Studies on UDPG-α-glucan Transglucosylase. IV. Purification and Characterization of Two Forms from Rabbit Skeletal Muscle*

M. ROSELL-PEREZ† AND J. LARNER

From the Department of Pharmacology, School of Medicine, Western Reserve University, Cleveland 6, Ohio

Received June 24, 1963

Previous papers of this series have dealt with the two different forms of UDPG-α-glucan transglucosylase prepared from rat skeletal muscle, and their interconversion. In the present paper, the purification and characterization of the two forms of transglucosylase from rabbit skeletal muscle are presented. Methods for the purification of both forms are included. The dependent or "*D*" form was purified over 600-fold, and the independent or "*I*" form was purified over 150-fold. Kinetic constants of these two purified forms are presented, together with some studies on activation by anions and cations.

Rosell-Perez and Larner (1961) and Rosell-Perez *et al.* (1962) demonstrated that two forms of UDPG-α-glucan transglucosylase (transglucosylase)[1] could be prepared from rat skeletal muscle by extracting frozen stored muscle (*D* form) or by incubating crude ammonium sulfate fractions with mercaptoethanol (*I* form). These two forms were differentiated from each other kinetically by their UDPG concentration dependence in the presence of glucose-6-P and Mg^{++}, as well as by their different sensitivities to inhibition by *p*-mercuribenzoate. Rosell-Perez (1962) and Rosell-Perez and Larner (1962, 1963, 1964) showed further that the enzyme obtained from muscle of toadfish and frog differed markedly in its sensitivity to glucose-6-P when compared to the enzyme from rat. In this respect, these enzymes appeared to resemble the dependent or *D* form of the enzyme more closely than the independent or *I* form in rat.

To extend these studies, the enzyme from rabbit skeletal muscle was investigated in order to determine whether two forms of the enzyme could be prepared and further purified from this species. Previous studies on transglucosylase from rabbit muscle have appeared (Hauk and Brown, 1959; Brown and Kornfeld, 1960; Kornfeld and Brown, 1962).

In the present paper, it is shown that two forms of transglucosylase can be prepared from rabbit skeletal muscle by procedures similar to those used with rat muscle. These two forms have been purified and studied. The K_m and V values were determined for UDPG in the presence and absence of glucose-6-P and Mg^{++} and were compared with the constants of the two forms of the enzyme from rat muscle. The *D* form was purified over 600-fold, and the *I* form was purified about 150-fold. A preliminary communication on the preparation of the two forms of the enzyme from rabbit muscle has already been presented (Rosell-Perez, 1962).

* This work was supported in part by a research grant (A-2366) from the National Institute of Arthritis and Metabolic Diseases of the United States Public Health Service and by a Public Health Service research career program award (AM-K6-985) from the National Institutes of Health.

† Present address: Catedra de Bioquimica, Facultad de Farmacia, Universidad de Barcelona, Pedralbes-Barcelona, Spain.

[1] Abbreviations used are: UDPG, uridine diphosphoglucose; Tris, tris(hydroxymethyl)aminomethane; glucose-6-P, glucose 6-phosphate; transglucosylase, UDPG-α-1,4-glucan transglucosylase; TPN, nicotinamide adenine dinucleotide phosphate; DEAE-cellulose, diethylaminoethylcellulose.

MATERIALS AND METHODS

Analytical.—Transglucosylase was measured as the radioactivity incorporated into glycogen from C^{14}-glucose-labeled UDPG (Villar-Palasi and Larner, 1961). The assay mixtures and conditions were as previously described (Rosell-Perez *et al.*, 1962). The UDPG, Mg^{++}, and glucose-6-P concentrations used in the kinetic studies are stated in the legends of the figures. Glucose-6-P was assayed spectrophotometrically with glucose-6-P dehydrogenase and TPN (Larner *et al.*, 1959). For resuspending the enzyme preparations, 0.05 M Tris–0.005 M Versene buffer, *p*H 7.8, was used. Mercaptoethanol (up to 0.05 M) was added in some of the steps to stabilize the enzyme.

Chemicals.—DEAE-cellulose (type 20, 0.86 meq/mg) obtained from the Brown Company was washed thoroughly with glass-distilled water and was equilibrated with 0.05 M Tris–0.005 M Versene buffer, *p*H 7.8 containing 0.05 M mercaptoethanol. Unlabeled UDPG was purchased from the Sigma Chemical Company. Glycogen (rabbit liver) and glucose-6-P were obtained from Nutritional Biochemicals Corporation; glycogen was reprecipitated once with ethanol before use. Versene was obtained from Fisher Scientific Co., mercaptoethanol from Eastman Kodak Co., Tris from J. Frederick Smith Chemical Co., and *p*-mercuribenzoate from Sigma Chemical Co.

