Journal of Cellular Biochemistry 39:185–195 (1989)

Comparative Study of Nuclear and Cytoplasmic Glycogen Isolated From Mutant HD33 Ascites Cells

Marijana Kopun, Christof Granzow, and Clara R. Krisman

Institut für Zell- und Tumorbiologie, Deutsches Krebsforschungszentrum, D-6900 Heidelberg, Federal Republic of Germany (M.K., C.G.); Instituto de Investigaciones Bioquímicas "Fundación Campomar," Facultad de Ciencias Exactas y Naturales and CONICET, 1405 Buenos Aires, Argentina (C.R.K.)

Mutant cells of the HD33 subline of the Ehrlich-Lettré ascites tumor synthesize and store glycogen mainly intranuclearly, when growing in vivo, and exclusively in the cytoplasm, when permanently cultivated as a suspension cell strain. To investigate whether there exist differences between glycogen of nuclear and cytoplasmic origin, the ultrastructure and the biophysical and biochemical properties of glycogen from in vivo and in vitro grown HD33 ascites cells were compared. Pronounced heterogeneity and differences in glycogen particle ultrastructure were evident in situ and after isolation of the native, high-molecular polysaccharide. Nuclear glycogen contains a fraction of heavier molecules (up to 2×10^9) and larger particles (up to 340 nm) which could not be found in the cytoplasmic preparations, which contained only particles smaller than 140 nm. The subparticles of β-type are similar in both nuclear and cytoplasmic glycogen. The absorption spectra and glucose analysis after degradation with phosphorylase and debranching enzyme indicate that nuclear glycogen has a higher degree of branching, associated with a decrease in the average chain length between the branching points, and shorter external polyglucosidic chains than cytoplasmic glycogen. This is the first report about the analysis and properties of isolated nuclear glycogen.

Key words: glycogen analysis; glycogen isolation; glycogen, neoplasm; glycogen, ultrastructure; cell nucleus; carcinoma, Ehrlich tumor

Intranuclear glycogen, which occurs under a variety of physiological and pathological conditions [1], has been characterized only at the ultrastructural level. Detailed comparisons of the size and arrangement of nuclear and cytoplasmic glycogen particles, based on electron micrographs of ultrathin tissue sections from manyfold sources are summarized in [2]. Until now, no nuclear glycogen was analyzed biochemically.

Received March 28, 1988; accepted August 9, 1988.

© 1989 Alan R. Liss, Inc.

The question of whether there exist special properties of nuclear glycogen appears particularly interesting if the intranuclear localization of the polysaccharide is due to nuclear glycogen synthesis, as has been demonstrated, for the first time [1], in cells of the mutant HD33 subline of the Ehrlich-Lettré mouse ascites tumor [3]. These cells synthesize and store intranuclearly excessive amounts of glycogen when growing in vivo, especially during the late phase of tumor growth [4]. In contrast, after conversion to growth conditions in vitro and permanent cultivation as a suspension cell strain [5], the rapidly proliferating HD33 ascites cells, whose origin is identical with that of their in vivo growing counterpart, permanently accumulate excessive amounts of cytoplasmic glycogen, whereas their nuclei proved to be free of both glycogen and glycogen synthase activity [6]. Nuclear glycogen deposits of in vivo grown ascites tumor cells were found to consist of α- and β-particles, cytoplasmic deposits of in vitro grown cells only of β-particles [7,8], suggesting possible differences in the processes leading to nuclear and cytoplasmic particle formation.

The unusually divergent pattern of glycogen synthesis and deposition in HD33 cells and its dependence on the growth conditions enabled us to isolate, to analyse, and to compare glycogen of nuclear and cytoplasmic origin. To our knowledge, the present report is the first evidence of the properties of the α1,4-α1,6-glucopolysaccharide isolated from intranuclear glycogen deposits. The results presented here are based on ultrastructural, biochemical, and physicochemical analyses of isolated nuclear and cytoplasmic glycogen. Two types of inhomogeneity of glycogen have been found. The first of these relates to the differences within a population of glycogen molecules with different molecular weight, resulting in glycogen particles of variable size and shape. Nuclear glycogen contains a fraction of heavier molecules and larger particles which could not be found in the cytoplasmic preparations. The second type relates to inhomogeneity within the molecule and reveals that the nuclear polysaccharide has a higher degree of branching, associated with a decrease in the average chain length between the branching points, and shorter external glucosyl chains. These inhomogeneities may suggest differing activities of glycogen branching enzyme inside and outside the ascites cell nuclei.

