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Kinetics of the Splitting of Hyaluronic Acid by Streptococcal Hyaluronidase

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The splitting of hyaluronic acid by streptococcal hyaluronidase was investigated viscometrically. This degradation can be described by either of the following two expressions:

$$Et = A(1/y-1) + B(1/y^3-1) \quad (1)$$
$$Et = C(e^{D/y}-e^{D}) \quad (2)$$

where y is a measure of the remaining viscosity, and E the total enzyme concentration. These expressions are of the same form as those previously found for the splitting of hyaluronic acid by a preparation of testicular enzyme. In the present case expression (2) by insertion of the experimental values yields a closer fit than expression (1), the constant D being 0.165.

The source of streptococcal hyaluronidase was a strain of group A hemolytic streptococcus type 24 (A. 24 N.W.).

The solution of enzyme was prepared by Dr. Viggo Faber, Statens serum-institut, Copenhagen, in the following way:

0.1 ml of an 18 hours culture was inocculated into 1 l of Todd-Hewitt substrate [1]. After incubation for 24 hours at 37°C the culture was centrifuged, the supernatant Seitz-filtered, and the pH adjusted to 6.0. The solution was stored at —20° C until use. This solution contains 25 turbidity-reducing units per ml as measured with a modification of the turbidity-reducing method described by Faber [2].

The experimental conditions of the kinetic investigations reported here were identical with those used by Andersen and Graae [3], with the only exception that the substrate concentration was lowered to 0.2 %.

The stability of the enzyme was checked as described by Andersen and Graae [3]. The result was that the enzyme was stable at 20 °C for more than 4 hours.

It was established that the time necessary to reach a given degree of reaction varies approximately inversely proportional to the enzyme concentration. A similar result was obtained by Hale [4], Madinaveitia and Quibell [5], and in this laboratory [3].

Table 1. a means degree of reaction; t_1, t_2 and t_3 times necessary to reach the given degree of reaction for three different enzyme concentrations. Times are determined by graphical interpolation from the measured figures indicated in Tables 2 and 3.

a	t_1 min	t_2 min	t_3 min	t_2/t_1	t_3/t_1	t_3/t_2
0.491	(4.7)	7.9	12.5	(1.68)	(2.65)	1.58
0.564	(6.3)	11.0	17.4	(1.75)	(2.76)	1.58
0.636	(8.9)	15.0	24.8	(1.69)	(2.79)	1.65
0.709	12.0	22.0	36.5	1.83	3.04	1.66
0.781	19.5	36.5	60	1.87	3.08	1.64
0.854	39.0	73.5	122	1.88	3.13	1.66

Mutually interdependent values of time and degree of reaction for three different enzyme concentrations, are recorded in Table 1. The figures given in parenthesis are estimated to be erroneous, because the time is determined under the wrong condition that a linear relationship exists between time and decrease in viscosity [3]. The constant value of the proportions seems to indicate that the chronometric integral is of the ordinary form $Et = f(y)$. The directly measured values are recorded in Table 2.

Table 2. The table gives the measured values of time and the difference Δ between the flow time for the solution and the flow time for water (45.0 sec.).

Expt. 1		Expt. 3	
t_1 min.	Δ flow time, sec.	t_3 min	Δ flow time, sec.
0	275	0	274
3.33	171	3.77	210
6.91	110	8.58	164
10.15	89	12.62	139
12.92	77	16.20	124
18.96	62	21.32	107
27.11	49.5	27.05	95
31.72	45.0	37.58	78.5
39.13	40.0	52.75	65.0
45.90	36.2	60.70	60.0
54.53	33.0	74.17	53.3
60.52	31.3	86.17	48.8
70.61	28.5	100.75	44.7
72.46	28.0	112.25	42.3
80.20	26.5	122.15	39.3
90.55	24.8	131.43	38.8
100.21	23.1	155.72	35.3
103.09	23.2	171.98	33.6
114.51	22.1	185.55	32.5
125.82	21.1	207.60	30.9
139.42	20.3	224.92	29.4
149.99	20.0	235.73	28.9
188.00	18.0	247.93	28.0
223.56	16.7	263.60	27.4

Table 3. The table indicates the measured values of time and Δ flow time for exp. No. 2. y is $\eta_{spc}/\eta^{\circ}_{spc}$ where η_{spc} is the specific viscosity at time t and η°_{spc} is the specific viscosity at zero time, and Δ_1 and Δ_2 is the difference between calculated and measured times for the chronometric integrals 1 and 2, respectively.

t min	Δ flow time sec	y	Δ_1	Δ_2
0	276	1	0	0
3.25	189	0.6848	0.13	0.29
7.65	137.5	0.4982	0.21	0.42
11.63	114.1	0.4134	0.05	0.10
14.92	100.0	0.3623	0.12	0.09
21.78	81.5	0.2953	0.01	−0.06
28.70	69.3	0.2511	0.18	−0.42
53.52	48.0	0.1739	0.29	−0.58
73.17	40.1	0.1453	−0.34	−0.20
86.15	36.6	0.1326	−1.14	0.24
98.80	34.0	0.1232	−2.53	0.64
114.17	33.0	0.1196	−12.89	−8.81
116.33	31.6	0.1145	−7.27	−1.47
117.10	31.0	0.1123	−4.36	2.33
144.08	28.1	0.1018	−10.69	2.16
173.28	25.9	0.0938	−19.71	1.24
211.97	24.0	0.0870	−37.03	−5.17
244.78	22.6	0.0819	−50.46	−6.49
255.07	22.0	0.0797	−51.24	−0.60
1 181	13.7	0.0496	−707	−175

As will be seen from Table 3, the two expressions earlier found [3], fit in this case too. These were

$$Et = A(1/y - 1) + B(1/y^2 - 1) \quad (1)$$

and

$$Et = C(e^{D/y} - e^{D}) \quad (2)$$

In the experiment calculated in Table 2 the values of the constants are: A = 5.05, B = 0.93, C = 37.71 and D = 0.165. It is obvious from these experiments that expression (2) is the best one. It should be noticed that it could be expected that the constant D only depends of the nature of the enzyme and neither of enzyme nor substrate concentration.

The amount of reducing sugar liberated from a given substrate is much greater with pneumococcal hyaluronidase than with testicular hyaluronidase as enzyme [6]. Other bacterial hyaluronidases, especially streptococcal enzymes, show a similar behaviour, *cf.* Rapport, Linker and Meyer [6].

The fact that chronometric integrals of the same form fit both testicular and streptococcal hyaluronidases strongly suggests that the pathways of the reactions are the same. However, in a viscometric investigation it is impossible to distinguish between a reaction resulting in a disaccharide as endproduct and one resulting in other oligosaccharides as endproducts. Therefore the experimental results reported here do not necessarily contrast those obtained by Rapport *et al.*[6]

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