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Biochem. J. (1963) **87**, 351

α -(1 $\rightarrow$ 4)-Glucosans

16. THE MOLECULAR STRUCTURE OF SOME PIG-MUSCLE GLYCOGENS*

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In recent years, increasing attention has been directed to a condition in the muscles of pigs that is superficially characterized by an absence of normal pigmentation and by marked exudation of fluid. This condition, the so-called 'white-muscle disease', has been related to the precipitation and denaturation of sarcoplasmic proteins (Bendall & Wismer-Pedersen, 1962) that occurs when a high temperature and a relatively low pH coincide, e.g. when the rate of fall of pH during post-mortem glycolysis is abnormally high (Ludvigsen, 1954; Wismer-Pedersen, 1959; Lawrie, 1960). The condition appears to affect pigs of the Landrace, Pietrain and Poland China breeds more than those of the Large White breed, which predominates in the United Kingdom (Briskey, Bray, Hoekstra, Phillips & Grummer, 1959; Lawrie & Gatherum, 1962). Notwithstanding possible differences in the glycolytic enzymes, it seemed desirable to investigate the structural characteristics of the muscle glycogen in these circumstances. Glycogens from longissimus dorsi muscles of normal British pigs of the Large White breed and of apparently normal and exudative muscle of American pigs of Poland China, Chester White and Hampshire breeds have accordingly been isolated and examined.

* Part 15: Kjølberg & Manners (1962).

MATERIALS AND METHODS

Preparation of glycogen for analysis. Samples of glycogen were prepared before the onset of *rigor mortis* from longissimus dorsi (lumbar) muscles of Large White pigs (of various ages), obtained in the United Kingdom within 1 hr. of slaughter, as described by Lawrie, Manners & Wright (1959). 'Pre-rigor' glycogens were prepared similarly from Poland China, Chester White and Hampshire pigs, samples of longissimus dorsi muscle being frozen in acetone and solid carbon dioxide immediately after slaughter and sent in the frozen condition by air from Madison, Wis., U.S.A., to the United Kingdom.

Analytical methods. Glycogen was determined by the method of Good, Kramer & Somogyi (1933); results are expressed as percentages of wet weight of tissue. The methods used for the determination of glucose content, iodine-staining, periodate oxidation and β -amylolysis limit were those described by Lawrie *et al.* (1959); the interaction with concanavalin-A and measurement of 'glycogen value' was carried out by the method of Manners & Wright (1962*b*). Average chain length was determined by α -amylolysis, as described by Manners & Wright (1962*a*), except that the relation between the apparent percentage conversion into maltose, P_M , and the degree of branching, $P_{(1\rightarrow6)}$, was obtained from a slightly different equation:

$$P_{(1\rightarrow6)} = 23.4 - 0.20P_M.$$

All analytical results are based on the observed glucose contents, which were all higher than 96.5%.

RESULTS AND DISCUSSION

Muscle glycogen from Large White pigs. The longissimus dorsi muscles of Large White pigs of various ages contained between 0.9 and 1.2 % of glycogen, the structural characteristics of which are shown in Table 1, where they are compared with those of other mammalian muscle glycogens.

A consistent feature of the glycogens was the greater iodine-staining power compared with liver glycogens. The $\lambda_{\max}$ values in Table 1 represent the mid-point of a wide absorption peak, and are accurate only to within $\pm 5 \text{ m}\mu$.

In general, the properties of the muscle glycogens of the Large White pig fall within the generally accepted range of 'normal' values (for details, see Manners, 1957), and there is no apparent change in structure with increasing age. However, there are small but significant differences between the muscle glycogens from different species. In a study (Lawrie *et al.* 1959) on horse- and ox-muscle glycogens, chain lengths of 17 were generally observed, whereas other work (Liddle, 1956; Wright, 1960; Kjølberg, 1962) on glycogen from the leg muscles of rabbit gave samples with chain lengths of 13 ± 2 and β -amylolysis limits of 38–45 %. The present results indicate a degree of branching in certain pig-muscle glycogens that is intermediate between the above values, and a corresponding increase in the β -amylolysis limits to 52–54 %. These structural differences presumably reflect some variation in the relative activity of uridine diphosphate glucose- α -glucan glucosyl-

transferase and 'branching enzyme' in the various tissues.

Muscle glycogen from Poland China, Chester White and Hampshire pigs. Glycogen was isolated from the longissimus dorsi muscles of Poland China, Chester White and Hampshire pigs, some of which were affected by white-muscle disease. The glycogen content of the latter was considerably lower than that from non-affected animals, but there was no significant difference in the molecular structure (see Table 2). The rapid rate of post-mortem glycolysis in the white-muscle condition does not therefore appear to involve an abnormality in the glycogenolytic enzyme system [phosphorylase, amylo-(1 $\rightarrow$ 6)-glucosidase and related amylo-(1 $\rightarrow$ 4)-glucosidase (cf. Walker & Whelan, 1960)]; whether the relative activity of other glycolytic enzymes is affected remains to be established.