EXPERIMENTAL

Enzyme Preparations.—As in rat, the two forms of the enzyme were prepared from rabbit muscle starting with fresh or freshly frozen muscle (*I* form), or from stored frozen muscle (*D* form).

Rabbits were killed by a blow on the head, and were exsanguinated by section of the neck vessels. Muscle from the hind legs was rapidly removed by dissection and immediately frozen in liquid N_2. The frozen muscle was stored at −20°. For the preparation of the *I* form, the muscle was used within 2 days.

Homogenates (1:10, w/v) of unfrozen or frozen muscle were made. When unfrozen muscle was used, it was weighed, minced with scissors, and then homogenized in a prechilled Waring Blendor with 5 volumes of 0.05 M Tris–0.005 M Versene buffer (*p*H 8.1) for 3 minutes. An additional 5 volumes of the same buffer was added, and the mixture was stirred thoroughly. When frozen muscle was used, it was first powdered in a stainless steel mortar chilled with liquid nitrogen. The frozen powder was weighed, homogenized in 5 volumes of the same buffer, and diluted with an additional 5 volumes of buffer (step 1, Tables I and II).

TABLE I
PURIFICATION OF RABBIT-MUSCLE TRANSGLUCOSYLASE
(*D* Form)

Step	Activity[a] (units/mg protein)		Purification *X*-Fold		Recovery
	−Glucose-6-P[b]	+Glucose-6-P[c]	−Glucose-6-P[b]	+Glucose-6-P[c]	+Glucose-6-P[c]
1. Homogenate (1:10, w/v)	0.005	0.009	—	—	—
2. Supernatant fluid (Lourdes)	0.005	0.020	—	2.2	39.8
3. 100,000 × *g* particulate fraction resuspended and incubated 30 min at 30°	0.019	0.192	3.7	21.4	27.7
4. DEAE fraction III (eluted with 0.25 M NaCl)	0.27	5.2	53.6	576.0	13.4[d]

[a] Unit and specific activity used as defined by the recommendations of the Commission on Enzymes of the International Union of Biochemistry. [b] Assayed in the absence of glucose-6-P. [c] Assayed in the presence of glucose-6-P. [d] Calculated from seven peak fractions containing the enzyme.

TABLE II
PURIFICATION OF RABBIT-MUSCLE TRANSGLUCOSYLASE (*I* FORM)

Step	Assay	Units/ml Activity	Total Units	Specific Activity (units/mg protein)	Recovery (%)
1. Homogenate	−G-6-P[a]	0.137	724.6	0.0051	—
(1:10, w/v)	+G-6-P[b]	0.185	974.9	0.0067	—
2. Supernatant fluid	−G-6-P	0.064	296.6	0.0065	41
(Lourdes)	+G-6-P	0.084	388.8	0.0086	53.6
3. $(NH_4)_2SO_4$, precipitate	−G-6-P	0.233	140	0.0105	19.3
resuspended (1:10)	+G-6-P	0.358	215	0.0156	22.1
4. Dialyzed fluid	−G-6-P	0.390	117	0.018	16.2
	+G-6-P	0.515	154.5	0.024	19.5
Lyophylized powder	10.3 g				
5. Suspension, 20 mg/ml	−G-6-P	0.28	143.3	0.028	19.8
	+G-6-P	0.33	170	0.033	17.5

[a] Assayed without glucose-6-P. G-6-P = glucose-6-P. [b] Assayed with glucose-6-P.

The homogenates were centrifuged at 7000 rpm for 20 minutes in the Lourdes refrigerated centrifuge. The precipitate was discarded (step 2, Tables I and II). Different steps were now used for the preparation of the *I* and *D* forms.

Preparation of the D Form.—For the third step, a high-speed centrifugation was used as described in the preparation of the glucose-6-P-dependent enzyme of toadfish and frog (Rosell-Perez and Larner, 1962). The homogenate (step 1) and extract (step 2) were obtained from frozen rabbit muscle stored in the deep freeze for a period of 2–3 months. The extract was centrifuged for 2 hours at 100,000 × *g* in the Spinco Model L ultracentrifuge. The pellet that was obtained had a ratio of activities determined with and without glucose-6-P over 10. The enzyme present in the pellet assayed with glucose-6-P was purified about 20-fold as compared to the homogenate (Table I).

