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Chemistry

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Abstract

Full Text

Chemistry

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Mechanism of Interaction of Free Radicals Formed from Inhibitors of Radical Processes with SH Groups of Proteins

The study of the action of inhibitors of radical processes on lactate dehydrogenase (LDH) showed that radicals arising from the inhibitor in the course of its oxidation possess an inactivating action ([1]). Since the most reactive groups of proteins are SH groups, it was natural to assume that the intermediate forms of oxidation of the inhibitor (propyl gallate (PG) was used in this work) would act specifically on SH groups.

The present work is devoted to clarifying this question.

We used a preparation of LDH isolated from rabbit muscle ([2]). Sulfhydryl groups in the enzyme preparation were determined by the method of amperometric titration in Tris buffer with $AgNO_3$ ([3]), and also in borate buffer and in a mixture of borate buffer with universal buffer at pH 7.6–7.8 with $HgCl_2$.

In our LDH preparation, per mole of protein with molecular weight 135,000, 13 SH groups were found, and after reduction with sodium sulfite, 2 –S–S–bonds (all determinations of –S–S–bonds were carried out with $AgNO_3$). According to literature data ([4]), LDH from rabbit muscle contains 14 SH groups and in the native state has no –S–S–bonds. Since the LDH preparation obtained by us could contain impurities of foreign SH-containing proteins, it was important to establish that the methods we used make it possible to determine the SH groups responsible for the enzymatic activity of LDH.

With this aim, determination was carried out of the change in relative activity (χ) in the course of binding of SH groups in the enzyme preparation by mercuric chloride. As can be seen from Fig. 1, the relative activity of LDH is proportional to the number of SH groups in the preparation.

Fig. 1. Relationship of lactate-dehydrogenase activity of the LDH preparation to the number of SH groups (%)

The kinetics of oxidation of PG was followed by the polarographic method ([5]). Samples were polarographed in borate buffer or in a mixture of borate and

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Fig. 1. Relationship of lactate-dehydrogenase activity of the LDH preparation to the number of SH groups (%)

Figure 1: Fig. 1. Relationship of lactate-dehydrogenase activity of the LDH preparation to the number of SH groups (%)

Figure 2

Figure 2: Figure 2

universal buffers at pH 7-7.2. It turned out that the protein lowers the height of the polarographic wave of PG to different extents depending on the degree of its nativity. Completely denatured protein does not affect the wave height; therefore, before polarography the protein in the sample was subjected to heat treatment for 1.5 min at 70° and then filtered off. Under these conditions, as control experiments show, no additional oxidation of PG occurred. Two series of experiments were carried out. In the first series, LDH (0.2 mg/ml) with PG ($10^{-3}M$) and without PG was kept in a stream of oxygen.

in borate buffer at pH 7.1 and 25°, and in the second series—in universal buffer at pH 9.7 and 26°.

At definite time intervals in all experiments, samples were taken for the determination of χ, the number of SH groups, and the PG concentration.

As can be seen from Fig. 2 (curve 1), PG at pH 7.0 and 26° is not oxidized. At the same time, no additional oxidation of SH groups or change in enzymatic activity is observed in the presence of PG as compared with the control. We determined the –S–S–bonds formed during the oxidation of the protein. It was found that the sum of SH groups and –S–S–bonds remains constant throughout the oxidation, i.e., all oxidized SH groups go into the formation of –S–S–bonds.

Fig. 2. Kinetic curves of PG oxidation (1), LDH inactivation (2), and consumption of SH groups (3) at 25° and pH 7.1 in borate buffer:
a—without PG, *b*—with PG

Special experiments showed that the observed decrease in the number of SH groups during oxidation is not connected with their "masking," since titration in 8 M urea gives no additional number of SH groups.

As can be seen from the kinetic curves shown in Fig. 3, in the presence of oxidizing PG (curve 1 is given as percentages of the initial PG), an accelerated consumption of SH groups is observed. In this case, as in the oxidation of the enzyme without PG, we reduced the oxidized protein and found that the sum of SH groups and –S–S–bonds remains constant within an accuracy of ±4%, which does not exceed the error limits of determination by the amperometric method. It follows from this that addition of PG oxidation products (radical or molecular) to the protein molecule does not occur.

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In this respect, inhibitors of radical processes of the PG type, according to the mechanism of their inactivating action on dehydrogenases, differ fundamentally from benzo- and naphthoquinones, which are capable of irreversibly adding to the SH groups of proteins ([4,6]).

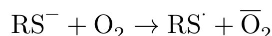
For LDH from rabbit muscle it was previously shown that the reaction of addition of *n*-benzoquinone to SH groups can even serve as a method for the quantitative determination of the SH groups of the enzyme ([4]). We repeated Pfliderer' s experiments on our enzyme preparation and obtained analogous results. In the product of addition of *n*-benzoquinone to the enzyme, after its reduction with Na_2SO_3, SH groups are not detected.

