ACTA CHEMICA SCANDINAVICA 2 (1948) 759—769

Hydrolysis of Glycogen with Hydrochloric Acid

BIRGER CARLQVIST

Institute of Organic Chemistry and Biochemistry, University of Stockholm, Sweden

Both amylopectin and glycogen are now regarded as polysaccharides built up of ramified glucose chains with α-glucosidic linkages. In most cases, methylated glycogen from different animals yields about 9 % of tetramethylglucose [1-4]. Bell [5] and Haworth [6a] found, however, certain samples containing only 6 % of end-groups. Among the hydrolysis products of methylated glycogen the only tri-derivative formed is 2,3,6-trimethylglucose. The yields of di- and tetramethylglucose should be equal, but the values for the former substance are unreliable since it cannot be obtained in a pure state. Haworth *et al.*[6a] found that nitric acid oxidized the dimethylglucose fraction mainly into *d*-dimethoxy succinic acid, which must have come from 2,3-dimethylglucose. In a recent paper, Bell [6b] states that trimethylglucose can also produce this compound, and since it is very difficult to separate the various methylglucoses the result obtained by Haworth is inconclusive as to the position of the interchain linkage (the point of ramification). Most of the linkages in glycogen are thus of the 1,4-type as in amylopectin, but there is a marked difference between these polysaccharides in the end-group content and the chain-length. Glycogen does not contain any »amylose fraction» with unbranched chains as in the case of starch [7].

In these experiments, a preparation of Glycogen Hoffmann-La Roche was used. A 5 % solution was prepared and precipitated by alcohol. The precipitate was centrifuged off and dried *in vacuo*. The ash content was 0.27 %. $[\alpha]_D = 196.5° - 197.5°$ ($c = 1$—2). The consumption of iodine, measured by Willstätter and Schudel's method, corresponded to a reducing power of about 1 %, calculated as glucose, and was the same as that of the unprecipitated preparation. The reducing power determined by means of a copper method was less than 0.1 %. (Different samples of starch also showed a small consumption of iodine corresponding to nearly 0.5 % glucose.)

ACID HYDROLYSIS

The hydrolysis of chain molecules has been studied by many investigators (summary by Sillén [17]). Freudenberg *et al.* [8, 9] found that maltose was more rapidly broken down with 50 % sulfuric acid than was starch. They showed also that the velocity of hydrolysis increased in the order cellulose < cellotetraose < cellotriose < cellobiose. In 1930 Kuhn [10] deduced some formulae for hydrolysis of chain molecules using two velocity constants. In an investigation of starch at this institute [11], we found that the best agreement was obtained if we assumed one of the two terminal linkages to be ruptured at the rate of 1.7 k and the other linkages at the rate of k. Myrbäck and co-workers [12] found that starch dextrins containing 1,6-linkages were hydrolyzed more slowly than starch itself.

Experiments

Glycogen was hydrolyzed with hydrochloric acid, and the progress of the reaction was measured by means of iodometric titration.

The reducing power of a dextrin or a sugar solution was determined by Willstätter and Schudel's method, modified by Myrbäck and Örtenblad [13]. The solution, which must be neutral, was mixed with about twice the necessary amount of 0.15 N iodine solution. A slight excess of 0.2 N NaOH was added drop by drop for five minutes. The solution was shaken continuously and, after 15 minutes in all, acidified with 4 N sulfuric acid and titrated with 0.1 N thiosulfate solution. A blank containing no sugar was treated in the same way.

41 g of glycogen, precipitated by alcohol, was dissolved in 1991 ml of water and boiled for one hour over a sand bath in a flask fitted with a reflux condenser. 8.7 ml of conc. HCl was added. At various intervals of time, samples of about 200 ml were withdrawn and neutralized with solid sodium carbonate, cooled and kept under toluene.

A sediment appearing after a few days was filtered off. (The amount was about 0.3 % of the total substance.)

The total amount of carbohydrate in each of these solutions was measured in three ways.

