ACTA CHEMICA SCANDINAVICA 9 (1955) 1656—1660

On the Catalytic Effect of Blood Serum on the Reaction between Colloidal Sulfur and Cyanide

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The catalytic effect of blood serum on the reaction between colloidal sulfur and cyanide has been investigated. The activity is confined to the albumin fraction, whereas the serum globulins are inactive. Low molecular weight sulfhydryl compounds are also active, although much less than the serum albumin when calculated on a molar basis. The reaction mechanism is discussed.

It was reported by Lang [1] that liver tissue catalyzed the formation of thiocyanate from colloidal sulfur and cyanide. The catalytic effect of the tissue was attributed to its content of rhodanese, an enzyme catalyzing the formation of thiocyanate from cyanide and thiosulfate. Investigations with purified rhodanese preparations showed [2], however, that this enzyme was without any effect on the reaction between colloidal sulfur and cyanide, but it was confirmed that different tissues contained a heat labile factor catalyzing this reaction. This factor, for which the name rhodanese S was suggested, was present also in blood serum in contrast to rhodanese. In this paper investigations on rhodanese S from blood serum will be reported.

METHODS

The test system described in the previous publication was abandoned, as no reproducible results were obtained due to the instability of the sulfur sol used. A new test system was developed in which a more stable sulfur sol was obtained by diluting a supersaturated solution of sulfur in ethylene glycol monoethylether with water. The assay was carried out as follows:

0.25 ml ethylene glycol monoethylether, containing 1.6 mg S per ml was added to 1.75 ml water in a small centrifuge tube, whereupon 0.5 ml sample solution, containing 0.10 M KCN and 0.04 M HCl was added. The pH obtained was 9.5. After 5 minutes at 20° C the reaction was stopped by the addition of 1 ml ferric nitrate reagent [2] and the mixture allowed to stand for 5 minutes in order to coagulate the sulfur. After the addition of 6.5 ml water and a subsequent centrifugation, the thiocyanate formed was obtained from the optical density determined at 460 mμ. In the case of some serum fractions which stabilized the sulfur sol against the effect of ferric nitrate, the optical density was corrected for remaining turbidity by adding to the contents in the cuvette 0.02 ml of a 30 %

mercuric nitrate solution. This caused the red thiocyanate color to disappear immediately but left the turbidity unaffected. A blank determination (the sample omitted) was always carried out and the corresponding correction was made. The test was calibrated with rabbit serum and the amount of CNS^- formed in the system was up to 2 μ-equivalents directly proportional to the amount of serum in the test. The activities of the different compounds are given as μ-equivalents of CNS^- formed per mg compound under the experimental conditions.

RESULTS

Using rabbit serum as a source of the factor it was observed in preliminary experiments that the pH-optimum for the catalyzed reaction was about pH 9.5, whereas the rate of the spontaneous reaction increased continuously with pH. About 50 % of the activity remained after heating the serum for 5 minutes at 60° C and only 15 % remained after 5 minutes at 70° C. Serum could be dialyzed against buffer or distilled water without significant loss of activity, and evidence was thus obtained for the protein nature of the factor. Determinations were consequently carried out on human serum (from retroplacentar blood) and protein fractions obtained thereof through a modified procedure according to Cohn *et al* [3]. From the results, shown in Table 1, it is evident that serum albumin is very active, whereas other fractions are less active or completely inactive. The activity found in these other fractions seemed to be proportional to their contamination with serum albumin, which is thus alone responsible for the serum activity, whereas the globulins are inactive. In the case of fractions IV-4 and VI the observed activities were lower than those calculated from the albumin content. In the case of fraction IV-4 it could be demonstrated that this fraction had an inhibiting effect on the serum albumin, but no such effect was obtained with fraction VI. This fraction, however, was obtained by freeze-drying the final supernatant remaining after precipitating out the other protein fractions. As this supernatant represents a very dilute (0.05 %) solution of the serum albumin it is possible that the albumin in fraction VI was to a large part denatured during the freeze-drying process. The activity and amount of protein found in fraction VI was also only about

Table 1. Catalytic activity of human serum fractions.

The albumin content was calculated from the activity by dividing the activity for each fraction by that of fraction V, assuming that the latter consisted of pure albumin.

Fraction	Activity μ-equiv.CNS^-/mg	[Albumin content] From electrophoresis *, %	[Albumin content] Calculated from the activity, %
Serum	0.60	43	42
II	0.045	5	3
III	0	Traces	0
IV–1	0.096	8–10	7.2
IV–4	0.18	30	13
V	1.40	100	100
VI	0.39	75	28

* Personal communication from Ingeniör H. Björling of AB Kabi.

Table 2. Heat stability of serum albumin.

Bovine serum albumin (Armour Fraction V) in 4 % solution of pH 7.7 heated at indicated temperature for 5 min. prior to assay.

Temperature, C°	Remaining activity, %
Control	100
40	101
45	96
50	95
55	72
60	44
70 *	12

* A slight turbidity appeared in this sample after the heat treatment whereas other samples were perfectly clear.

a few per cent of their part in the original serum. The catalytic activity of the albumin was also verified on Fraction IV from bovine serum and on crystalline bovine serum albumin, which had activities of 1.08 and 1.01 μ-equivalents CNS^{-1}/mg, respectively. That the activity was confined to the native protein and destroyed by denaturation was demonstrated by the effect of heat treatment and pH on the activity of bovine albumin (Table 2 and 3). Similarly tryptic digestion of the albumin for 14 hours at 37° C (2.5 mg trypsin and 19 mg albumin per ml at pH 9.0) destroyed 73 % of the activity. Furthermore, the activity was entirely destroyed by dodecyl sulfate at 0.001 M concentration.

