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UDPG-Glucan-Glucosyl-Transferase in Human Polymorphonuclear Leukocytes

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The UDPG:α-1,4-glucan α-4-glucosyl-transferase (transferase) of normal human polymorphonuclear leukocytes was assayed and characterized. The enzyme showed an absolute dependence on the presence of glucose-6-phosphate(G-6-P) and was further stimulated by Mg ions.

The K_m for the substrate UDPG (with G-6-P present) was 2×10^{-3} M without Mg^{2+} and 6×10^{-4} M with Mg^{2+} in the assay.

The activation constant (K_a) for G-6-P was 1.5×10^{-2} M without Mg^{2+} and 1×10^{-3} M with Mg^{2+} present.

Normal human polymorphonuclear leukocytes lack the mechanisms for interconversion of the D (G-6-P dependent) and I (G-6-P independent) forms of transferase.

The activity of transferase in the presence of G-6-P and Mg^{2+} ranged from 3.1 to 7.04 μmoles of glucose incorporated from UDPG into glycogen per 10^8 leukocytes in one hour.

Villar-Palasi and Larner [1,2] demonstrated that insulin stimulated, in rat diaphragmatic muscle, the activity of UDPG:α-1,4-glucan α-4-glucosyl-transferase (transferase) that does not require glucose-6-phosphate (G-6-P) for its action. Recently two forms of transferase were demonstrated in rat,[3] rabbit,[4] dog,[5] and human skeletal muscles [6] and a scheme of interconversion of the two forms was established.[5-8] The I form (independent) does not require G-6-P for activity whereas the D form (dependent) has an absolute or almost absolute requirement for this cofactor.[3-5] Esmann [9,10] showed that insulin has no influence on glycogen formation *in vitro* of leukocytes from normal and diabetic subjects, but that prolonged administration of insulin *in vivo* increased the capacity of leukocytes to synthetize glycogen.

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The presence of transferase activity in human leukocytes was suggested by histochemical studies.[11] Direct evidence of transferase activity has been obtained by several authors,[12–14] and recently [14] it has been claimed that transferase activity is also present in human erythrocytes. So far no details about the type and characteristics of the enzyme in blood cells are available.

The present report deals with some of the characteristics of the transferase found in polymorphonuclear leukocytes from normal subjects. The form of transferase detectable is absolutely dependent on G-6-P for activity.

METHODS

Leukocytes from normal human blood, containing 2–4 erythrocytes for each leukocyte, were prepared as described by Esmann.[9] The cells were washed twice in saline and suspended in 0.05 M Tris — 0.005 M EDTA, pH 7.8, to a final concentration of 1:10 w/v on the basis of 10^9 leukocytes = 1 gram.

The cells (> 90 % polymorphonuclear leukocytes) were completely disrupted by sonication (Mullard Ultrasonic Power Unit, 10 Kc) at 4°C for two or three periods of one minute each and cellular debris eliminated by centrifugating at 3000 × *g* for 10 min in an International refrigerated centrifuge. The supernatant was assayed for transferase activity about two hours after withdrawal of the blood.

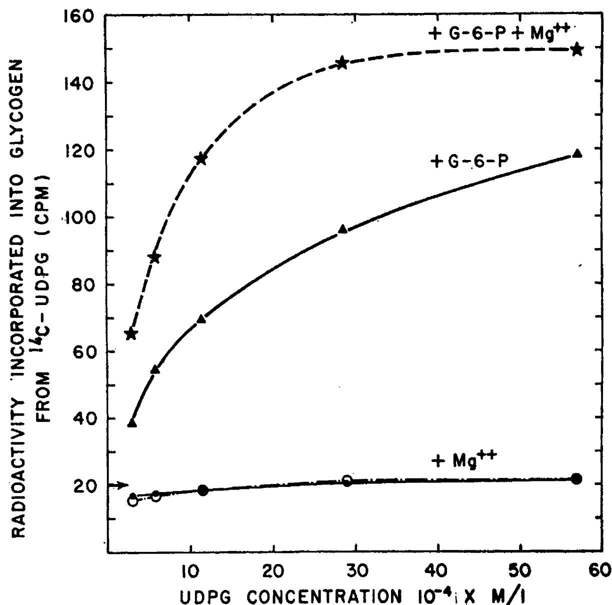

Fig. 1. A leukocyte homogenate was prepared by sonicating 8.45 × 10^8 polymorphonuclear leukocytes suspended in 8.5 ml 0.05 M tris — 0.005 M EDTA buffer, pH 7.8 and freed of cellular debris. The transferase activity of 0.05 ml was assayed at different concentrations of UDPG by measuring the incorporation of ^{14}C-glucose-labeled UDPG into glycogen in 10 min incubations. The concentrations (when present) of glucose-6-phosphate (G-6-P) and Mg^{2+} were 1 × 10^{-2} M and 8 × 10^{-3} M, respectively. The arrow on the left side indicates the background or incorporation without added enzyme.