1. The optical rotation was measured. Before the addition of acid the rotation was 3.58° (1 dm tube) and at the end of the reaction it was 1.09°. These values correspond to concentrations of 2.02 % and 2.08 % resp. as the specific rotation of glycogen is 177° (calculated for the hydrate) and that of glucose 52.5°. In this paper, the concentration of a solution is calculated for the hydrate (maltose as $C_{12}H_{22}O_{11} + H_2O$, glycogen as $C_6H_{10}O_5 + H_2O$ etc.).

2. 5 or 10 ml of a solution was evaporated to dryness, and after one day at 105°, the residue was weighed. As the substance is obtained in anhydrous form the measured values must be multiplied by a factor, which is 1 for glucose, 1.052 for maltose, 1.11 for glycogen etc. In this case, the values must also be corrected for the amount of salt. This method gave values between 2.00 % and 2.12 %.

3. The solution was hydrolyzed completely with N HCl for 3 hours at 100°, and the amount of glucose thus formed was titrated iodometrically. In this case, values between 2.03 % and 2.07 % were obtained. It can thus be considered the most accurate method.

The reducing power of the various solutions was determined. Finally, the degree of hydrolysis, α, was calculated (*i. e.* the ratio between the number of free, reducing groups and the total amount of glucose units).

At the beginning of the hydrolysis, the concentration of acid was 0.049 N, but this value had increased to 0.052 N after 23 hours. This may be due to formation of laevulinic acid or other acids. However, evaporation of water would have the same effect.

The unimolecular constant, k, was calculated from the formula

$$k = \frac{1}{t_2 - t_1} 2.303 \log_{10} \frac{1 - \alpha_1}{1 - \alpha_2}$$

The values of a and of k are given in Table 1.

Table 1. Hydrolysis of 2.05 % glycogen solution with 0.049 N HCl at 100°.

Fraction no.	t hours	Degree of hydrolysis %	k hours $^{-1}$
1	0.33	4.0	0.122
2	0.67	8.0	0.128
3	1.00	11.9	0.133
4	1.77	22.0	0.157
5	2.50	30.1	0.150
6	4.65	57.0	0.226
7	8.67	83.8	0.241
8	17.3	96.5	0.202
9	22.9	98.5	0.151
10	41.0	(101)	
		Extrapolated value $k_0 = 0.120$	

As can be seen from the table, the values of k increase at first, and at the end of the reaction they tend to decrease. During the course of hydrolysis, there are formed low-molecular dextrins which are broken down more rapidly, thus causing the increase in k. Branched dextrins containing more stable linkages than the 1,4-type must amount to a relatively larger portion at the end of the reaction, thus accounting for the decrease in the k values.

Freudenberg *et al.* [8] and Myrbäck *et al.* [11, 12] have obtained similar results in the case of starch. On the other hand, Swanson and Cori [14] found that k was nearly constant all the time on hydrolysis of amylose, amylopectin and glycogen. In those experiments, however, the temperature was lower and the concentration of the acid was much higher than in this work.

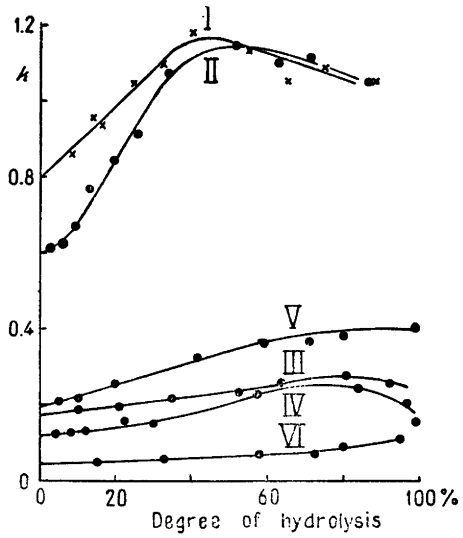

Fig. 1. The variation of the unimolecular constant, k, with the degree of hydrolysis.

I: Starch, 0.215 N HCl.
II: Glycogen, 0.215 » »
III: Starch, 0.049 » »
IV: Glycogen, 0.050 » »
V: Dextran, 0.21 » »
VI: » 0.05 » »

A hydrolysis experiment similar to the first one mentioned was carried out with slightly stronger acid (Table 2).

Table 2. Hydrolysis of 2.2 % glycogen solution with 0.063 N HCl at 100°.

