM) gluthathione respectively. It will be clear from these data that by reacting with NaOCl, amino acids and gluthathione in a comparatively low concentration strongly inhibit destruction of bilirubin; sulfur-containing compounds react most effectively with NaOCl.

For quantitative evaluation of the ratios of the velocity constants of interaction between the chemical groups chosen for testing and NaOCl, it is assumed that reactions in the mixture are described by the scheme:

$$A + NaOCl \xrightarrow{k_1} \text{products};$$
$$BR + NaOCl \xrightarrow{k_2} \text{products},$$

where A stands for amino acid or peptide, BR for bilirubin; k_1 and k_2 the velocity constants of the reactions. NaOCl was added to the mixture in a limited amount and was completely utilized. A small quantity of bilirubin was destroyed in the mixture, signifying observance of the inequality $k_1[A] >> k_2[BR]$ ([A] denotes the concentration of active chemical groups of amino acids or peptides). Under this condition a change in the NaOCl concentration was due mainly to the first reaction. From the usual formulas of second-order reaction kinetics we find that at the completion of the reaction

$$\log ([BR]/[BR]_0) = [k_2/k_1] \log \{([A_0] - [A])/[A]_0\}.$$

In this equation $[BR]_0$ and [BR] stand for the initial and final concentrations of bilirubin; $[A]_0$ and $[NaOCl]_0$ denote the initial concentrations of active groups in compound A and hypochlorite. It was assumed that in oxidized glutathione the three active groups (one disulfide and two amino groups) are characterized by the same value of k_1. A similar assumption was made in the case of k_1 for the amino group and sulfhydryl group of reduced glutathione. Calculation by the equation, using values of [BR] measured as optical density (see above), showed that the values of k_1 for reactions of the active groups of reduced glutathione, oxidized glutathione, alanine, serine, and bilirubin with NaOCl stand in the ratio of approximately 48:6.9:5.5:2.4:1. The ratio obtained between the values of k_1 for alanine and serine are in good agreement with data in the literature (in the case of alanine k_1 is 2.8 times higher [5]). We know that k_1 for amino groups of amino acids is 10^7-10^8 liters/mole·sec) [5]. The result is that NaOCl can react comparatively quickly not only with amino groups, but also with sulfhydryl and disulfide groups and with bilirubin. Destruction of bilirubin in PEP when NaOCl is present in a concentration of a few millimoles ought not to be significant. This is shown by the results of experiments on the model system. Only about 15% of

bilirubin was destroyed in a mixture containing bilirubin in a concentration of 0.02 mM and bovine serum albumin (0.06 mM) on the addition of NaOCl in a concentration about 2 mM, whereas in the absence of the protein destruction was complete. The reason for the weak degree of destruction of bilirubin in the presence of protein is that the concentration of active groups of the albumin was far greater than the concentration of bilirubin (one molecule of albumin contains several tens of amino groups), and the main mass of the NaOCl reacts with protein chemical groups. This is the situation in the case of PEP.

Active chemical groups reacting with NaOCl (mainly amino groups and sulfur-containing groups) are present in the composition of both platelets and plasma. The next experiments to study the direct and indirect antiaggregating action of NaOCl were carried out in accordance with three basic assumptions: 1) not every reaction can cause weakening of platelet aggregation; 2) most of the NaOCl in PEP reacts with plasma because it contains more active chemical groups than cells; 3) hypochlorite is completely utilized in the test objects. The basis for this last assumption is: against the background of the high reactivity of NaOCl within the concentration range used, platelet aggregation was weakened only partially.

If plasma alone was treated beforehand with NaOCl the degree of platelet aggregation in a mixture with this plasma was reduced by 60% if NaOCl was present in a concentration of 1 mM (Table 1). About the same antiaggregating effect (70%) was found as a result of the action of NaOCl on a previously prepared mixture of platelets and plasma (on reconstituted PEP). It might be concluded from these data that the antiaggregating effect of hypochlorite in PEP is mainly indirect in character. However, it is important to take note of the results of variation of the plasma concentration in reconstituted PEP and the amount of added NaOCl. The antiaggregating action of NaOCl on reconstituted PEP was reduced by about half when hypochlorite was reduced by half. However, a twofold dilution of the plasma with physiological saline, accompanied by a twofold reduction in the concentration of added NaOCl was followed by only a very slight change in the antiaggregating effect: it was about 60% (Table 1). This could be explained, on the other hand, by assuming that the antiaggregating action of NaOCl is basically direct; in this case the action of NaOCl can be described quantitatively by the formula given above, the only modification being that the bilirubin concentration is replaced by the degree of aggregation and the concentration of compound A by the concentration of active chemical groups of plasma. The whole set of data can thus be interpreted as follows. Irrespective of the chemical groups (whether groups in platelets or in plasma) with which NaOCl reacts initially, a reversible reaction proceeds between the active groups in the platelets (Pt) and plasma (Pm), in accordance with the scheme: $Pt + Pm^* \rightleftarrows Pt^* + Pm$, where Pm* and Pt* stand for modification products of active groups in plasma and platelets respectively. It is easy to see that if assumption 2 is satisfied, equivalent to $[Pm^*] \approx [NaOCl]_0$, the concentration of platelets remaining capable of aggregating and, consequently, the final degree of their aggregation, will not change in the presence of simultaneous and equal variation of the plasma concentration in PEP and the amount of added NaOCl.

NaOCl may thus have an indirect antiaggregating action on the platelets in PEP. This state of affairs is probably based on the reversible reaction between platelets and modification products of plasma. Not only amino groups, but also the sulfhydryl and disulfide groups of glutathione and bilrubin itself are characterized by high velocity constants of rection with NaOCl.

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