PRESENCE OF HYALURONATE-LYASE* IN HUMAN BLOOD SERUM

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During acidification of mixtures containing hyaluronic acid and proteins, mucin-like clots are precipitated (hyaluronate-protein complexes). It has been shown that if human serum or the serum of albino rats is used as the protein component of such mixtures, the mucin-like clots are quickly destroyed [2].

This action of the serum may be associated with the enzyme hyaluronate-lyase (H-lyase), one of the properties of which is the ability to prevent the formation of hyaluronate-protein complexes (the mucinase function). At the same time, reports in the literature [10, 12] and the results of the authors' experiment show that donors' serum cannot depolymerize hyaluronic acid at pH 6.0-7.2. This meant that the mucinase factor of the serum could not be identified with testicular H-lyase [2].

According to Gibian, serum H-lyase is active only in an acid medium [8]. He considers that serum H-lyase is depressed by the inhibitors accompanying it, which makes it difficult to find. The effect of the inhibitors may be weakened by diluting the serum. Following Gibian's recommendation, Berlepsch [5], diluted sera 1:10 with buffer solution (pH 4.0-4.6). In these conditions he found that they possessed H-lyase activity, as judged by the appearance of N-acetylglucosamine—one of the hydrolysis products of hyaluronic acid—in the test solution. No details are given in the paper about the determination of N-acetylglucosamine. Berlepsch noted that the detection of this substance in hyaluronate-protein mixtures at pH 4.0-4.6 is very difficult, and that his results were therefore only approximate. Meanwhile, the only reliable criterion by which the presence of H-lyase can be detected in an unstudied substrate is the discovery of hydrolysis products of hyaluronic acid (N-acetylglucosamine or glucuronic acid) in test mixtures containing this mucopolysaccharide.

EXPERIMENTAL METHOD AND RESULTS

The preparations of hyaluronic acid were isolated from human umbilical cords by the method of McClean, Rogers, and Williams [7].

The proteins were separated with chloroform by Sevag's method [13]. Preparations of streptococcal H-lyase were isolated from filtrates of group G cultures by means of ammonium sulfate [4]. The H-lyase activity was determined from the accumulations of N-acetylglycosamine in the test mixtures. N-acetylglucosamine was determined quantitatively by the method of Morgan and Elson [11].

The experimental mixtures consisted of 0.5-1 ml whole or diluted serum, 1.0-1.5 ml of 0.3-0.5% hyaluronic acid solution (in acetate buffer), and 0.1 ml toluene. The initial pH of the mixtures was between 5.0-7.2 and 4.3-4.5. The mixtures were incubated at 37° for 20 and 40 h.

Samples, each of 1 ml, were taken from the incubated mixtures and treated with an equal volume of 10% trichloroacetic acid solution. The precipitate of protein was removed by centrifugation. A solution of alkali was added to the supernatant fluid to neutralize the trichloroacetic acid (the amount of alkali needed was titrated accurately; it was 0.63-0.67 ml of a 1 N solution). A sample of 1 ml was taken from the neutralized mixture for the determination of N-acetylglucosamine.

All the reagents were prepared and added in accordance with the original formula. Samples were placed in test tubes (with ground-glass stoppers), which were immersed in a boiling water bath for 10 min. The optical density was measured after 45 min (the time for the color to develop) in a photocolorimeter at 536 mμ in cells with distances of 10 mm between the working surfaces. A calibration curve was plotted with a standard solution (from

*This enzyme was formerly known as hyaluronidase. See: "Classification and nomenclature of enzymes," 4.2.99.1 (IL, Moscow, 1962).

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Action of Donors' Blood Clots on Hyaluronic Acid in a Reaction Mixture of 0.5 ml Serum and 1.5 ml Hyaluronate at pH 4.3-4.5

Expt. No.	Serum №	Serum 1:10	Serum whole
		N-acetylglucosamine (in μg)	
1	900	6,2	47,2
2	62	4,5	14,9
3	63	4,0	24,3
4	64	5,4	29,7
5	65	5,4	33,8
6	66	3,0	32,4
7	67	3,0	29,0
8	68	2,5	46,0
9	21	4,0	32,4
10	22	4,0	28,4
11	69	—	33,2 (33,2)
12	70	—	28,4 (28,7)
13	71	—	33,0 (32,6)
14	72	—	28,0 (28,0)
15	73	—	20,3 (20,1)
16	74	—	16,2 (16,4)
17	1—11	—	18,9 (18,8)
18	2—11	—	17,5 (15,6)
19	3—11	—	16,5 (18,0)
20	4—11	—	17,0 (17,0)
21	5—11	—	17,0 (17,0)

Note. The results of analysis of parallel samples to which heparin was added immediately before determination of the N-acetylglucosamine are shown in parentheses.