MATERIALS AND METHODS

Ascites Tumor Cells

The mutant subline HD33 [3] of the Ehrlich-Lettré mouse ascites tumor was propagated in vivo as previously described [9]. NMRI mice bearing tumors 7–12 days after transplantation were sacrificed by cervical dislocation, and the tumor cells were aspirated. The almost exclusively nuclear glycogen deposition typical for this phase of tumor growth [4] was ascertained by light microscopy after iodine staining of smears of the individual, native tumor specimens. The cells were washed three times in cold Earle's balanced salt solution (EBSS), and either suspended in it to a density of about 10^8 cells/ml, frozen in liquid nitrogen, and kept at $-20°C$ until use, or suspended in 0.2 M glycine buffer, pH 10.5, and used immediately for the isolation of glycogen.

Suspension cultures of the corresponding ascites cell strain HD33 were maintained as described previously [5]. The cells from 3 L of suspension with 8×10^5 cells/ml were washed with EBSS, suspended in 0.2 M glycine buffer, (pH 10.5), and used for isolating cytoplasmic glycogen.

Chemicals and Reagents

Glycogen from bovine liver was obtained from Calbiochem (Frankfurt, FRG); glycogen from rabbit liver, type III, from Sigma (München, FRG); glycogen from rabbit liver and various enzymes from Boehringer (Mannheim, FRG); and agarose from Bio-Rad Laboratories (München, FRG). Proteinase K and other chemicals and reagents of analytical grade were products of Merck (Darmstadt, FRG).

Electron Microscopic Preparations

In ultrathin sections of whole cells, selective staining of glycogen particles with phosphotungstic acid (PTA) was performed according to Kopun et al. [6]. For negative staining of isolated glycogen particles on Formvar-covered, carbon-coated copper grids, 2% uranyl acetate was used. Electron micrographs were taken with a Zeiss EM 10 A electron microscope operated at 60 or 80 kV.

Isolation of Glycogen

Two methods were used for the extraction of native undegraded, high molecular glycogen from HD33 ascites cells:

a. Extraction with 2.5% $HgCl_2$ according to Mordoh et al. [10]. Briefly, frozen cells were well mixed with an equal volume of 5% $HgCl_2$, or washed cells were suspended in 2.5% $HgCl_2$, and left in the cold room overnight. After centrifugation for 20 min at 800*g*, three volumes of 96% ethanol were added to the supernatant fluid. The glycogen precipitate recovered by centrifugation (4,300*g*, 20 min) was redissolved in a small volume of water and centrifuged as before. The supernatant fluid was dialysed overnight against 500 volumes of 25 mM EDTA, pH 7, at 4°C before precipitating the glycogen with 3 volumes of 96% ethanol. The pellet was reprecipitated with ethanol and the final sediment was twice washed with ethanol, acetone, ether, and dried in vacuo.

b. Extraction with phenol. One volume of frozen cell suspension was mixed with 1 volume of 0.2 M glycine buffer, pH 10.5, and extracted with two volumes of phenol (80%, neutralized with KOH). When fresh cells were used, they were suspended in the glycine buffer, homogenized in the Parr cell disruption bomb as described [1], and the homogenate was extracted with an equal volume of phenol by shaking for 20 min at room temperature. After low-speed centrifugation, the upper layer was removed and the phenol was reextracted with an equal volume of distilled water, shaken, and centrifuged. The combined upper layers were mixed with 3 volumes of 96% ethanol, the precipitate was dissolved in a small volume of water, and either reprecipitated with ethanol, washed and dried as above, or treated with $HgCl_2$ (final concentration 2.5%), dialysed against 25 mM EDTA (pH 7), precipitated with ethanol, and processed as above. Alternatively, the phenol extracts were centrifuged for 20 min at 4,300*g* and the supernatant was dialyzed overnight against 25 mM EDTA (pH 7) and subsequently ultracentrifuged at 75,000*g* and 1°C for 20 h in an SW27 rotor [11]. The pellet was resuspended in water, recentrifuged at 100,000*g* for 7 h, and the new sediment was dissolved in water before precipitating the glycogen with 96% ethanol. After centrifugation at 4,300*g* and 4°C for 25 min, the precipitate was washed with ethanol and acetone, and air dried.

