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Studies on Glycogen Metabolism in Human Leukemic Cells. II. Kinetic Parameters of the Two Forms of Glycogen-Synthetase *

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Two forms of glycogen synthetase from leukemic leucocytes have been obtained in the 100,000 × *g* glycogen particulate fraction. About 90 % of the enzyme was normally found in the D form. I form was produced only after preincubation of the crude extract at 30° C, in the presence of mercaptoethanol and Mg^{2+}.

In spite of the lower glycogen levels and the lower glycogen synthetase activity found in leukemic leucocytes as compared to normal leucocytes, the kinetic studies of the two forms of glycogen synthetase showed that the enzyme seems to have similar characteristics to those reported for the same enzyme in normal leucocytes and in other tissues. The K_m values for UDPG were in the range of 10^{-4} M.

The activation constant for glucose-6-P was found to be lower in magnitude in leukemic leucocytes of the lymphoid series (10^{-4} M) than in leucocytes of myeloid series (10^{-3} M).

It is known that glycogen levels and glycogen synthetase activity in leukemic leucocytes are lower that in normal leucocytes (1-3, 6). Lymphocytes from normal subjects have glycogen synthetase D phosphatase and glycogen synthetase I kinase activities easily demonstrable *in vitro* (4) but polymorphonuclears don't show these activities (5). We have previously reported that leucocytes from acute monoblastic and undifferenciated leukemias possess the system for interconversion of glycogen synthetase D form and glycogen synthetase I form. It was not possible, however, to demonstrate interconversion in leucocytes from chronic myelocytic leukemias (6).

Kinetic constans of normal polymorphonuclear leucocytes have been described by NAHAS *et al.* (7) and PLESNER *et al.*

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(8) in a purified enzyme preparation. The same parameters have been studied in normal lymphocytes (4).

In the present paper, kinetic characteristics for both forms of glycogen synthetase in leukemic cells are given. The K_m for the substrate UDPG and activation constant (K_a) for glucose-6-P have been calculated. The effects of glucose-6-P, inorganic orthophosphate, magnesium, ATP and ADP were also studied in the purified 100,000 × *g* particulate fractions obtained from the 14,000 × *g* crude extracts.

Materials and Methods

Leucocytes from patients affected by acute myeloblastic, chronic myelocytic, acute lymphoblastic, chronic lymphocytic, acute monoblastic and undifferentiated leukemias were prepared as described by Cusso *et al.* (6). Leucocytes were homogenized in 50 mM Tris-1 mM EDTA (pH 7.8) and then centrifuged at 14,000 × *g* for 15 minutes at 2° C. The supernatant (crude extract) was recentrifuged at 100,000 × *g* for 60 minutes. The last centrifugation sedimented glycogen and glycogen synthetase activity as a pellet. The 100,000 × *g* pellet was resuspended in Tris-EDTA buffer, manually homogenized and used as source of enzyme in the D form.

In order to obtain glycogen synthetase I form, the 14,000 × *g* supernatant was preincubated with 50 mM mercaptoethanol and 10 mM $MgCl_2$ at 30° C for 60 minutes. When a yield of 90 % in I form was obtained the homogenate was centrifuged at 100,000 × *g* for 60 minutes. The pellet, after resuspension in Tris-EDTA buffer, was the source of enzyme in its I form.

Glycogen synthetase was determined by the filter paper method of Thomas *et al.* (9), measuring the radioactivity incorporated into glycogen from ^{14}C glucose labeled UDPG.

Chemicals. UDP-glucose and rabbit liver glycogen were purchased from Sigma Chemical Co. UDP-(^{14}C) glucose was obtained from the Radiochemical Center (Amersham, England). Tris-hydroxymethylamine methane (Tris) and EDTA were obtained from Merck. ATP, ADP and Glucose-6-P were from Boehringer Manheim.

Results

After the centrifugation at 100,000 × *g* described in Methods, the enzyme in its D or I form was recovered in the remaining glycogen pellet with about a 5 to 10-fold purification.