The particulate nature of the enzyme in the pellet made it unsuitable for column chromatography. It was therefore suspended in a buffer consisting of 0.05 M Tris–0.005 M Versene–0.05 M mercaptoethanol (*p*H 7.8), incubated at 30° for 30 minutes, and then centrifuged for 15 minutes at 15,000 rpm in a Servall centrifuge. Activity was assayed during the incubation. After 30 minutes almost all the activity of the suspension was recovered in the Servall supernatant fluid. The ratio of activities with and without glucose-6-P was essentially unchanged (9.7 as compared to 10.2). The glycogen content of the supernatant fluid increased during the time of incubation, although there was an over-all loss of about 50% as compared to the initial suspension. The enzyme, visibly clarified, was then used for chromatography over DEAE-cellulose.

DEAE-cellulose columns of 1.0–1.2 cm in diameter and 9–10 cm in height (bed volumes 7.1–11.3 ml) were equilibrated overnight in the cold room with a continuous flow of 0.05 M Tris–0.005 M Versene (*p*H 7.8). The ratio of cellulose to protein varied from 35 to 50 in different preparations. Fractions of 3–4 ml were collected. A variable gradient device similar to that described by Peterson and Sober (1962) was used. The two mixing chambers produced a convex gradient elution. It was found that the phosphorylase was eluted at concentrations of NaCl between 0.05 and 0.1 M, whereas the transglucosylase was eluted at a concentration of about 0.23 M NaCl. However, the transglucosylase was spread over a rather wide area. Better results were obtained when the column was first eluted with 0.1 M NaCl to remove the phosphorylase and then with 0.25 M NaCl to elute the transglucosylase. In Figure 1, the results of an experiment are presented. It can be seen that a sharp peak of transglucosylase activity was obtained after elution with 0.25 M NaCl. Table I shows the purification obtained. The transglucosylase activity measured with glucose-6-P was 19.2 times higher than that measured without. The purification of the enzyme measured with and without

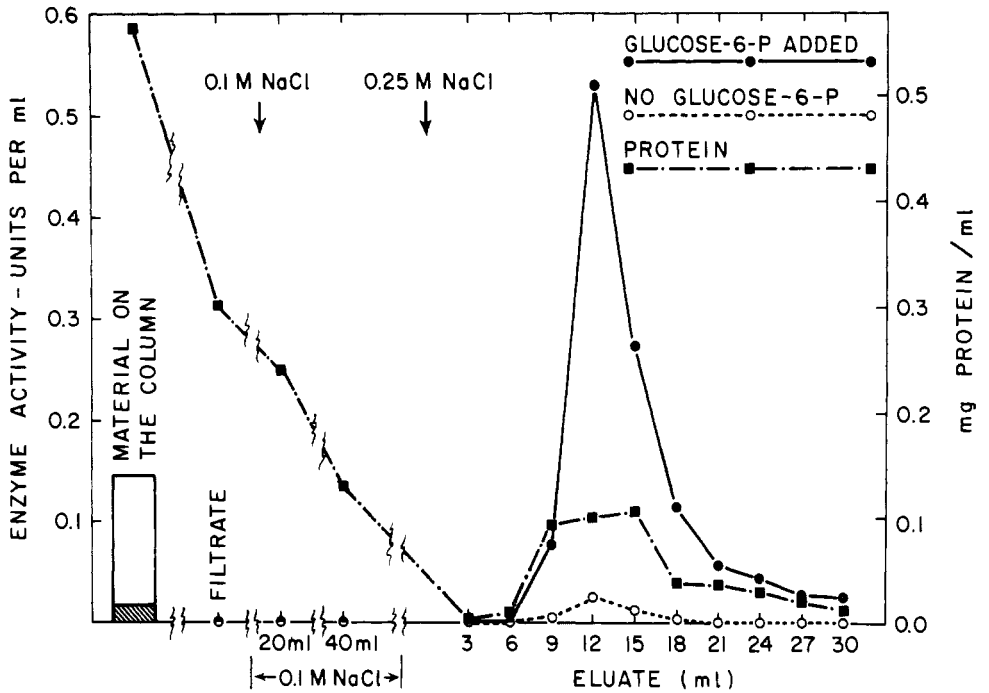

FIG. 1.—Elution diagram of DEAE-cellulose column of the *D*-form preparation. The solid line shows the peak of transglucosylase activity. The dashed line shows the activity detected in the absence of glucose-6-P. No enzyme activity was detected in the first 20 ml.

glucose-6-P was quite different: 53.6 for the *I* (-glucose-6-P) form as compared to over 500-fold for the *D* form (+ glucose-6-P).[2]

The column fractionation produced a 10- to 20-fold purification of the enzyme applied. Attempts made to separate the two enzyme forms by gradient elution were not successful.