Analytical Procedures

The size distribution of isolated glycogen was measured by sedimentation rate on linear gradients of 10–40% sucrose as previously described [10]. The centrifugation was carried out at 15,000 rpm for 33 min under standard conditions.

Polysaccharide was determined either as total sugars by the phenol-sulphuric acid method [12] or by enzymatic glucose determination [13] after hydrolysis of glycogen with amyloglucosidase according to Keppler and Decker [14] using rabbit liver glycogen as a standard.

The colorimetric determination of the glycogen-iodine complex was carried out by the method of Krisman [15] with minor modifications: NH_4Cl was omitted, and to ensure the stability of the color, 150 μl of 5 N HCl was added to the 50 μl of sample. After mixing with 1.5 ml of Krisman's reagent the spectra were recorded between 380 and 540 nm.

Total acid hydrolysis of isolated glycogen was carried out in sealed ampoules with 1 N HCl at 100°C for 6 h. The liberated sugars were identified by descending paper chromatography on Whatman No. 1 paper. The solvent used was butanol-pyridine-water (6:4:3), and the reducing substances were located with the silver nitrate reagent [16].

The degree of branching points based on the assay of total glucose residues and those specifically linked (α1,6) were determined after treatment with debranching enzyme and phosphorylase b as described in [17].

Protein was determined by an improved Lowry method according to Bensadoun and Weinstein [18], DNA was determined according to Schneider [19].

RNA was identified on a 1.5% agarose gel stained with ethidium bromide after electrophoresis in 0.89 M Tris, 0.002 M EDTA, 0.89 M borate buffer, pH 8.0.

Mild acid treatment was performed with 0.1 N HCl, and the hydrolysis was followed by the decrease in turbidity of glycogen solutions at 500 nm as described [20].

Samples of isolated glycogen (2 ml) were incubated for 1 h/37°C with 1 μg of RNase A in 4 mM Mg-acetate, 50 mM Tris-HCl buffer (pH 7.0), and subsequently with 3 mg proteinase K for 2 h/37°C, or with 15 mg of proteinase K for 18 h/37°C in 100 mM acetate buffer, pH 6.0. Both enzymes were tested and found to be free of amylolytic activity.

RESULTS

Electron Microscopic Observations

PTA staining of glycogen particles in situ revealed different patterns in HD33 ascites cells grown in vivo or in cell culture. The cytoplasm of the latter contained large accumulations of densely packed particles of variable size, most of which were apparently subdivided into few uniform subparticles (Fig. 1a,b). In contrast, in cells of in vivo grown ascites tumors, the glycogen deposits resided mostly inside the nuclei and consisted of groups of distinct, large, frequently rosette-shaped particles assembled of multiple subparticles (Fig. 1c,d).

Similar differences were also visible in negatively stained preparations of glycogen which was extracted from these cell types. Most of the glycogen particles of in vitro grown cells appeared as compound structures and displayed a low number of distinct subparticles. Rosette-like particles were rare. The particle size varied between