The Michaelis constants for the D and I forms of glycogen synthetase in leukemic leucocytes. The effect of UDPG concentration upon enzyme activities in the

Table I. *Kinetic data of glycogen-synthetase in leukemic leucocytes.* In parentheses number of experiments.

Diagnosis	K_m for UDPG (mM)		K_a for G-6-P (mM)	
	I-form	D-form	I-form	D-form
Chronic myelocytic leukemia		0.23±0.10 (7)		5.40±2.40 (6)
Acute myeloblastic leukemia	0.17±0.08 (3)	0.26±0.10 (4)	0.025 (1)	1.17±0.18 (2)
Chronic lymphocytic leukemia	0.09 (1)	0.25±0.27 (3)		0.36±0.16 (3)
Acute lymphoblastic leukemia	0.26 (1)	0.09±0.04 (2)		0.22 (1)
Acute monoblastic leukemia	0.38 (1)			
Undifferentiate leukemia		0.42 (1)		1.65±0.91 (2)

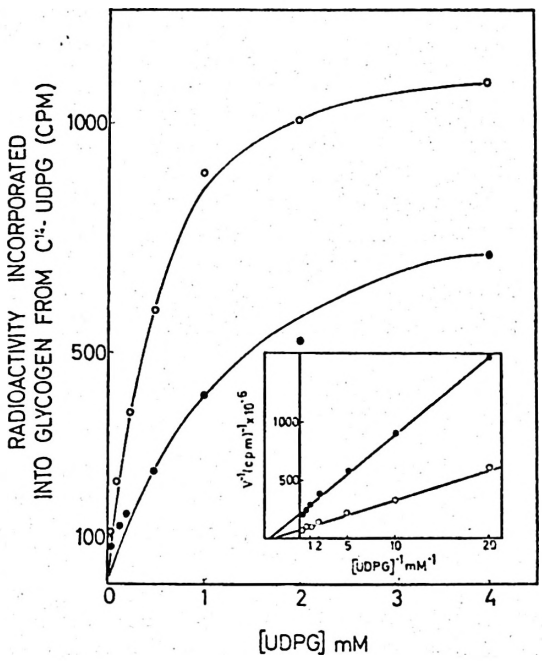

Fig. 1. *Effect of various concentrations of UDPG on the glycogen synthetase I activity assayed with and without Mg^{2+}.*
The standard reaction mixture with no G-6-P was incubated at 30° C with the amounts of UDPG-glucose indicated in the figure; without Mg^{2+} (●) and with 10 mM $MgCl_2$ (○). Figures are plotted according to Lineweaver-Burk in the insert.

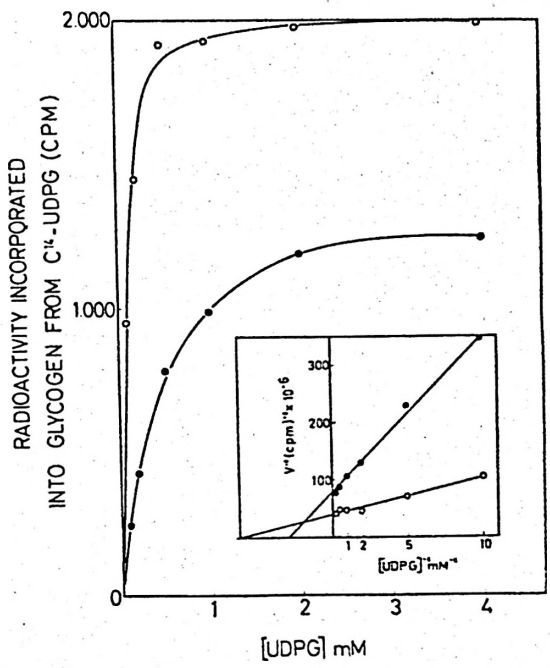

Fig. 2. *Effect of UDPG glucose concentration on glycogen synthetase D form measured with and without Mg^{2+}.*
The standard reaction mixture was incubated in the same conditions as for the I form but in the presence of 10 mM glucose-6-P, without (●) and with 10 mM $MgCl_2$ (○). Figures are plotted according to Lineweaver-Burk in the insert.