Preparation of the I Form.—Crude stable preparations of *I* form could be obtained easily by steps already described for the rat-muscle enzyme (Rosell-Perez *et al.*, 1962). A stable powder with a very low ratio of activites measured with and without glucose-6-P was obtained as starting material by precipitating the enzyme from crude extracts with ammonium sulfate, followed by dialysis and lyophilization.

This powder (10–20 mg), suspended in the buffer mentioned under Materials and Methods, was used for the transformation to the *I* form (Rosell-Perez *et al.*, 1962). Mercaptoethanol was added to a concentration of 0.05 M, and the enzyme suspension was incubated for 60 minutes at 30°. At the end of this period, no stimulation by glucose-6-P was detected.[3] In Table II, the results of a preparation are presented. The recovery of enzyme measured in the absence and presence of glucose-6-P (19.8 and 17.5%) was lower than that obtained with similar preparations obtained from rat muscle.

Further Purification of the I Form.—After incubation with mercaptoethanol for 1 hour at 30°, the enzyme was purified on a DEAE-cellulose column. The peak of activity was eluted with a concentration of NaCl similar to that used for eluting the *D* form. The purification obtained on the column was 4- to 6-fold. The total purification of the *I* form was about 30- to 50-fold compared to the homogenate.

[2] These values are not corrected for the *I* activity initially present in homogenates. If corrected, the purification of the *D* form would be over 1000-fold.

[3] On the basis of the kinetics of the *I* form, a small stimulation by glucose-6-P would have been expected. Traces of sulfate ions present in this preparation could conceivably mask this stimulation.

In order to prepare the *I* form in a more highly purified state, the enzyme which had been incubated in mercaptoethanol was first centrifuged for 2 hours in the Spinco Model L ultracentrifuge at 100,000 × *g* without added NaCl. The activity was found in the 100,000 × *g* particulate fraction. This fraction was resuspended in a buffer consisting of 0.05 M Tris, 0.005 M Versene, and 0.05 M mercaptoethanol (*p*H 7.8), and was incubated for 15 minutes at 30°. The insoluble protein was removed by centrifugation for 20 minutes at 3000 × *g*, and the supernatant fluid that contained the enzyme was then chromatographed on a DEAE-cellulose column as described above for the purification of the *D* form.

Figure 2 shows a typical chromatogram. The column step produced a 10- to 20-fold purification making the total purification about 170-fold in the peak tubes. The enzyme activities measured with and without glucose-6-P were eluted together, and there was a small stimulation in the presence of the sugar phosphate. The ratio of activities with and without glucose-6-P remained constant in all the fractions. The small stimulation observed in the presence of this activator may be due to the concentration of the UDPG in the test mixture. As will be seen below in the kinetic studies, no change in *V* was demonstrated with glucose-6-P. Table III shows the purification obtained in this procedure.

Kinetic Characterization of Both Forms.—The two forms of rat muscle transglucosylase were characterized (Rosell-Perez *et al.*, 1962) by the kinetic differences of the UDPG concentration dependence in the absence and presence of glucose-6-P and Mg^{++}.

Initial experiments with rather crude preparations of the enzyme from rabbit muscle showed marked kinetic differences consistent with those of the two forms from rat muscle. It was therefore of interest to determine whether these kinetic differences were retained with purification.

Figure 3 shows the Lineweaver-Burk plot obtained with a highly purified *D* form of the enzyme from rabbit muscle. In the absence of added glucose-6-P, very low