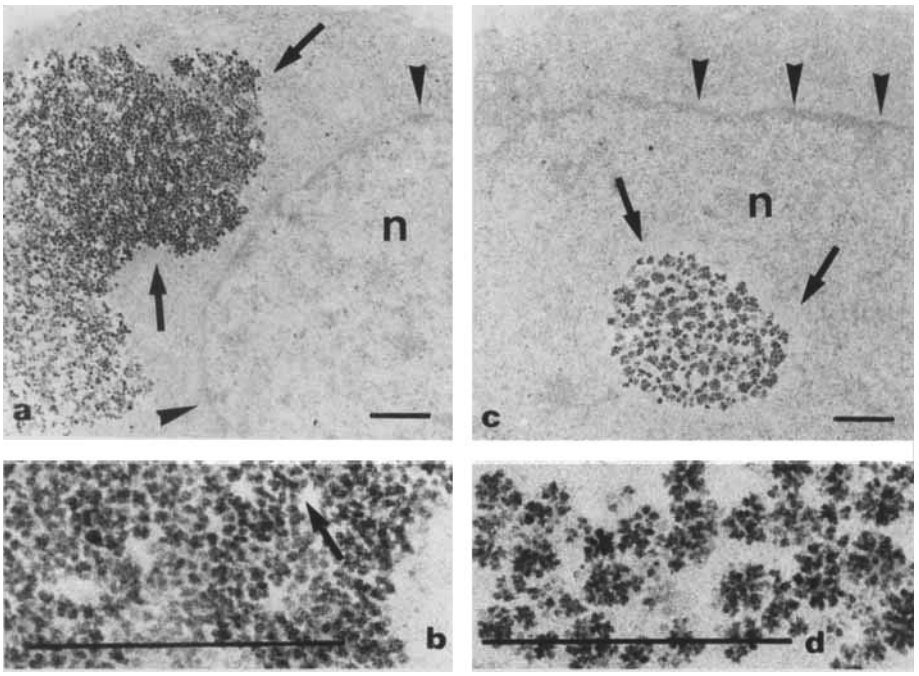

Fig. 1. PTA stained glycogen deposits, in situ, of HD33 ascites cells grown in vitro (a,b) and of HD33 ascites tumor cells grown in vivo (c,d). Other cellular structures remain unstained. n = nucleus; arrowheads = nuclear envelope. Bar, 1 μm. **a:** Large, cytoplasmic deposit (arrows) of densely packed glycogen particles. ×8,000. **b:** Irregular subparticle arrangement of cytoplasmic glycogen particles. Structures resembling α-particles (arrow) are rare. ×40,000. **c:** Intranuclear accumulation (arrows) of α-particles varying in size. ×8,000. **d:** Rosette-shaped subparticle arrangement of intranuclear glycogen particles. ×40,000

25 and, at the most, 136 nm, with the majority of the particles measuring between 48 and 68 nm (Fig. 2a–c). The same classes of glycogen particles were also present in preparations extracted from in vivo grown tumor cells (Fig. 2d–f). In addition, however, a class of very large, spherical or elongated particles made up from numerous subparticles was observed, ranging in diameter from 90 up to 338 nm and being except for their size, morphologically indistinguishable from glycogen α-particles extracted from rat liver [21] (Fig. 2d,e). In both of the cell types, the subparticle dimensions were identical (23–25 nm), which corresponds to the size of liver glycogen β-particles [21].

The described particle size and structure remained almost unchanged after RNAse/proteinase K-treatment (Fig. 2c,f).

Biochemical and Physicochemical Properties of Isolated Glycogen

Only glucose was identified from acid hydrolysed nuclear or cytoplasmic glycogen by paper chromatography. The yield of glucose after acid hydrolysis accounted for more than 98% of the polysaccharide.

In some preparations RNA could be identified; in the samples extracted with phenol and subsequently ultracentrifuged, no RNA was detected.