No.	t hours	Degree of hydrolysis %	k hours $^{-1}$
I	0.38	5.6	0.151
II	1.00	14.8	0.173
III	2.20	31.8	0.185
IV	4.29	61.8	0.295
V	7.24	83.0	0.275
		Extrapolated value k_0	= 0.145

HYDROLYSIS OF MALTOSE, STARCH AND DEXTRAN

In order to study the relation between the velocity constant and different types of bonds, hydrolysis experiments on maltose, starch and dextran were also carried out under the same conditions. If the values of k are plotted against the degree of hydrolysis, curves of characteristic form are obtained (see Fig. 1). There is a marked difference between starch and glycogen (curves I—II and III—IV). In the beginning, the k values of glycogen increase more rapidly than those of starch. In the middle of the hydrolysis, there is only a small difference, thus indicating that the low-molecular dextrins from both

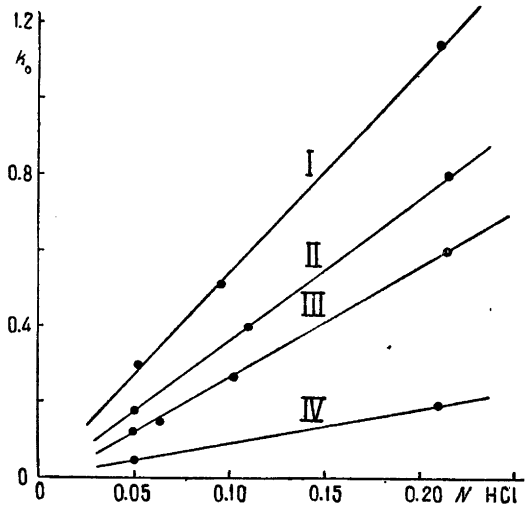

Fig. 2. The relation between the initial rate constant, k_0, and the acid strength.

I: Maltose.
II: Starch.
III: Glycogen.
IV: Dextran.

glycogen and starch are hydrolyzed with nearly the same velocity. The k values of dextran increase steadily during the course of the reaction (curves V and VI). In the case of maltose, k is nearly constant all the time.

The extrapolated value, k_0, from the curves in Fig. 1 is a measure of the initial velocity. From Fig. 2, it appears that k_0 is proportional to the concentration of acid. When the values of α in Table 1 or 2 are plotted against the time, a practically straight line is obtained up to about 50 % of breakdown. The slope of this line has a value that is only slightly higher than that of k_0. Similar results were obtained on hydrolysis of starch and dextran. The first phase of the hydrolysis seems thus to follow a zero-order reaction, but it is impossible to explain such a mechanism. Successive, unimolecular reactions with increasing velocity constants can also show the same effect.

The ratio between the k_0 of maltose and that of starch, glycogen and dextran is calculated for 0.050 N and 0.200 N acid (Table 3).

Table 3. The ratio between various k_0 values.

Concentration of the acid	$\frac{k_0 \text{ maltose}}{k_0 \text{ starch}}$	$\frac{k_0 \text{ maltose}}{k_0 \text{ glycogen}}$	$\frac{k_0 \text{ maltose}}{k_0 \text{ dextran}}$
0.050 N	1.54	2.24	6.00
0.200 N	1.48	1.93	6.00

Dextran formed by certain species of *Leuconostoc* contains mainly α-glucosidic 1,6-linkages. According to Table 3, dextran is hydrolyzed six times

more slowly than maltose. Under quite different conditions, Swanson and Cori [14] found the rate to be 11—15 % of that of maltose. It may be assumed that the 1,4-linkages in the interior parts of a long chain are split with the initial rate of hydrolysis of starch, and the same relation between the 1,6-linkages and dextran is also assumed. On these assumptions, the rate constant of a 1,4-linkage is about $6/1.5 = 4$ times greater than that of a 1,6-linkage. In the present investigation, I have reckoned with interchain linkages of the 1,6-type, since the rate constants have not been determined for any other type. The possibility of the presence of 1,3 and 1,2-linkages is not out of the question; in any case, however, the interchain linkages of glycogen are probably more stable than the 1,4-linkage. The number of interchain linkages must be almost the same as of the end-groups, *viz.* 9 % for glycogen and 4.5 % for starch. Hence one would expect the difference in the k_0 values to be $(9—4.5) \cdot 0.75$ or about 3 %, as the relative difference in the rate of hydrolysis between the two types of bonds is 1—0.25. The effect observed is, however, much greater, about 30 %.