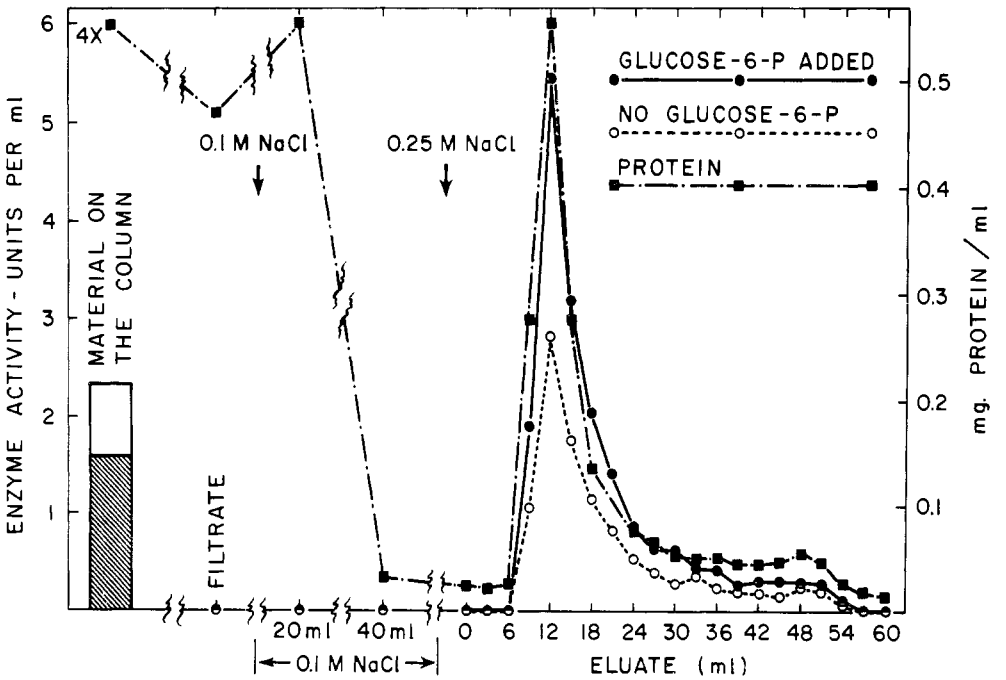

FIG. 2.—Elution diagram of the DEAE-cellulose columns of the *I*-form preparation. The solid and dashed lines represent the activity measured with and without glucose-6-P, respectively. The ratio of activity with and without glucose-6-P was constant. The difference in the stimulation can be accounted for by the UDPG concentration used (4.2×10^{-3} M) (K_m without glucose-6-P = 3.3×10^{-3} M and with glucose-6-P = 4.2×10^{-4} M).

TABLE III
PURIFICATION OF RABBIT-MUSCLE TRANSGLUCOSYLASE (*I* FORM)

Step	Specific Activity (units/mg protein)	Purification *X*-Fold
1. Suspension 20 mg/ml	0.028	5.5[a]
2. Suspension after 60 min incubation	0.029	5.7
3. 100,000 × *g* particulate fraction (resuspended)	0.040	7.9
4. Supernatant fluid after 15 min incubation and low centrifugation	0.067	13.2
5. DEAE fractions (from elution with 0.25 M NaCl		
FRACTIONS—		
III	0.38	75
IV	0.51	100
V	0.63	124
VI	0.84	165
VII	0.85	167
VIII	0.73	144

[a] Referred to the homogenate in Table II.

activity was observed even in the presence of Mg^{++}. As in rat, Mg^{++} depressed the activity of this form as measured without glucose-6-P. The addition of glucose-6-P produced a 50-fold rise in the V at saturating concentrations of UDPG. With this enzyme preparation, a decrease in the K_m for UDPG was also observed in the presence of added glucose-6-P.

Figure 4 shows a similar plot obtained with a purified (150-fold) *I* form of the enzyme. It can be observed that no change in V occurred with added activators. The effect of glucose-6-P was to decrease the K_m of the enzyme for UDPG. Thus a stimulation was detected only at nonsaturating concentrations of UDPG. Mg^{++} alone, as observed with the rat-muscle *I* form, brought about a decrease of the K_m for the UDPG. In the rabbit preparations this effect was less evident and was observed only with certain concentrations of Mg^{++}. In Table IV the K_m values for UDPG determined for both forms of the enzyme are presented.

The Effect of Ions on the Two Forms of the Enzyme.—Ca^{++} and Mg^{++} depressed the activity of the *D* form of the enzyme measured in the presence of glucose-6-P (Table V). When tested on the *I* form, Ca^{++} in the range of concentration of 5×10^{-3} to 1.5×10^{-2} M increased the activity measured without or with added

TABLE IV
KINETIC CONSTANTS DETERMINED FOR THE TWO FORMS OF RABBIT-MUSCLE TRANSGLUCOSYLASE[a]

Enzyme Forms	Constants	Additions to the Test Mixtures: None	Glucose-6-P	Mg^{++}	Mg^{++} + Glucose-6-P
D (576-fold purified)	K_m (moles/liter)	5×10^{-3}	2.6×10^{-4}	7.1×10^{-3}	2.7×10^{-4}
$R^b = 50$	*V* (units/mg protein)	0.07	3.3	0.07	2.8
I (150-fold purified)	K_m (moles/liter)	3.3×10^{-3}	4.2×10^{-4}	2.5×10^{-3}	5×10^{-4}
$R = 1.0$	*V* (units/mg protein)	1.2	1.2	1.2	1.2

[a] Determinations were done with the enzyme obtained after elution from DEAE-cellulose columns. [b] Ratio of activities with and without glucose 6-phosphate.