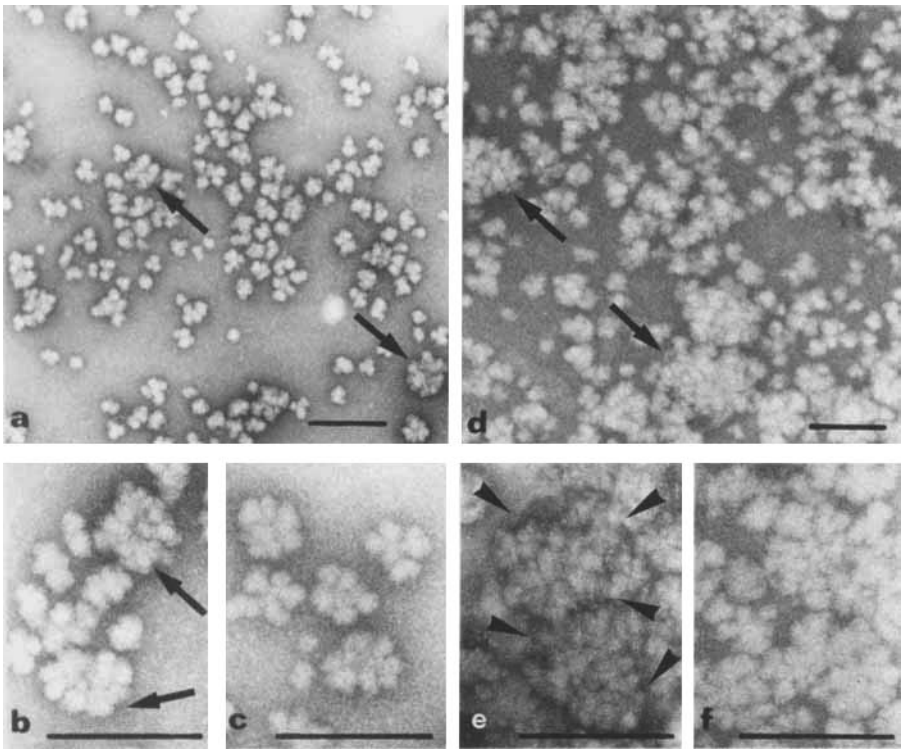

Fig. 2. Negatively stained glycogen particles, isolated from HD33 ascites cells grown in vitro (a–c) and from HD33 ascites tumor cells grown in vivo (d–f). Bar, 0.2 μm. **a:** Phenol-extracted preparation showing moderate variability in particle size and subparticle number. Some α-particles (arrows) are present. ×50,000. **b:** Two examples (arrows) of the largest class of particles observed. ×100,000. **c:** Unchanged particle structure after RNAse/proteinase K treatment. ×100,000. **d:** Phenol extracted preparation with considerable particle size variability, containing a subpopulation of unusually large particles (arrows). ×50,000. **e:** Two large glycogen particles demarcated by arrowheads. ×100,000. **f:** Unchanged particle structure after RNAse/proteinase K treatment. ×100,000.

DNA contamination was absent from the preparations of extracted glycogen. All glycogen samples isolated with $HgCl_2$ were contaminated with 16–20% protein, but phenol-extracted glycogens contained only 0–0.3% protein. Again, the combination of phenol-extraction and ultracentrifugations yielded superior results. Therefore, phenol extracted glycogen was taken for further analyses.

The spectra measured in the presence of Krisman's reagent showed typical α1,4-α1,6-glucopolysaccharide patterns. Nuclear glycogen from in vivo grown cells showed almost a plateau between two very similar maxima, at 410 nm and 450 nm (Fig. 3). However, cytoplasmic glycogen from in vitro grown cells shows one sharp maximum at 470 nm and a shoulder at 390 nm (Fig. 3).

As previously described [12] the color developed with iodine depends on chain length, the wavelength of maximum absorption (λ max) being related to the degree of branching of the polysaccharide. On the other hand, the phenol-sulphuric acid method as well as amyloglucosidase hydrolysis measure the total amount of glucoses disregarding polysaccharide structure. The R value is the ratio between the amounts of polysaccharide as determined, in parallel samples, by the iodine reagent and by

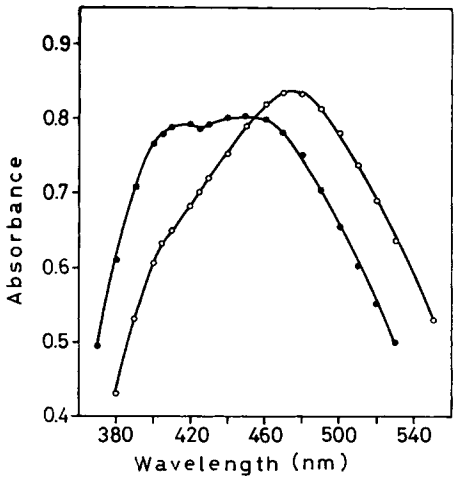

Fig. 3. Absorption spectra after reaction with Krisman's reagent of nuclear (●---●) glycogen isolated from in vivo growing HD33 ascites tumor cells and cytoplasmic (○-----○) glycogen extracted from cultured HD33 ascites cells.

complete acid or enzymatic hydrolysis. It indicates that the external branches are longer or shorter than in the standard polysaccharide used, when the R values are higher or lower than 1 [17,22].

According to Tolmasky and Krisman [22] the ratio between the absorbances of the iodine-polysaccharide complex at λmax and at 390 nm (A ratio) changes with the length of the external polyglucosidic chains. This ratio is more sensitive than R and gives a similar, but more rapid measure of polysaccharide structure [22]. The values for A ratio higher or lower than 1.4 show that the external branches are longer or shorter than those of the standard polysaccharide.

As shown in Table I, the values for R and A clearly indicate that the outer branches correspond to undegraded glycogen. The values for cytoplasmic glycogen from in vitro growing cells are slightly higher than for the rabbit liver glycogen indicating that the external branches are longer than in the standard. However, nuclear glycogen has unequivocally shorter external branches as compared to the cytoplasmic or to the rabbit liver glycogen standards.

Both samples of α1,4-α1,6-glucopolysaccharide were highly branched, as can be concluded from values of 10% and 11.5% for branching points obtained under the conditions described by Krisman et al. [17]. The difference between the values for nuclear and cytoplasmic glycogen is statistically significant ($P < 0.01$).

