THE ISOLATION FROM RAT LIVER OF A GLYCOGEN COMPLEX WHICH CONTAINS RNA FRAGMENTS

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The drastic treatments used to prepare highly purified glycogen permit limited inference concerning the composition of the intracellular material. Two recent brief reports (Lowe, 1959; Roe et al., 1960) suggest that native glycogen is not simply a polymer of glucose.

Glycogen has been isolated by the following procedure: Livers from male Wistar rats weighing between 125-175 g. were homogenized in 0.25 M sucrose containing .00018 M $CaCl_2$. Nuclei were removed from the 10% homogenate by centrifugation at 800 x g. for 10 minutes. The cytoplasmic supernate was made 10% with respect to NaCl and heated at 100° for 30 minutes. The NaCl extract was freed from the amorphous precipitate, 2 volumes of ethanol were added and the precipitate allowed to form for at least 2 hours at 30° (Barnum and Huseby, 1950). The precipitate (sodium ribonucleate and glycogen) was digested in 1 N NaOH at 100° for 30 minutes. The alkaline extract was cooled, 2 volumes of ethanol were added and the precipitate was allowed to form for 2 hours at 30°. This latter precipitate was dissolved in ice water and any insoluble material was removed by centrifuging. It was subsequently made 1 N with HCl at 0° and promptly neutralized with $NaHCO_3$. Two volumes of ethanol were added to precipitate the

glycogen. This supernate was designated AS; the glycogen precipitate, GP. With each isolation by centrifugation, appropriate washes were used.

The optical density at 260 mμ (OD) of hydrolyzed cytoplasmic sodium ribonucleate isolated by the initial steps of the above procedure amounts to about 1,000 OD units per liver (Lowe et al., publication pending). The AS after hydrolysis with 1 N HCl at 100° for 15 minutes was found to contain 15-25 OD units per liver for fasted animals (glycogen $<$0.02 g./liver). With animals fed ad lib. prior to sacrifice (glycogen, 0.6-0.8 g./liver), AS contained 56-92 OD units/liver. Paper chromatography (descending, HCl-H_2O-iso-propanol) of hydrolyzed samples of AS yielded spots corresponding to adenine, guanine, and uridine, or uridylic acid. Two spots were unidentified. A cytidylic acid spot was absent, probably due to deamination by the 1N NaOH (Hurst and Kuksis, 1958). Carbohydrate content was determined by paper chromatography (ascending, pyridine-n-butanol-H_2O) and showed that after hydrolysis AS contained ribose and glucose.

After extensive dialysis, GP was hydrolyzed with 1 N HCl at 100° for 15 minutes. The optical density ranged from 2-20 OD units/liver regardless of whether GP was obtained from fed or fasted animals. Acid hydrolyzed GP was concentrated and freed of glucose on a Dowex-1 chloride column. Paper chromatography of the eluate revealed only adenine and guanine with an A/G ratio of about 3:2. Carbohydrate resolution of hydrolyzed GP by chromatography revealed glucose but no ribose in a system which would have detected a molar ribose content if greater than 1/400 of that of the purines.

The purines were not dislodged from GP by prolonged dialysis against H_2O, acetate buffer, or 40% urea solutions. Experiments on synthetic mixtures of adenine, guanine, adenosine, guanosine, adenylic acid

and guanylic acid with rat liver glycogen prepared by 30% KOH did not disclose any co-precipitation of the purines when glycogen was precipitated from solution with 66% EtOH.

Table I: Hydrolysis of Glycogen (GP).

Time[a]	TOTAL SAMPLE			EtOH SUPERNATE		
	OD[b]	Carbohydrate[c] Reducing	Total	OD[b]	Carbohydrate[c] Reducing	Total
0	9.2	117	6112	0.72	4	206
30	4.8	43	6952	4.1	13	482
60	6.2	62	6784	5.3	25	761
180	7.4	175	6848	6.5	134	1970
400	7.6	382	6408	7.2	312	3624
700	8.4	573	6512	7.9	624	5184
1440	8.8	639	7072	7.9	707	5520

a in minutes

b at 260 mμ

c μg glucose

The hydrolysis of GP showed that the purine-glycogen binding was more labile than the inter-glucose bonds (Table I). Aliquots of one preparation of GP were hydrolyzed with 0.05 N HCl at 80°. The aliquots were assayed for reducing (Nelson, 1944) and total carbohydrate (Mokrasch, 1954) before and after removal of unhydrolyzed glycogen by addition of 2 volumes of ethanol. Prior to measurement of OD, aliquots were completely hydrolyzed in 1 N HCl at 100° for 15 minutes.

The nature of AS and GP is the subject of current studies. While

the resistance of GP to dialysis is indicative of strong binding which could be covalent, the only possible configurations which would yield on hydrolysis adenine, guanine, and glucose require that the purine bases be attached by the primary amino group or the 9-N as glucosamines to the 2, 3, or 6 carbons of the glucose monomer. A glucosyl purine bond is excluded as the purine found is far in excess of the aldehydic groups available for such binding. Other covalent structures which are consistant with the products of hydrolysis involve bridging with ultraviolet transparent moieties.

Our findings to date point to strong irreversible adsorption phenomena which may be indicative of functional association of RNA and glycogen in native tissue.

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