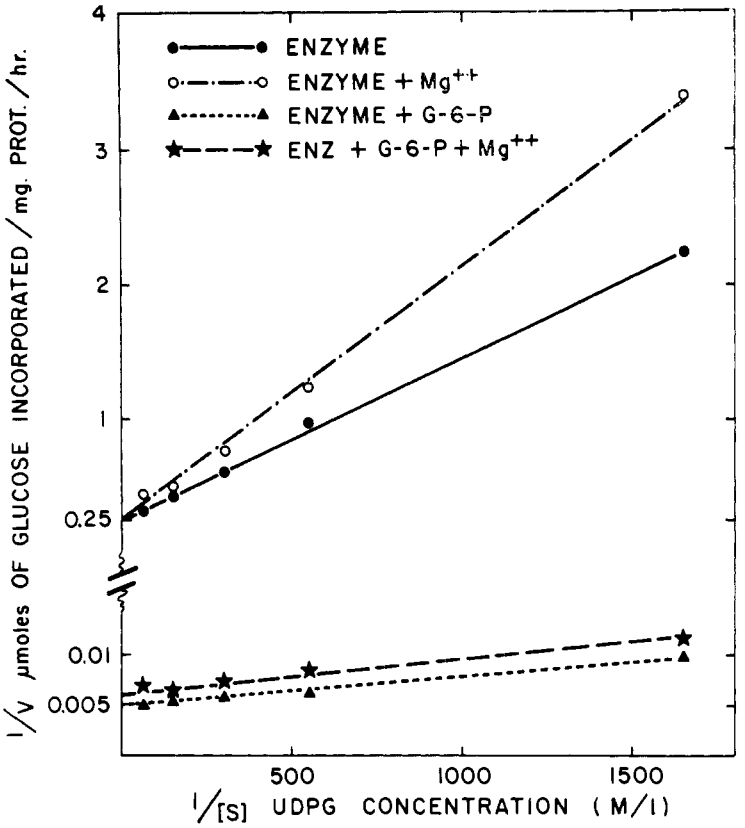

FIG. 3.—Reciprocal plot of the UDPG concentration dependence with a purified *D*-form preparation. The sugar phosphate concentration was 1×10^{-2} M and $MgCl_2$ concentration was 3×10^{-3} M. The differences in *V* at saturating concentrations of UDPG are 50-fold in the presence of glucose-6-P

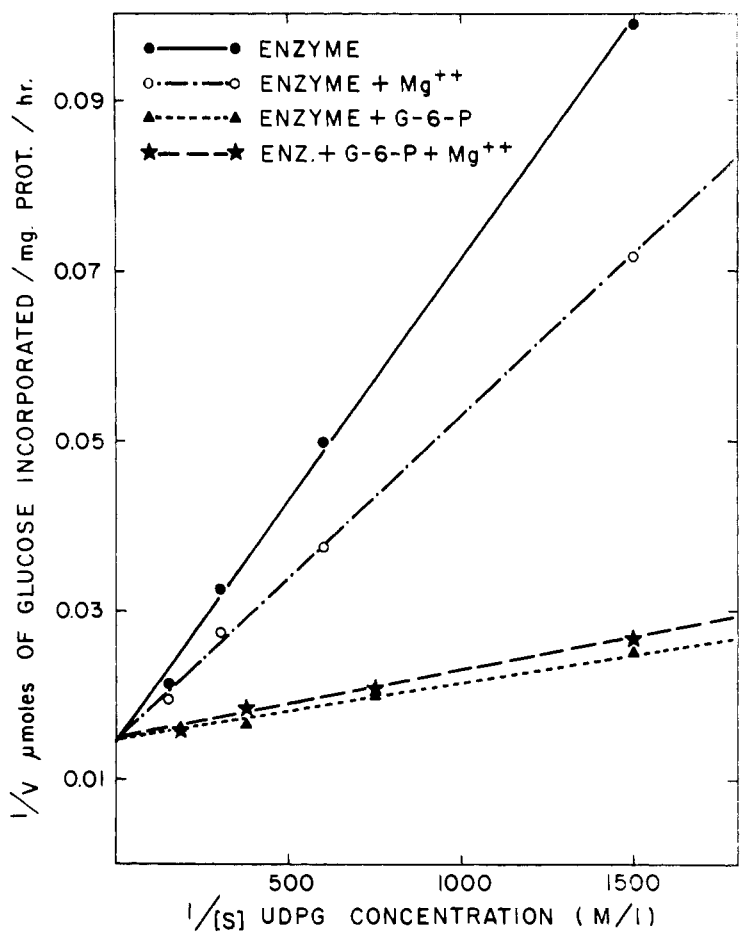

FIG. 4.—Reciprocal plot of the UDPG concentration dependence with a purified *I*-form preparation. Glucose-6-P concentration was 1×10^{-2} M; $MgCl_2$ concentration was 6.6×10^{-3} M. No changes in *V* were detected at saturating concentrations of UDPG.