CALCULATIONS BY MEANS OF TWO VELOCITY CONSTANTS

At first, it is convenient to consider only long, straight chains, assuming the one terminal linkage (as in maltose) to be hydrolyzed at the rate of k_0 $(1 + p)$ and the other linkages at the rate of k_0. The values 1.93 and 2.24 in Table 3 would then mean that p is about 1 in the case of glycogen. According to Sillén [17], there is the following relation between the degree of hydrolysis, α, and $k_0 t$

$$1 - \alpha = e^{p(1-x)}\, x^{1+p} \quad \text{where } x = e^{-k_0 t}$$

The values of $1 - \alpha$ obtained by means of this equation are compared with the experimental ones in Table 1. The agreement is good only when $p = 2$ (see Table 4), and from this fact one can conclude that the velocity »constant», k, increases more rapidly than the rate of formation of new terminal linkages. If we now take into consideration the lower rate of splitting the 1,6-linkages, this correction becomes $9 \cdot 0.75$ or about 7 %. Instead of k_0 the value $1.07\ k_0$ ought to be used, but even after this correction, the agreement is still not good when $p = 1$, which should be expected from the ratio of the k_0 values in Table 3.

Table 4. Comparison between the observed and the calculated values of 1—a.

No.	k_0t	1—a			
		Obs.	Calc. for		
			$p = 2$	$p = 1$	$p = 0.5$
2	0.081	0.920	0.914	0.915	0.920
3	0.120	0.881	0.880	0.881	0.885
4	0.212	0.780	0.780	0.789	0.798
5	0.300	0.699	0.685	0.715	0.721
6	0.558	0.430	0.431	0.508	0.542
7	1.04	0.162	0.161	0.238	0.290
8	2.08	0.035	0.018	0.042	0.085

FERMENTATION EXPERIMENTS

Glucose and maltose can be determined separately by fermentation with Swedish baker's yeast. At a pH of about 5, glucose is fermented much more rapidly than maltose. Myrbäck and Leissner [15] have shown that also maltotriose, containing two 1,4-linkages, is fermented with about the same velocity as maltose.

Method

The sugar solution was evaporated on a water bath to remove all traces of toluene. Small tubes were used, each containing the solution thus obtained (about 2 ml) and 1 ml of a yeast suspension. This was prepared by suspending 40 g of baker's yeast in 100 ml of a phosphate buffer (1/15 *M*, pH = 5.3). The tubes were connected with a gas buret, immersed into a thermostat at 30° and shaken mechanically. Readings were made after 5 minute intervals, and the amount of CO_2 was plotted against the time (Fig. 3). Parallel experiments with known amounts of glucose and maltose must be carried out, because the formation of carbon dioxide is not stoichiometric. (Many examples of differential fermentation in [11, 15, 16]).

Sillén [17] has derived the following equations relating to the change in the amount of fermentable sugar with the degree of hydrolysis:

$$\text{Glucose} = 1-(1-\alpha)\ (2-x) \text{ where } 1-\alpha = e^{p(1-x)}x^{1+p}$$
$$\text{Maltose} = 2(1-x)^2\ (1-\alpha)$$
$$\text{Maltotriose} = 3(1-x)^2\ (1-\alpha)x$$

The experimental values of fermentable sugar (Table 5) were plotted against the degree of hydrolysis, and the corresponding curves for the values calculated

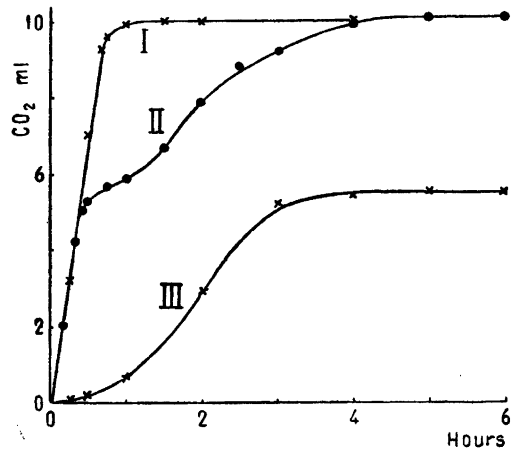

Fig. 3. Fermentation diagram. Fermentation of

I: 50.5 mg of glucose,
II: 10 ml of solution no. 4,
III: 29.4 mg of maltose hydrate.

by means of Sillén's formulae were drawn in the same figure. A few values from this graph are listed in Table 6.