The molecular weight distribution of the isolated cytoplasmic and nuclear glycogen is shown in Figure 4a,b. Both glycogens presented a relatively sharp peak around 500 S, which represents 58% of the cytoplasmic and 37% of the nuclear glycogen, indicating a molecular weight of about 100 million. Nuclear glycogen is heavier than the cytoplasmic one as can be seen on an extended peak between 1,600 S and 3,200 S representing 30% glycogen with the molecular weight of 5–14 $\times$ 10^8 (Fig. 4b).

After mild acid degradation, the change in the molecular weight was registered by sucrose gradient centrifugation in both types of isolated glycogen (Fig. 4a,b). The mean molecular weight of acid-degraded cytoplasmic glycogen was about 30 million;

TABLE I. Comparative Values for Glycogen Extracted From Various Sources

Source of glycogen	λ max (nm)[a]	R[b]	A[c]	Degree of branching[d] points: Glc (α1,6) (%)[d]
Rabbit liver	460	0.97 (0.94)	1,4	9.4
Corn	475	1.51	1.6	7.5
Cytoplasmic (HD33 in vitro)	470	1.01 ± 0.01 (n = 4)	1.52 ± 0.06 (n = 4)	10.03 ± 0.25 (n = 3)
Nuclear (HD33 in vivo)	410–450	0.89 ± 0.09 (n = 4) (0.84 ± 0.05) (n = 5)	1.08 ± 0.07 (n = 7)	11.53 ± 0.16 (n = 3)

[a]The maximum absorption peak was measured in the presence of Krisman's reagent [15].
[b]Ratio between the amount of polysaccharides obtained by Krisman's reagent and either by phenol-sulphuric acid or amyloglucosidase hydrolysis (values in parentheses) as defined in [22].
[c]Ratio between the absorbances at the wavelength of maximum absorption and at 390 nm as defined previously [22].
[d]The branching points (Glcα1,6) were measured as the glucose molecules involved in the α1,6 linkages specifically liberated by the method previously described [17].

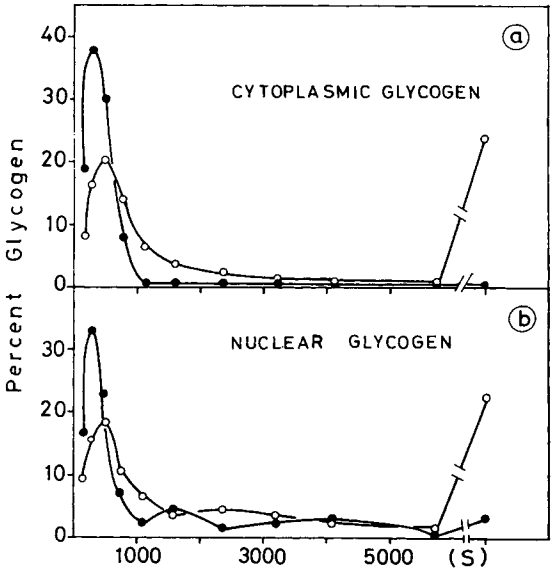

Fig. 4. Sedimentation curves of isolated glycogen (○-----○) and size distribution after treatment of the samples with 0.1 N HCl for 20 min at room temperature and subsequent neutralization with NaOH (●----●). Centrifugation was carried out under the conditions described previously [25]. **a:** Glycogen extracted from in vitro growing HD33 ascites cells. **b:** Glycogen extracted from in vivo growing HD33 ascites tumor cells.

the value of 200×10^6 is not exceeded (Fig. 4a). However, the nuclear glycogen particles of high molecular weight are more resistant to acid hydrolysis, as can be seen from the smaller peak of heavy particles with a molecular weight of 500×10^6; in addition significantly heavier molecules (up to 2×10^9) are also present (Fig. 4b). The treatment of nuclear glycogen with 0.1 N HCl is separately shown in Figure 5; the decrease of the absorption at 500 nm with a $t_{1/2}$ of 4.5 min indicates the destroying of bonds by which the subparticles are linked to form the large particles.

DISCUSSION

The present comparison of nuclear and cytoplasmic glycogen of HD33 ascites cells is based on the native polysaccharide which was obtained by various extraction procedures and is most closely related to the state of glycogen in living cells.