TABLE V
ACTION OF CATIONS ON RABBIT-MUSCLE TRANSGLUCOSYLASE

Enzyme Preparation	Cations	Glucose-6-P[a]	C^{14}-Glucose Incorporated into Glycogen (cpm)[b] Concentration Tested (Control) 0	5×10^{-3} M	1×10^{-2} M	1.5×10^{-2} M
D form (purified 650-fold)	Ca^{++}	+	77	45	40	23
		−	9	6	6	8
$R^c = 8.5$	Mg^{++}	+	77	47	57	58
		−	9	7	7	9
I form (purified 50-fold)	Ca^{++}	+	116	111	183	157
		−	59	59	106	94
$R = 1.5$	Mg^{++}	+	116	187	127	118
		−	59	74	77	32

[a] The signs + (plus) and − (minus) indicate presence or absence of glucose 6-phosphate in the test. [b] Counts per minute. [c] Ratio of activities with and without glucose 6-phosphate.

TABLE VI
ACTION OF ANIONS $PO_4^{\equiv}$ AND $SO_4^{=}$ ON RABBIT-MUSCLE TRANSGLUCOSYLASE

Enzyme Preparation	Anions	Glucose-6-P[a]	C^{14}-Glucose Incorporated into Glycogen (cpm)[b] Concentration Tested (Control) 0	2.5×10^{-3} M	5×10^{-3} M	1×10^{-2} M	1.5×10^{-2} M
D (100,000 × *g* fraction (Purified 30-fold) $R^c = 5.9$	$PO_4^{\equiv}$	+	770	765	752	730	710
		−	130	217	265	285	290
D (purified 650-fold)	$SO_4^{=}$	+	77	59	58	79	77
		−	9	18	26	29	32
$R = 8.5$	$PO_4^{\equiv}$	+	77	67	66	65	78
		−	9	23	29	33	18
I (purified 50-fold)	$SO_4^{=}$	+	116	114	119	131	149
		−	79	107	107	113	116
$R = 1.5$	$PO_4^{\equiv}$	+	116	127	135	140	130
		−	79	113	122	131	125

[a] The signs + (plus) and − (minus) indicate presence or absence of glucose 6-phosphate in the test. [b] Counts per minute. [c] Ratio of activities with and without glucose 6-phosphate.

glucose-6-P. For Mg^{++}, the range was between 5×10^{-3} M and 1×10^{-2} M. The range of stimulation of the *I* form by Mg^{++} and Ca^{++} was very narrow. The activation by Mg^{++} presented in Figure 4 (Mg^{++} concentration 6.6×10^{-3} M) was reversed by doubling this concentration.

The action of $PO^{\equiv}$ and $SO_4^{=}$ in the range of concentration of 2.5×10^{-3} to 1.5×10^{-2} M on transglucosylase activity was also examined (Table VI). At the concentrations tested, the *I* form of the enzyme was found to be stimulated both with and without glucose-6-P, whereas the *D* form was inhibited in the presence of glucose-6-P and was stimulated in the absence of this cofactor.

DISCUSSION

Two forms of UDPG-α-glucan transglucosylase were prepared and purified from rabbit skeletal muscle. The early steps were similar to those previously used for the preparation of the two forms from rat skeletal muscle (Rosell-Perez *et al.*, 1962). For further purification, chromatography on DEAE-cellulose was found to be useful.

Both forms were characterized by the kinetics of the UDPG concentration dependence with and without glucose-6-P and Mg^{++}. The *D* form had a relatively low affinity for UDPG when glucose-6-P was absent. The addition of glucose-6-P produced a slight decrease in K_m and a large increase in V. Mg^{++} was slightly inhibitory in the absence of glucose-6-P. The *I* form was stimulated by glucose-6-P only at nonsaturating levels of UDPG. The K_m for UDPG was decreased in the presence of glucose-6-P or Mg^{++}, whereas no change in V was detected with either of these activators.

Kornfeld and Brown (1962) have described the preparation and purification of a transglucosylase from rabbit skeletal muscle. The enzyme studied by them appeared to be similar to the *I* form in that it showed a decrease in the K_m for UDPG in the presence of glucose-6-P with only a slight or small change in V. The change in V is consistent with the interpretation that there may be some additional *D* form present in their preparation.