It is known from the literature that quinones having strong electron-donor groups in a neighboring position to an unsubstituted hydrogen in the ring are not capable of adding to a mercapto group ([6]). In our case, orthooxyquinone, apparently formed during the oxidation of PG ([7–9]), has, next to an unsubstituted hydrogen in the ring, an OH group—a strong electron-donor substituent. This may explain why in our experiments we practically did not observe products of addition to the protein at the SH group. However, in the general case one cannot exclude the possibility that some inhibitors of radical processes whose oxidation gives quinones capable of adding to mercapto groups will act on SH-containing enzymes both by oxidation of SH groups by free radicals formed during oxidation of the inhibitor and by addition of the quinone to the protein molecule at SH groups.

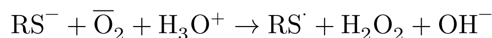
Oxidation of SH groups is apparently accompanied by the formation of intermolecular —S—S—bonds and sulfide-disulfide interaction.

([10,11]), since after reduction of the oxidized enzyme with sodium sulfite (Na_2SO_3) and glutathione the initial activity is not restored.

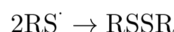
It may be imagined that oxidation of the SH groups of the protein in the absence of PG proceeds according to the following scheme:



$$\mathrm{RS^- + O_2 \rightarrow RS^{\cdot} + \overline{O}_2}$$



$$\mathrm{RS^- + \overline{O}_2 + H_3O^+ \rightarrow RS^{\cdot} + H_2O_2 + OH^-}$$



$$\mathrm{2RS^{\cdot} \rightarrow RSSR}$$

where $\mathrm{RS^-}$ is the protein.

Then

$$\frac{d[\mathrm{RS^-}]}{dt} = -K[\mathrm{RS^-}],$$

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Fig. 3. Kinetic curves of PG oxidation (1) and consumption of SH groups (2)—without PG, (3)—with PG, at 26° and pH 9.7 in universal buffer.

Figure 3: Fig. 3. Kinetic curves of PG oxidation (1) and consumption of SH groups (2)—without PG, (3)—with PG, at 26° and pH 9.7 in universal buffer.

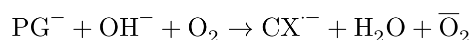
i.e., the consumption of the SH groups of the protein should obey a first-order equation. As is seen from the semilogarithmic transformations shown in Figs. 2 and 3, such a dependence does indeed occur.

From the literature it is known ([12]) that many proteins are synergists with respect to antioxidants—phenols. The mechanism of the synergistic action of proteins has not been clarified; however, it may be assumed that, like other synergists (for example, SH-containing compounds), proteins are capable of reducing radicals formed from antioxidants in the course of inhibition.

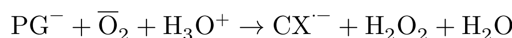
Fig. 3. Kinetic curves of PG oxidation (1) and consumption of SH groups (2)—without PG, (3)—with PG, at 26° and pH 9.7 in universal buffer.

Attention is drawn to the presence of an induction period on the kinetic curve of PG oxidation (Fig. 3, curve 1), after the end of which the reaction develops at a constant rate. During oxidation of PG without protein, there is no induction period, and the initial rate of the reaction remains constant to at least 50% conversion ([5]). It should be noted that during oxidation of PG in the presence of protein, the stationary rate established after the end of the induction period is higher than without protein.

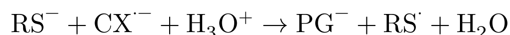
If the induction period on the kinetic curve of PG oxidation is associated with reduction of semiquinone radicals by the SH groups of the protein back to the initial PG, then the processes occurring during the induction period may be represented by the following scheme:



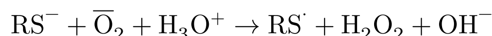
$$PG^- + OH^- + O_2 \rightarrow CX^{\cdot -} + H_2O + \overline{O}_2$$



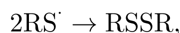
$$PG^- + \overline{O}_2 + H_3O^+ \rightarrow CX^{\cdot -} + H_2O_2 + H_2O$$



$$RS^- + CX^{\cdot -} + H_3O^+ \rightarrow PG^- + RS^{\cdot} + H_2O$$



$$RS^- + \overline{O}_2 + H_3O^+ \rightarrow RS^{\cdot} + H_2O_2 + OH^-$$



$$2RS^{\cdot} \rightarrow RSSR,$$

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where PG^- and $CX^{\cdot -}$ are ionized PG and the semiquinone, respectively. In this case, in the presence of semiquinones, as a first approximation we do not take into account the consumption of SH groups by autoxidation.

It follows from the scheme that

$$\frac{d[\mathrm{RS}^-]}{dt} = -K'[\mathrm{PG}^-],$$

i.e., during the induction period the SH groups should be consumed according to a linear law. As is seen from curve 2 of Fig. 3, a linear consumption of SH groups takes place during the first 25 minutes of oxidation. The end of the induction period coincides with the consumptio-

by the destruction of 50% of the SH groups. The nature of the above-noted accelerated consumption of PG after the end of the induction period remains unclear.

The possibility cannot be excluded that the antitumor action of inhibitors of radical processes (phenols) is connected with the ability we have found of intermediate oxidation forms of the inhibitors to inactivate enzymes of cancer cells.

In this case, inactivation may occur both through destruction of the active groups of the enzyme and through structural changes in the protein.

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