100,000 × *g* particulate fraction from different types of leukemic leucocytes was studied. When enzyme preparations of I form were assayed (in the absence of glucose-6-P), the K_m for UDPG varied from 0.09 to 0.38 (table I), the lowest constant being that obtained from chronic lymphocytic leucocytes. K_m from other leukemic leucocytes did not differ substancially. In the presence of 10 mM magnesium the V_{max} increased but the K_m for the substrate remained roughly the same (fig. 1). The effect of magnesium was similar in several I forms assayed from different leukemic leucocytes.

The K_m for the substrate UDPG assayed on the D forms (in presence of 10 mM glucose-6-P) ranged from 0.09 mM to 0.42 mM (table I). It can be observed in figure 2 that maximal velocity was increased and the K_m decreased from 0.40 to 0.15 mM with 10 mM magnesium. This effect of magnesium was the same in all D forms from the different types of leukemic leucocytes.

In one case (an acute myeloblastic leukemia) it was possible to calculate the K_m for UDPG for both forms of glycogen synthetase obtained from the same enzyme source. The kinetic constants of K_m for UDPG found were 0.28 mM for the I form and 0.25 mM for the D form. There was not a significant difference between both constants.

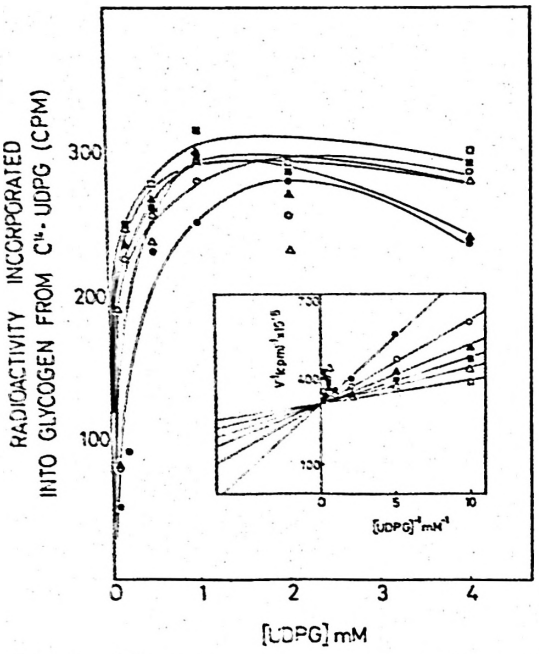

Fig. 3. *Effect to various concentrations of glucose-6-P on the K_m for UDPG glucose synthetase I form.*
The figure is plotted according to Lineweaver-Burk in the insert. Glucose-6-P concentration used were: 0 mM (●), 0.05 mM (○), 0.1 mM (▲), 0.25 mM (△), 1 mM (■), 2 mM (□).

The effect of glucose-6-P on the kinetic constants for the two glycogen synthetase forms was also studied. It was evident that the presence of glucose-6-P in increasing concentration acting on the I form (fig. 3) was followed by a decrease of K_m for UDPG. The K_m was 0.12 mM without glucose-6-P and decreased up to 0.04 with 2 mM glucose-6-P. No change in V_{max} could be observed. High UDPG concentration caused a slight inhibition. The D form in all type of cells studied had practically no activity in the absence of added glucose-6 P (fig. 4).

Constants of activation by glucose-6-phosphate. The influence of the concentration of glucose-6-P was studied in different types of leukemic leucocytes. The activation constants obtained for these forms of glycogen synthetase at saturing concentration of UDPG are shown in table I. The K_a for the I form in an acute monoblastic leukemia was also determined and was found to be 0.025 mM The K_a values for glucose-6-P in the D forms were of different magnitude depending on the type of leucocytes. The activation cons-