Table 5. The amount of fermentable sugar in the solutions no. 1—8 and no. I—V. (Average of three similar experiments.)

No.	Glucose %	Maltose fraction %	No.	Glucose %	Maltose fraction %
1	1.0	—	8	93.1	—
2	(2.0)	3.1	I	1.2	2.0
3	1.8	6.8	II	6.3	7.4
4	11.1	12.0	III	18.0	15.6
5	19.0	16.9	IV	47.0	15.0
6	43.1	16.1	V	(78.1)	6.1
7	72.9	5.0			

Table 6. Comparison between the observed and the calculated amounts of fermentable sugar.

Degree of hydrolysis %	Glucose %			Maltose fraction %		
	Obs.	Calc. for		Obs.	Calc. for	
		$p = 2$	$p = 1$		$p = 2$	$p = 1$
20	9.0	6.3	5.0	10.5	10.9	11.6
30	16.1	13.0	11.0	16.4	18.1	20.6
40	24.8	21.0	19.0	19.9	24.5	28.9
50	34.5	30.4	28.5	19.6	28.5	34.2
60	44.9	42.0	39.6	16.0	30.0	36.0
70	57.0	54.0	52.5	11.4	28.5	34.0
80	69.4	67.6	66.8	7.2	23.6	27.2
90	83.9	83.1	82.9	3.1	15.0	15.9

Considering first the values of glucose, the agreement is best when $p = 2$. The experimental values of maltose + maltotriose are, however, much smaller than the calculated ones. This must be due to the branched, unfermentable dextrins.

As an example, the calculation of the chain-length for these branched dextrins is made in the case of solution no. 7. The unfermentable sugar amounts to 100—72.9—5.0 = 22.1 %, and the degree of hydrolysis is 83.8 % (Tables 1 and 5). An estimate of the ratio maltose/maltotriose by means of the above-mentioned formulae gives a value of about 2. The reducing power of the maltose fraction is then $2/3 \cdot 1/2 + 1/3 \cdot 1/3 = 4/9$ of the same amount of glucose. The reducing power of the unfermentable part is thus 83.8—72.9—$4/9 \cdot 5.0 = 8.7$ %, and the average degree of polymerization is $\bar{n} = 22.1/8.7 = 2.54$. There must therefore be a large amount of di and trisaccharides with linkages other than the 1,4-type at high degrees of hydrolysis.

CALCULATIONS BY MEANS OF THREE VELOCITY CONSTANTS

Sillén has also deduced more complicated formulae for branched molecules (not published), assuming the interchain linkages to be broken with a lower velocity constant, $q \cdot k_0$. As mentioned before, the 1,4-linkages are hydrolyzed about 4 times more rapidly than the 1,6-bonds, which means that the value of q is about 0.25. Numerical calculations of $1-a$ and of the fermentable sugar have been made for different values of p and q (Tables 7 and 8).

On comparison between Tables 6 and 8, it is clear that the effect of introducing the constant q is negligible in the case of glucose, but the calculated values of the maltose fraction are lowered considerably. By these calculations the amounts of all types of saccharides can be computed so that only unbranched di- and trisaccharides are considered. The calculated amounts of the

Table 7. Comparison between the observed and the calculated values of 1—a. Formulae with two or three velocity constants are used.

No.	$k_0 t$	$1-a$				
		Obs.	Calc. for			
			$p = 2,\ q = 0.2$	$p = 2$	$p = 1,\ q = 0.2$	$p = 1$
III	0.319	0.682	0.670	0.672	0.708	0.695
IV	0.622	0.382	0.400	0.382	0.505	0.458
V	1.05	0.170	0.224	0.165	0.302	0.234

Table 8. Comparison between the observed and the calculated amounts of fermentable sugar. Formulae with three constants are used.