A common ultrastructural finding in negatively stained glycogen preparations extracted from both nuclear and cytoplasmic deposits is the coexistence of glycogen α- and β-particles with numerous intermediate size classes of particles, seemingly representing distinct degrees of subparticle assembly. Except for this inhomogeneity, the glycogen particles extracted from these highly malignant cells appeared morphologically normal. In particular, the largely irregular, strangely shaped particles as described for the AH 13 rat ascites hepatoma [23], were completely absent from the mouse ascites cells regardless of the growth conditions. However, the presence of very large α-particles measuring up to 350 nm in diameter distinguishes glycogen of nuclear origin from cytoplasmic preparations, and is reflected in the differing ultrastructure of nuclear and cytoplasmic glycogen deposits in situ, as shown after specific PTA staining (cf. Fig. 1). In conventionally stained preparations, intranuclear glycogen α-particles and cytoplasmic β-particles have been described in HD33 ascites cells [7,8]. According to the present findings on isolated glycogen, however, the bulk of the cytoplasmic particles represent higher orders of subparticle assembly. Neither RNA nor protein, being accessible to the RNase or proteolytic activity, are required for maintaining the structure of the glycogen particles. The described class of extra-large α-particles is also causing the peak around 2,400 S present in sedimentation

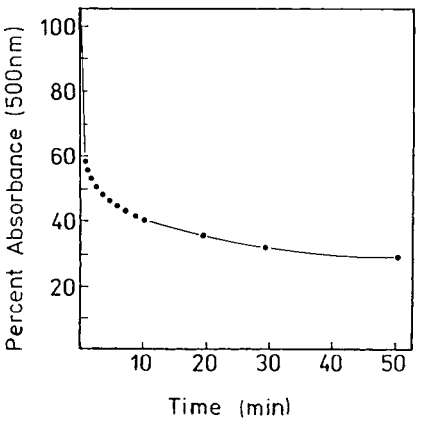

Fig. 5. Changes of turbidity at 500 nm after treatment of nuclear glycogen with 0.1 N HCl at room temperature. The absorbance before acid addition was taken as 100%.

profiles of nuclear but missing in profiles of cytoplasmic glycogen. A similar molecular weight distribution as in nuclear glycogen of HD33 ascites tumor cells was registered for glycogen extracted from the liver of refed rats [10] or synthesized from uridine diphosphate glucose using glycogen synthase in vitro [24,25]. The peak persists, although at slightly lower S values, after mild acid treatment, suggesting a relatively high acid resistance of these aggregates. Glycogen particles of comparable size have been isolated from the liver of well-fed rats [23] or as overgrown products of in vitro glycogen synthesis through phosphorylase [26], suggesting that their formation results from stimulated glycogen synthesis in the absence of glycogen degradation. Analogously, their occurrence in the HD33 cell nuclei may be due to a coincidence of uninterrupted, very active glycogen synthesis during the extended intermitotic intervals typical for ascites tumors with reduced growth rate, with the absence of glycogen degradation from the nuclear compartment of the tumor cells [4].

The reaction with the iodine reagent [15] uncovered, in addition to the described variety at the ultrastructural level, significant intramolecular differences between nuclear and cytoplasmic glycogen. Both the absorption spectra (cf. Fig. 3, Table I) and the results from glucose analysis after degradation with phosphorylase and debranching enzyme indicate that nuclear glycogen has a higher degree of branching, associated with a decrease in the average chain length between the branching points and shorter outer polyglucosidic chains. A reduction in chain length has been described in liver glycogen from a patient with type III glycogen storage disease [27,28], where amylo-1,6-glucosidase enzyme is missing. In striking contrast to the nuclear glycogen of HD33 cells, round to ovoid β-particle subunits of the pathological liver and muscle glycogen measuring about 45–60 nm and 35–50 nm, respectively, and beeing associated in large, unstructured areas were seen in patients with this enzyme deficiency [29,30]. In HD33 ascites tumor cells, nuclear glycogen is accumulated in the intermitotic period, and its degradation takes place mainly in the cytoplasm after the disintegration of the nuclear membrane during mitosis. Furthermore, phosphorylase activity was found to be missing in isolated nuclei of in vivo growing HD33 cells (unpublished data). Only under extremely unphysiological conditions, namely, when the ascites tumor cells were incubated in vitro and maintained for 18 h in glucose-free medium, a degradation of nuclear glycogen could be achieved. This fact of normally absent degradation of glycogen in the nuclear compartment might be related to its changed structure.