These are some small but unexpected differences between the structure of the muscle glycogens from Large White pigs, on the one hand, and the structure of those from the three American breeds, on the other. Direct comparisons of the results require caution since those in Table 1 are for 'pre-rigor' samples whereas those in Table 2 represent partially 'post-rigor' material. Nevertheless, with the possible exception of the sternocephalicus muscle of the ox, glycogens from various 'pre-rigor' and 'post-rigor' horse and ox muscles are very similar (Lawrie *et al.* 1959), so that the present inter-breed differences in average chain length (about 2 glucose residues), β -amylolysis limit (about 10 %) and glycogen value (about 0.1) are probably valid.

Table 1. *Comparison of the properties of pig longissimus dorsi and other mammalian muscle glycogens*

Experimental methods are indicated in the text. Chain lengths are expressed in terms of D-glucose residues. Results are given for glycogens from the longissimus dorsi of pig (the present work) and horse (Lawrie *et al.* 1959), the psoas of ox (Lawrie *et al.*) and leg muscle of rabbit (Liddle, 1956; Wright, 1960; Kjølberg, 1962). P_M , Apparent percentage conversion into maltose.

Sample	Chain length (periodate oxidation)*	P_M	Chain length (α -amylolysis)	β -Amylo- lysis limit (%)	Exterior chain length	Interior chain length	Iodine-staining properties		Glycogen value
							$\lambda_{\max}$. (m μ)	$E_{\max}$.	
Pig (Large White cross)									
4 months	15	85	16	52	10	4	495	0.35	0.95
4-5 months	15	84	15	53	11	3-4	485	0.33	0.94
5 months	15	84	15	54	10-11	3-4	495	0.45	0.94
6 months	15	84	15	53	10-11	3-4	495	0.38	0.94
7-8 months	—	84	15	52	10	4	495	0.38	0.96
Pig (Essex sow)									
18 months	15	82	14	53	10	3-4	495	0.35	0.94
Horse	17	83	17	53	11-12	4-5	480	0.34	0.87
Ox	16-17	82	16	50	11	4-5	490	0.34	0.86
Rabbit									
I	13	—	—	45	8	4	490	0.4	—
II	11	—	—	38	6-7	3-4	460	0.23	—
III	13	—	—	46	8	4	490	0.3	—
IV	15	82	14	44	9	4-5	460	0.38	0.96

Table 2. *Comparison of the properties of glycogen from the longissimus dorsi muscles of healthy and 'white-muscle'-affected pigs*

Experimental methods are indicated in the text. Glycogen contents were determined (a) at slaughter in the U.S.A., and (b) after receipt in the United Kingdom. Chain lengths are expressed in terms of D-glucose residues. P_M , Apparent percentage conversion into maltose.

Breed and identification	Glycogen content		Chain length (periodate oxidation)	P_M	Chain length (α -amylol- ysis)	β -Amylo- lysis limit (%)	Exterior chain length	Interior chain length	Iodine-staining properties		Glycogen value
	(a)	(b)							λ_{max} (m μ)	E_{max}	
Healthy											
Hampshire A	1.96	0.67	13	78	13	42	8	4	465	0.36	1.08
Hampshire B	1.32	0.26	13	78	13	43	8	4	460	0.40	1.00
Chester White A	1.20	0.19	13	78	13	42	8	4	470	0.36	1.02
Poland China A	0.47	0.11	14	80	14	40	8	5	465	0.38	1.05
Affected											
Poland China B	0.42	0.06	—	76	12	39	7	4	470	0.41	1.08
Chester White B	0.52	0.07	—	76	12	37	7	4	470	0.38	0.99
Hampshire C	—	0.09	14	79	13	35	7	4-5	465	0.39	1.04
Poland China C	0.39	0.08	14	81	14	41	8	5	465	0.41	1.06

They go together with other biochemical differences between breeds, e.g. in serum and sarcoplasmic proteins (R. K. Scopes, unpublished work) and in ultimate pH and colour of muscle (Lawrie & Gatherum, 1962), and may reflect an inter-breed differentiation in the detailed pattern of glycogen synthesis rather than a metabolic or chemical heterogeneity in the glycogens.

SUMMARY

1. Glycogen isolated from the longissimus dorsi muscles of Large White pigs of various ages has an average chain length of 15 glucose residues. This represents a degree of branching intermediate between that of glycogens from horse longissimus dorsi and rabbit-leg muscle glycogens.

2. Muscle glycogen from three American breeds of pigs has been analysed; these differ slightly in branching characteristics from the samples from the Large White breed.

3. American pigs suffering from white-muscle disease have a lower glycogen content than non-affected animals, but the molecular structures of the polysaccharides are identical.

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