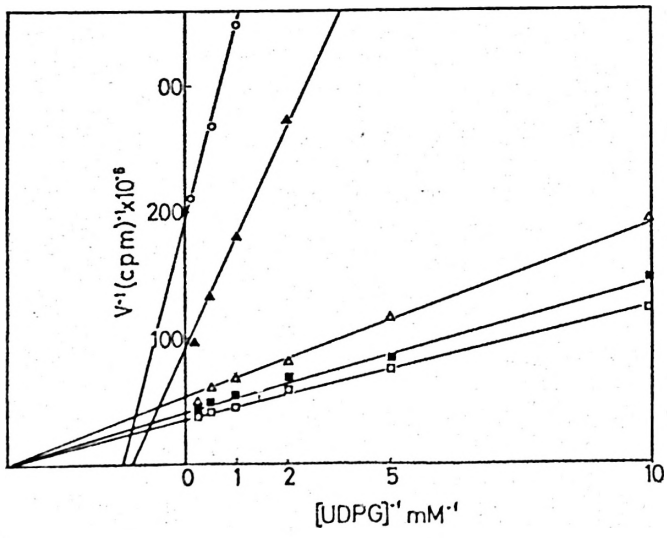

Fig. 4. *Effect of various concentrations of glucose-6-P on the K_m for UDPG-glucose of glycogen synthetase D form.*
The figure is plotted according to Lineweaver-Burk. Glucose-6-P concentrations used were: 0.5 mM (●), 1 mM (▲), 5 mM (△), 10 mM (■) and 25 mM (□).

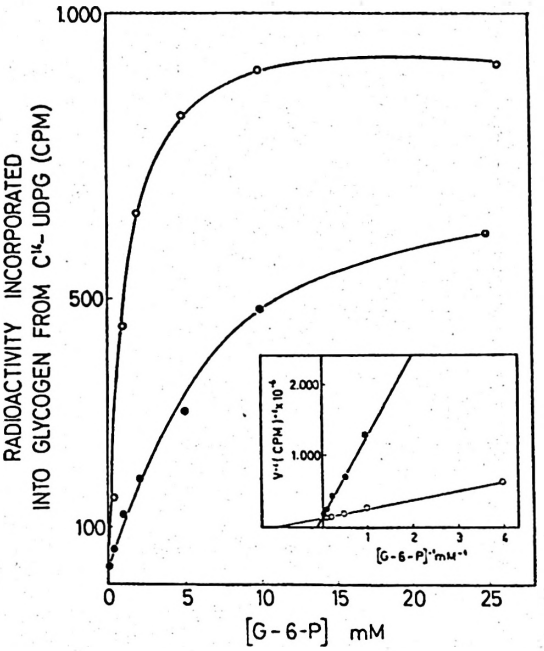

Fig. 5. *Effect of glucose-6-P concentrations on glycogen synthetase D form and its modification by Mg^{2+}.*
The standard reaction mixture was incubated at 30° C with 4 mM UDP-glucose and the amounts of glucose-6-P indicated in the figure: without Mg^{2+} (●) and with 10 mM Mg^{2+} (○). Figures are plotted according to Lineweaver-Burk in the insert.

tants found in acute myeloblastic, chronic myelocytic and undifferentiated leukemic leucocytes were in the order of 10^{-3} M whilst the K_a in acute lymphoblastic and chronic lymphocytic was around 10^{-4} M. Figure 5 shows the effect of magnesium on the activation constant. With 10 mM Mg^{2+} the K_a decreased by a factor of approximately 10 without any change in V_{max}.

Influence of different cellular metabolites on both activities. Phosphate ions were found to be inhibitory for the glycogen synthetase D form, but not for the I form in leukemic leucocytes (fig. 6). The enzyme was not found to be stimulated by P_i but the latter together with 0.1 mM glucose-6-P increased slightly the enzyme activity (fig. 6 on the rigth). P_i inhibited glycogen sythetase D activity at low glucose-6-P concentrations, but glucose-6-P reversed the inhibition at concentrations aproaching 5 mM (fig. 6 on the left).