However, differences in the degree of branching have been shown by Tolmasky and Krisman to result from the differing intrinsic properties of the branching enzymes involved in glycogen synthesis [22]. Similarly, the observed altered composition of nuclear glycogen of in vivo growing HD33 ascites cells could have resulted from an alteration of the nuclear branching enzyme activities. This possibility is presently being investigated in our laboratory.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the valuable technical assistance of Mrs. Barbara Liebetrau. The authors are obliged to Dr. Herbert Spring for advice in the electron microscopic part of this investigation and to Mrs. Ursula Joa for typing the manuscript. This investigation was supported in part by the Deutsche Forschungsge-

meinschaft (Gr. 715/1-2), and by a guest scientist grant of the Deutsches Krebsforschungszentrum to C.R.K., who is a member of the Research Career of the Consejo Nacional de Investigaciones Cientificas y Tecnicas (CONICET), Argentina.

This work is dedicated to the memory of Dr. Luis F. Leloir, deceased December 2, 1987.

REFERENCES

1. Granzow C, Kopun M, Zimmermann H-P: J Cell Biol 89:475–484, 1981.
2. Ferrans VJ, Maron BJ, Buja LM, Ali N, Roberts WC: J Mol Cell Cardiol 7:373–386, 1975.
3. Granzow C, Nielsén K: Hereditas 100:93–110, 1984.
4. Kopun M, Granzow C: Cell Mol Biol 31:181–193, 1985.
5. Granzow C, Granzow V, Edler L, Sauer W: Cell Tissue Kinet 19:245–255, 1986.
6. Kopun M, Spring H, Granzow C: FEBS Lett 147:207–210, 1982.
7. Zimmermann H-P, Granzow V, Granzow C: J Ultrastruct Res 57:140–149, 1976.
8. Zimmermann H-P, Granzow V, Granzow C: J Ultrastruct Res 54:115–123, 1976a.
9. Granzow C: J Cancer Res Clin Oncol 102:57–69, 1981.
10. Mordoh J, Krisman CR, Leloir LF: Arch Biochem Biophys 113:265–272, 1966.
11. Bueding E, Orrell SA: J Biol Chem 239:4018–4020, 1964.
12. Montgomery R: Arch Biochem Biophys 67:378–386, 1957.
13. Bergmeyer HU, Bernt E, Schmidt F, Stork H: In Bergmeyer HU (ed): "Methoden der enzymatischen Analyse." Weinheim/Bergstr.: Verlag Chemie 1974, Vol 2, pp 1241–1246.
14. Keppler D, Decker K: In Bergmeyer HU (ed): "Methoden der enzymatischen Analyse." Weinheim/Bergstr.: Verlag Chemie 1974, Vol 2, pp 1171–1176.
15. Krisman CR: Anal Biochem 4:17–23, 1962.
16. Trevelyan WE, Procter DP, Harrison JS: Nature 166:444–445, 1950.
17. Krisman CR, Tolmasky DS, Raffo S: Anal Biochem 147:491–496, 1985.
18. Bensadoun A, Weinstein D: Anal Biochem 70:241–250, 1976.
19. Schneider WC: In Colowick SP, Kaplan NO (eds): "Methods in Enzymology." New York: Academic Press, 1957, Vol 3, pp 680–689.
20. Parodi AJ, Krisman CR, Leloir LF, Mordoh J: Arch Biochem Biophys 121:769–778, 1967.
21. Drochmans P: J Ultrastruct Res 6:141–163, 1962.
22. Tolmasky DS, Krisman CR: Eur J Biochem 168:393–397, 1987.
23. Iwamasa T, Hamada T, Fukuda S, Ninomiya N, Takeuchi T: Acta Pathol Jpn 32:461–471, 1982.
24. Parodi AJ, Krisman CR, Mordoh J: Arch Biochem Biophys 141:219–227, 1970.
25. Krisman CR, Parodi AJ: Arch Biochem Biophys 141:228–235 1970.
26. Mordoh J, Leloir LF, Krisman CR: Proc Natl Acad Sci USA 53:86–91, 1965.
27. Gunja-Smith Z, Marshall JJ, Smith EE: FEBS Lett 13:309–311, 1971.
28. Carter JH, Lee EYC: Anal Biochem 39:373–386, 1971.
29. Garancis JC, Panares RR, Good TA, Kuzma JF: Lab Invest 22:468–477, 1970.
30. Benkoel L, Gulian JM, Chamlian A: Cell Mol Biol 30:145–152, 1984.