Some other proteins, egg albumin, β-lactoglobulin and myoglobin, were also assayed and found to be completely inactive. Investigations on some amino acids and related compounds (Table 4) demonstrated, however, that compounds containing a sulfhydryl or a disulfide group had a certain activity, although calculated on a molar basis much less than that of serum albumin. Under the conditions of the test, cysteine thus had an activity of 1.21 equivalents CNS^-/mole which is only 1.3 % of the corresponding figure for human

Table 3. pH-Stability of serum albumin.

Bovine serum albumin (Armour Fraction V) in 2 % solution exposed to the indicated pH for 30 min. The sample neutralized prior to assay. Activity at pH 7.8 taken as 100 %.

pH	Remaining activity, %
1.8	86
3.4	97
4.1	100
5.8	101
7.8	100
9.4	101
10.7	98
11.5	70
11.9	46

Table 4. Activity of low molecular weight compounds.

Compound	Specific activity μ-equiv.CNS^-/mg	Molar activity equiv.CNS^-/mole
Cysteine	10.0	1.21
Cystine	5.85	1.40
Reduced glutathione	3.70	1.16
Oxidized »	1.48	0.91
» methionine	0	0
Glycine	0	0

serum albumin, 97 equivalent CNS^-/mole (taking 68 000 as the molecular weight for serum albumin). The activity of the disulfide compounds (cystine, oxidized glutathione) was about the same as for the corresponding sulfhydryl compounds (calculated on equimolar basis), but preincubation with cyanide was necessary in this case in order to reach this activity. As one mole of a sulfhydryl compound was formed from one mole of disulfide under these conditions, it was concluded that the activity was due to the sulfhydryl compound formed. In accordance with these findings it was observed that the other reaction product obtained in the cyanolysis of cystine, 2-amino-thiazoline-4-carboxylic acid, was completely inactive. The association of the activity with an intact sulfhydryl groups was demonstrated by blocking the sulfhydryl group in glutathione with iodoacetate, which destroyed the activity, as did oxidation of cysteine to cysteic acid. Other sulfhydryl compounds as sodium sulfide, thioglycolic acid, dithiobiuret and thiophenol were also active, although less than cysteine or glutathione. The catalytic effect of sulfhydryl compounds on the reaction between colloidal sulfur and cyanide might be related to their known ability to react with free sulfur [4]. The end products are a disulfide and hydrogen sulfide in this reaction but probably a persulfide RSSH is initially formed. In the presence of cyanide this persulfide will probably decompose giving thiocyanate and the original sulfhydryl compound. The reaction system can thus be depicted as follows:

(1) $RSH + S \rightarrow RSSH$ followed by
(2) $RSSH + HCN \rightarrow RSH + HCNS$

But at the same time reaction (1) is followed by

(3) $RSSH + RSH \rightarrow RSSR + H_2S$ which is followed by
(4) $RSSR + HCN \rightarrow RSCN + RSH$

Reactions III(3) and (4) can be summarized as

(5) $RSH + S + HCN \rightarrow RSCN + H_2S$.

The active sulfhydryl compound is thus removed from the system through reaction (5) and in accordance with this we observed that the catalytic activity of cysteine decreased with reaction time.

The catalytic effect of serum albumin, on the other hand, was not due to the sulfhydryl groups [5] present in the preparation, as blocking of the latter with N-ethyl-maleimide did not affect the activity. (The complete blocking of the sulfhydryl groups was checked by amperometric titration with mercury

ions according to Kolthoff *et al.*[6]). A preincubation with cyanide in the presence of iodoacetate did not decrease the activity, indicating that the activity was not due to the disulfide groups present in the albumin.

DISCUSSION

The present investigation has only been concerned with rhodanese S from blood serum, although it was previously [2] reported that liver and muscle tissue contains a similar factor. No further experiments have as yet been carried out with these tissues. The catalytic reaction is probably without any physiological significance, as free sulfur has not been demonstrated in tissues from higher animals, and cyanide occurs only in trace amounts [7]. The presence of rhodanese S activity in blood serum may, however, explain the established antidote effect of collidal sulfur in cyanide poisoning [8]. The activity in serum is, as demonstrated in the present work, associated with the serum albumin fraction, and it appears possible to determine the albumin concentration in serum from its rhodanese S activity. A comparative study of the albumin content and rhodanese S activity of different normal and pathological sera is planned. It must, however, be kept in mind that even purified serum albumin preparations have been reported to be inhomogeneous [9], but any attempts at further characterization of rhodanese S in serum albumin have as yet not been made.

The present investigation has not offered any explanation for the catalytic activity of serum albumin. In an attempt to elucidate the reaction mechanism further, the spontaneous reaction between free sulfur and cyanide was studied in a homogeneous system, obtained by replacing water in the standard test system with methanol. The reaction between sulfur and cyanide was then spontaneous and went to completion in less than one minute in confirmation of previous reports in the literature [10]. This may indicate that the effect of the catalyst on the colloidal sulfur-cyanide system is simply due to a facilitation of the contact possibilities between the free sulfur in the solid state and the cyanide.

The human serum protein fractions were a generous gift from Ingeniör H. Björling of AB Kabi to whom the author wishes to express his sincere gratitude.

This work is part of an investigation supported by *H. M. Konung Gustaf VI Adolfs 70-års fond för svensk kultur* and *Magn. Bergvalls Stiftelse.*

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Received September 10, 1955.