The inhibitions produced by ATP and ADP on the D form were surmounted by additions of increasing glucose-6-P concentrations and were of a competitive type. ATP was a more effective inhibitor

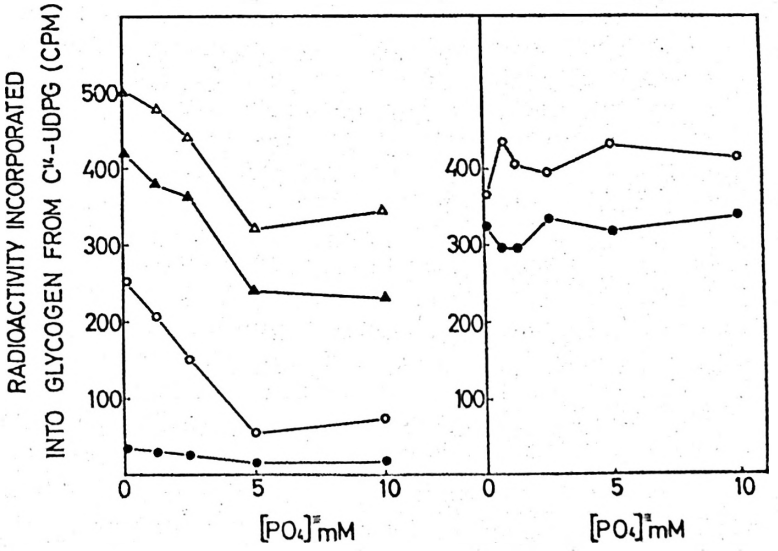

Fig. 6. *Effect of phosphate anions on bot forms of glycogen synthetase.*
Right: The I form was assayed at various phosphate concentrations in presence of 1.0 mM glucose-6-P (○) and in absence of it (●). Left: A D form was assayed at different glucose-6-P concentrations: without (●), 1 mM (○), 5 mM (▲) and 10 mM (△).

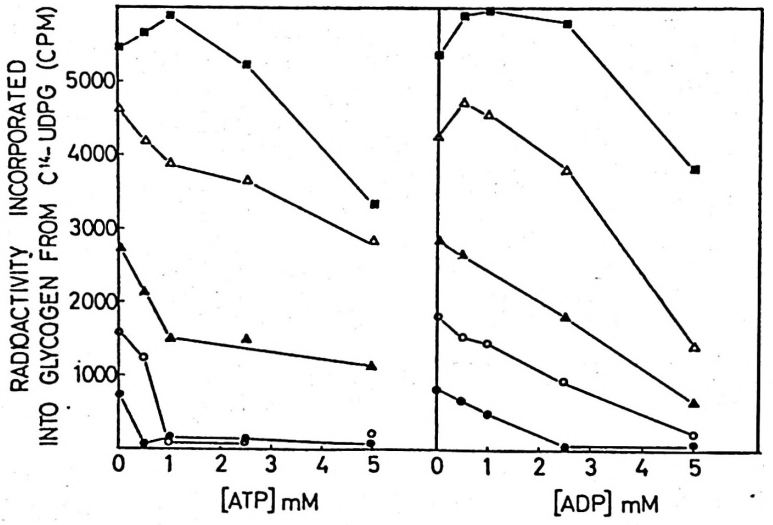

Fig. 7. *Effect of various ATP and ADP concentrations on glycogen synthetase D form.* The enzyme activity was assayed with 0 mM (●), 1 mM (○), 2.5 mM (▲), 5 mM (△) and 10 mM glucose-6-P (■).

than ADP, both competing for the same site, that of glucose-6-P (fig. 7).

Discussion

Two types of glycogen synthetase activities were partially purified from leucocytes of acute myeloblastic, acute lymphoblastic, chronic lymphocytic, acute monoblastic and undifferentiated leukemias. Only one form was obtained from leucocytes of chronic myelocytic leukemias. The purification obtained for the preparations after 100,000 × *g* centrifugation was of 5 to 10 fold. Higher purifications were not obtained because of the scarcity of the biological material, but these preparations allowed us to make some kinetic studies. The preparation of the glucose-6-P independent activity *in vitro* was possible because the D to I form transformation was produced *in vitro* when the crude enzyme was incubated at 30° C with mercaptoethanol and magnesium. The same manipulations, however, did not produce this transformation in the above mentioned chronic myelocytic leucocytes (6).