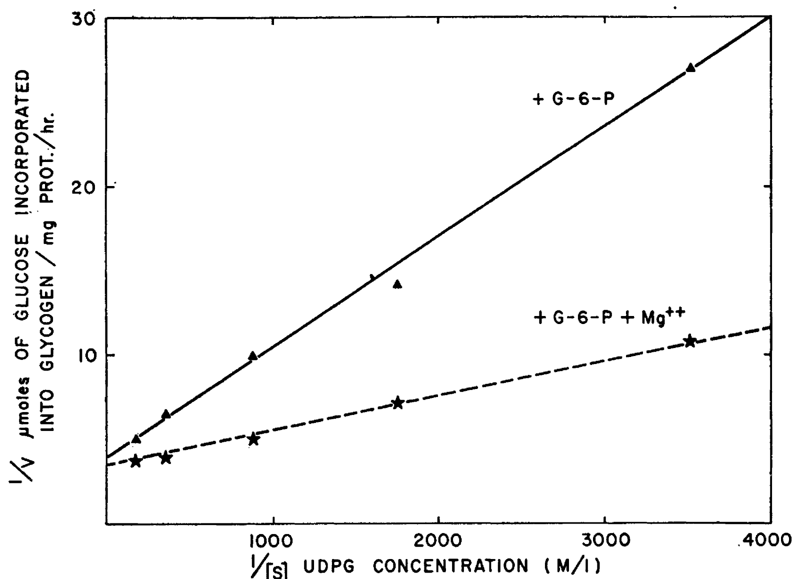

Fig. 2. The Lineweaver-Burk diagram of the dependence of leukocyte transferase on substrate concentration (UDPG). See also legend to Fig. 1.

Transferase activity was measured as the radioactivity incorporated into glycogen from ^{14}C-glucose-labeled UDPG [2] during 5—10 min periods of incubation at 30°C. The incorporation of activity was linear with time for the first 15 min of incubation. The assay mixtures with (when necessary) the addition of Mg^{2+} or G-6-P have already been described.[3-5]

EXPERIMENTAL RESULTS

Under these conditions the detection of transferase activity in normal human polymorphonuclear leukocytes was absolutely dependent on the addition of G-6-P. With a substrate concentration of 5.7×10^{-3} M UDPG and in the presence of 1×10^{-2} M G-6-P the transferase activity ranged from 2.28 to 4.73 μmoles of glucose incorporated into glycogen per 10^8 leukocytes in one hour. When Mg^{2+} was added at a concentration of 8×10^{-3} M the transferase activity almost doubled and ranged from 3.1 to 7.04 μmoles of glucose incorporated per 10^8 leukocytes in one hour. Addition of Mg^{2+} without the presence of G-6-P did not give rise to any transferase activity.

The UDPG dependence of these preparations was studied. Fig. 1 shows the radioactivity incorporated into glycogen in dependence of the UDPG concentration under different conditions. The arrow at the left side of the figure indicates the background or radioactivity incorporated without added enzyme. No activity was detected without G-6-P at any concentration of UDPG, even in the presence of Mg^{2+} (8×10^{-3} M). Activity appeared in the presence of G-6-P (1×10^{-2} M) and the addition of Mg^{2+} greatly stimulated the activity at any concentration of UDPG tested.

Fig. 2 shows the reciprocal plots of the activity-concentration curves. In three experiments the K_m for UDPG determined in the presence of G-6-P was 2×10^{-3} M (range 3×10^{-3} to 1×10^{-3} M). With Mg^{2+} present the value of K_m decreased to 6×10^{-4} M (range 4.2×10^{-4} to 7.5×10^{-4} M).

The influence of the G-6-P concentration on the activity of transferase from normal human leukocytes is shown in Fig. 3. Mg^{2+} (8×10^{-3} M) again markedly stimulated the activity. The activation constant K_a (the concentration of G-6-P that gave half maximal activation) was calculated from the reciprocal plot representation of the activity at different concentrations of G-6-P. Without Mg^{2+} K_a was 1.5×10^{-2} M. The addition of Mg^{2+} (8×10^{-3} M) decreased this value to 1×10^{-3} M (15-fold).

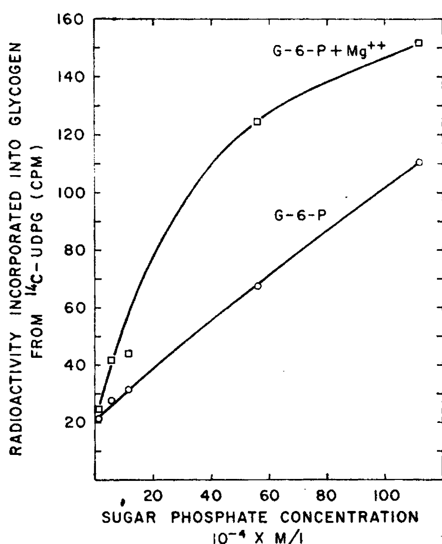

Fig. 3. Leukocyte transferase activity was assayed in the presence of 5.7×10^{-3} M UDPG and with different concentrations of glucose-6-phosphate (G-6-P). The concentration of Mg^{2+} (when present) was 8×10^{-3} M.