Degree of hydrolysis %	Glucose %			Maltose fraction %			
	Obs.	Calc. for		Obs.	Calc. for		
		$p = 2$ $q = 0.2$	$p = 1$ $q = 0.2$		$p = 2$ $q = 0.2$	$p = 1$ $q = 0.2$	$p = 1$ $q = 0.5$
30	16.1	13.9	13.0	16.4	17.1	17.4	17.7
50	34.5	31.5	29.5	19.6	21.6	25.2	27.0
70	57.0	55.0	53.0	11.4	16.1	22.0	24.4
90	83.9	83.1	83.0	3.1	4.0	5.1	9.0

maltose fraction in Table 8 are only slightly larger than the observed ones when $p = 2$ and $q = 0.2$. Concerning the values of $1-a$, the agreement is, however, not so good for $p = 2$ and $q = 0.2$ as for $p = 2$ alone (Table 7).

These experiments show that the simple formulae that seem to be valid for starch do not hold good in the case of glycogen. The velocity of hydrolysis increases more rapidly than the rate of formation of the new, terminal linkages, and this shows that the other bonds are not split at the same rate as could be assumed in the case of starch. It is possible that, for instance, the linkages in the short interior chains, *i. e.* the chains between two ramifications, are more protected. (The average length of these chains is, according to Meyer and Fuld [7], only about 3 units.) These effects must be more obvious in the case of glycogen than of starch. It is of little value to introduce more complex formulae since there is so little known of the hydrolysis of different types of linkages.

On hydrolysis of glycogen, starch and dextran, the optical rotation was measured, and the specific rotation was plotted graphically against the degree of hydrolysis. Practically straight and coincident lines were then obtained so that, in this way, no difference between the linkages could be proved.

In a subsequent paper, the enzymatic experiments with glycogen will be described.

SUMMARY

The hydrolysis of glycogen, starch, dextran and maltose with hydrochloric acid has been studied. Maltose is hydrolyzed about twice as rapid as glycogen under the same conditions. Different formulae for hydrolysis of straight and branched chain molecules deduced by Sillén have been discussed.

REFERENCES

1. Haworth, W. N., and Percival, E. *J. Chem. Soc.* (1932) 2277.
2. Bell, D. J. *Biochem. J.* **31** (1937) 1683.
3. Meyer, K. H. *Naturwissenschaften* **29** (1941) 287.
4. Hassid, W. Z., and Chaikoff, J. L. *J. Biol. Chem.* **123** (1938) 755.
5. Bell, D. J. *Biochem. J.* **30** (1936) 1612, 2144.

6a. Haworth, W. N., Hirst, E. L., and Isherwood, F. A. *J. Chem. Soc.* (1937) 577.

6b. Bell, D. J. *Biol. Rev.* **23** (1948) 256.

7. Meyer, K. H., and Fuld, M. *Helv. Chim. Acta* **24** (1941) 375.
8. Freudenberg, K., Kuhn, W., Dürr, W., Bolz, F., and Steinbrunn, G. *Ber.* **63** (1930) 1510.
9. Freudenberg, K., and Blomqvist, G. *Ber.* **68** (1935) 2070.
10. Kuhn, W. *Ber.* **63** (1930) 1503.
11. Myrbäck, K., and Magnusson, B. *Arkiv Kemi, Mineral. Geol.* **A 20** (1945) no. 14.
12. Myrbäck, K., Örtenblad, B., and Ahlborg, K. *Biochem. Z.* **307** (1940) 53.
13. Myrbäck, K., and Örtenblad, B. *Svensk Kem. Tid.* **50** (1938) 72.
14. Swanson, M. A., and Cori, C. F. *J. Biol. Chem.* **172** (1948) 797.
15. Myrbäck, K., and Leissner, E. *Arkiv Kemi, Mineral. Geol.* **A 17** (1943) no. 18.
16. Myrbäck, K. *Arch. Biochem.* **14** (1947) 53.
17. Sillén, L. G. *Svensk Kem. Tid.* **55** (1943) 221.

Received September 16, 1948.