3.1 to 54.0 μg of N-acetylglucosamine). In 52 experiments in which the pH of the test mixtures (donors' serum and hyaluronic acid) varied within limits of 5.0-7.2, the donors' serum did not produce hydrolysis of the hyaluronic acid.

In another series of experiments the action of donors' serum was studied on hyaluronic acid in a medium of pH 4.3-4.5. Mucin-like clots were precipitated in the mixtures, but they did not prevent the reaction or the analysis of the reaction products.

The table shows that N-acetylglucosamine was found in all the test mixtures. The results of these experiments thus showed that the optimum of action of the sera on the hyaluronic acid lay in the acid pH zone. Meanwhile, the results of these experiments confirmed the views of Berlepsch [6] and Gibian regarding the suppression of H-lyase activity by corresponding inhibitors in whole serum.

On the other hand, in some samples, containing diluted sera, for example, in experiments 1 and 2, the amount of N-acetylglucosamine was higher than the corresponding mixtures with whole serum (when calculated in relation to whole serum).

It was decided to determine in model experiments whether donors' serum could depress the activity of H-lyase. For this purpose, a preparation of H-lyase isolated from filtrates of streptococci of group G was used, for human blood contains no specific antibody against the enzyme from cultures of this group [3]. The donors' serum was mixed at the same time with hyaluronic acid and a solution of the bacterial enzyme. The initial pH of the mixtures was 5.0-5.2, and in the parallel experiments 6.8-7.1. At these pH values the H-lyase of the sera themselves was inactive, but the hypothetical nonspecific enzyme inhibitor must have been fully active, in accordance with Berlepsch's findings.

The results of these experiments showed that donors' serum does not depress the activity of streptococcal H-lyase.

The question of the H-lyase nature of the tissue factors acting on hyaluronic acid has been discussed in the literature [9]. In order to answer this question more completely, the effect of heparin on the serum factor was studied, because heparin is known to have the property of depressing the activity of H-lyase of different origin [1]. A series of two-stage experiments was carried out: in stage one the serum was mixed with heparin (pH 4.4-4.6), and the mixture was incubated for 100 min at 37°. In stage 2 a solution of hyaluronic acid (pH 4.4-4.6) was added to the mixture, and the tubes were allowed to stand at 37° for a further 18-20 h. It was found that no N-acetylglucosamine appeared in any of the mixtures incubated with heparin. On the other hand, in the control experiments in which heparin was replaced by acetate buffer, as a rule N-acetylglucosamine was found. Hence, the results of these experiments showed that the activity of the serum factor is neutralized by heparin, and this is very characteristic of H-lyase.

However, a different interpretation may be placed on these experimental results: heparin did not prevent the splitting of hyaluronate by the serum factor, but the N-acetylglucosamine liberated in these circumstances could not be detected in the presence of heparin. The results of control experiments also clarified this question: samples of test sera were incubated with hyaluronate in the absence of heparin. At the end of incubation, when liberated N-acetylglucosamine had accumulated in the mixtures, immediately before it was determined in the samples, heparin solution was added to them.

As the table shows, heparin did not prevent the detection of N-acetylglucosamine in the test mixtures, to which it was added at the end of incubation, i.e., when the substrate in these mixtures was already hydrolyzed.

Hence, donors' blood serum contains the enzyme H-lyase. This enzyme is active in vitro only in an acid medium, at pH 4.0-4.5, thus differing from testicular H-lyase, the optimal pH of which is 6.0-7.0. Like H-lyase of testicular and bacterial origin, serum H-lyase is inactivated by heparin. Its physiological importance will be the subject of future investigation.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of the first issue of this year.