The absolute dependence of transferase from human white cells on the presence of G-6-P could not be changed as in the case of the particulate enzyme from dog muscle.[5] Fig. 4 shows the enzyme activity in dependence of the duration of preincubation at 30°C with or without added mercaptoethanol (0.05 M). No rise in activity measured in the absence of added G-6-P could be detected during the time of preincubation. Addition of ATP-Mg had no effect.

DISCUSSION

The data presented here on the transferase activity found in human polymorphonuclear leukocytes indicate that the enzyme present in these cells is a D form.[3-5] This is proved by the absolute dependence of the addition of G-6-P for activity, and by the absence of any stimulatory effect of Mg^{2+} when added without G-6-P. Mg^{2+}, however, definitely enhanced the activity of

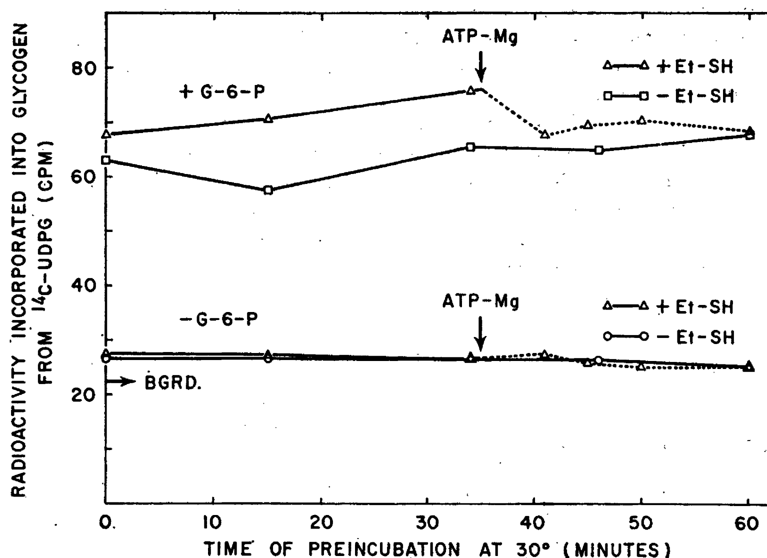

Fig. 4. Leukocyte transferase was assayed at different time intervals after preincubation without and with 0.05 M mercaptoethanol (Et—SH). Concentration of glucose-6-phosphate (G-6-P), when present, was 1×10^{-2} M. At time 35 min ATP—Mg (0.005—0.01 M) was added to the reaction mixture. The assays of enzyme activity were of 6 min duration after the period of preincubation. No rise in activity was observed in mixtures without G-6-P and with Et—SH. No significant fall of activity upon addition of ATP—Mg was observed in mixtures with G-6-P activated enzyme. No transformation of transferase D to transferase I was therefore observed.

leukocyte transferase in the presence of G-6-P. This resembles the action of Mg^{2+} on the D form of the enzyme found in frog skeletal muscle.[15]

The levels of activity of the transferase reported here, although slightly lower, are in agreement with those reported by Miller and Vander Wende.[13] Likewise the transferase values observed in leukocytes by Huiging [16] also coincide with ours. Cornblath *et al.*[14], on the other hand, reported in abstract form values a hundred times or more higher than ours. Some tentative assays done by us on leukocytes from different species seem to indicate that, under our conditions, the range of activity lies under 10 μmoles of glucose incorporated into glycogen per 10^8 leukocytes in one hour. These values are in agreement with direct measurements of the net synthesis of glycogen by intact leukocytes *in vitro* done by Esmann [9] and others.

It is interesting to notice that the transferase present in white blood cells (D form) should not be stimulated by insulin in accordance with data reported by Villar Palasí and Larner [1,2] in rat diaphragm. Previous work by Esmann [9] and recent data by Sasso and Dell' Amore [17] demonstrated that insulin *in vitro* does not stimulate glycogen synthesis in the leukocytes of normal subjects. These findings appear to be in agreement with the type of transferase here

reported and with the lack of systems of interconversion showed in Fig. 4, which would produce the insulin-sensitive I form of the enzyme.

The concentration at which G-6-P causes half maximal activation is high compared to that found for transferases from other sources. The marked positive influence of Mg^{2+} in increasing the affinity for the activator makes more physiological the level of activation by G-6-P and stresses the role of Mg^{2+} in glycogen synthesis of human leukocytes. During conditions of abundant glucose supply the levels of G-6-P may rise sufficiently to stimulate the transferase D, since leukocytes have no glucose-6-phosphatase.[18] With a limited access to glucose, as in an exudate, the low level of G-6-P would favour glycogen degradation and energy release.

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