Both forms were characterized by their kinetics on the UDPG dependence with and without glucose-6-P and Mg^{2+}. The K_m for UDPG has been found to be of the same order of magnitude (10^{-4} M) in all types of leukemic leucocytes. These constants were similar for the independent and dependent form of glycogen synthetase and they are in agreement with the K_m values found for normal leucocytes (5, 12) and lymphocytes (4). In other words, the glycogen synthetase of leukemic leucocytes does not seem to differ from that of normal white blood cells in its dependence on the substrate concentration.

The magnesium effect on the UDPG concentration dependence was different in the I and D form. The V_{max} of the I form increased with magnesium but the K_m did not change. Both K_m and V_{max} were slightly modified by this cation in the D form. This is characteristic behavior in these enzyme forms from many sources.

The glycogen synthetase I was stimulated by glucose-6-P. The K_m for UDPG was significantly decreased in the presence of glucose-6-P, whereas no change in V_{max}

was detected with this activator. The D form had a low affinity for UDPG when glucose-6-P was absent and the addition of the activator produced a large increase in V_{max} and no change in K_m. The glycogen synthetase found in these cells was always a D form and therefore the question persists as to which form is active in the circulating cells.

The concentration at wich glucose-6-P caused half maximal activation on I form was similar to that reported by NAHAS *et al.* (12) in normal polymorphonuclear cells.

The clear differences between the K_a's for glucose-6-P in the D forms of glycogen synthetase from leukemic leucocytes of myeloid type and lymphoid type could be useful to differentiate both types of leukemias. We calculated the activation constants in two undifferentiated leukemias and found that they were similar to those of myeloid cells. The clinical behavior of both patients responded to a myeloid treatment, in accordance with our prediction.

The Mg^{2+} effect was to increase affinity of the enzyme for glucose-6-P, decreasing K_a in a large proportion. No effect of Mg^{2+} on V_{max} was observed. This could signify that the D-form could ve active at least in some type of leukemic leucocytes specially those of myeloid origin.

Inorganic phosphate was an inhibitor of glycogen synthetase D form from leukemic leucocytes as has been described (10, 11). However it was not an inhibitor of glycogen synthetase I. In this case phosphate and glucose-6-P increased the activity slightly. ATP and ADP were inhibitors of the D form of glycogen synthetase in these cells and this inactivation was surmounted by addition of glucose-6-P as it occurs in the dog muscle enzyme (13), and in the enzyme D-form from other sources.

We can conclude that the two forms of glycogen synthetase from leukemic leucocytes seem to have, in general, characteristics similar to the glycogen synthetase from normal leucocytes and also to the same enzyme from other tissues. However, the different order of magnitude in the K_a's found for the myeloid and lymphoid cells, and the glycogen synthetase D phosphatase activity found in all types of cells studied, except the chronic myelocytic (6), could be of help for the diagnosis and medical treatment in some cases.

Resumen

Se han obtenido dos formas de glucógeno sintetasa en la fracción de glucógeno particulado de 100.000 × *g* a partir de leucocitos de pacientes leucémicos. En el momento de aislar los leucocitos el 90 % del enzima se halló en forma D. La forma I se obtuvo preicubando el extracto crudo a 30° C en presencia de mercaptoetanol y Mg^{2+}.

A pesar de los bajos niveles de glucógeno y la escasa actividad glucógeno sintetasa halladas en los leucocitos leucémicos comparados con los normales, los estudios cinéticos de las dos formas de glucógeno sintetasa mostraron que el enzima posee unas caracteristicas similares a las halladas para el mismo enzima en leucocitos normales y en otros tejidos. Los valores de K_m para el substrato UDPG fueron del orden de 10^{-4} M.

La constante de activación para la glucosa-6-P es de un orden de magnitud menor en los leucocitos leucémicos de la serie linfoide (10^{-4} M) que en los leucocitos de la serie mieloide (10^{-3} M).

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