An inorganic activator was found in the supernatant of rat-muscle preparations (Rosell-Perez, 1962) that was identified as inorganic phosphate. Also, previous work demonstrated that rat-muscle transglucosylase was stimulated by sulfate ions. It was previously found that Mg^{++} stimulated only the *I* form of the transglucosylase from rat. The results obtained with the effect of anions and cations in rabbit-muscle preparations are in accordance with the work done on rat-muscle transglucosylase. The bivalent cations, Ca^{++} and Mg^{++}, in a narrow range of concentrations, stimulated the *I* form of transglucosylase slightly and inhibited the *D* form. $SO_4^{=}$ and $PO_4^{\equiv}$ stimulated the activity of the *D* form in the absence of glucose-6-P. When these anions were tested in the presence of glucose-6-P, the activity was depressed. When tested on an *I*-form preparation, both with and without glucose-6-P, the activity was increased. Therefore

it seems reasonable to establish this stimulation by $PO_4^{=}$ and $SO_4^{=}$ as occurring on either the *I* or the *D* form of transglucosylase. On the other hand, Mg^{++} and Ca^{++} appear to stimulate only the *I* form. In paper V it will be demonstrated that phosphate and sulfate ions competitively inhibit the activation by glucose-6-P of the particulate *D* form of transglucosylase from dog muscle (Rosell-Perez and Larner, paper in preparation).

This study on the preparation of two forms of transglucosylase from rabbit muscle, together with the previous one on rat-skeletal muscle (Rosell-Perez *et al.*, 1962), support the hypothesis of the existence of both forms in these mammalian species. This hypothesis was proposed to explain the action of insulin on this system (Villar-Palasi and Larner, 1960).

ACKNOWLEDGMENT

The authors wish to thank Miss Helene Sasko and Mr. Norman Brown for technical assistance in these studies.

REFERENCES

Brown, D. H., and Kornfeld, R. (1960), Abstracts of Papers, 135th Meeting of the American Chemical Society, Sept., 1960, p. 78 c.
Hauk, R., and Brown, D. H. (1959), *Biochim. Biophys. Acta 33*, 556.
Kornfeld, R., and Brown, D. H. (1962), *J. Biol. Chem. 237*, 1772.
Larner, J., Villar-Palasi, C., and Richman, D. J. (1959), *Ann. New York Acad. Sci. 82*, 345.
Peterson, E. A., and Sober, H. A. (1962), *Methods Enzymol. 5*, 3.
Rosell-Perez, M. (1962), *Fed. Proc. 21*, 206.
Rosell-Perez, M., and Larner, J. (1961), *Fed. Proc. 20*, 193.
Rosell-Perez, M., and Larner, J. (1962), *Biochemistry 1*, 769.
Rosell-Perez, M., and Larner, J. (1963), *Fed. Proc. 22*, 463.
Rosell-Perez, M., and Larner, J. (1964), *Biochemistry 3*, 81 (this issue).
Rosell-Perez, M., Villar-Palasi, C., and Larner, J. (1962), *Biochemistry 1*, 763.
Villar-Palasi, C., and Larner, J. (1960), *Biochim. Biophys. Acta 39*, 171.
Villar-Palasi, C., and Larner, J. (1961), *Arch. Biochem. Biophys. 94*, 436.

Studies on UDPG-α-glucan Transglucosylase. V. Two Forms of the Enzyme in Dog Skeletal Muscle and Their Interconversion*

M. ROSELL-PEREZ† AND J. LARNER

From the Department of Pharmacology, School of Medicine, Western Reserve University, Cleveland 6, Ohio

Received June 24, 1963

UDPG-α-glucan transglucosylase (transglucosylase) extracted from fresh skeletal muscle of dog is highly dependent on glucose-6-phosphate for activity. High-speed centrifugation yielded a particulate fraction that had an absolute requirement for the sugar phosphate. It behaved kinetically like the dependent or *D* forms that had been prepared previously from stored frozen rat- and rabbit-skeletal muscle. The *D* form was converted totally to an independent or *I* form by incubating crude extracts for 40–60 minutes at 30° in the presence of mercaptoethanol. This sulfhydryl reagent increased the rate and the yield of the *I* form in the *D*-to-*I* conversion. The kinetic behavior of the *I* form obtained after transformation was similar to the *I* forms of other species studied. The reverse conversion of the *I* to the *D* form was obtained after adding ATP-Mg^{++}. This conversion was enhanced markedly by the addition of adenosine3′, 5′-cyclophosphate to the ATP-Mg^{++} system. UTP-Mg^{++}, in similar concentrations, produced an effect that was three times greater than ATP-Mg^{++}. However, the effect of UTP-Mg^{++} was accompanied by a parallel decrease in the activity measured with glucose-6-phosphate. Because of these and other facts, a scheme of interconversion through an inactive intermediate is postulated. Studies of the inhibitory action of *p*-mercuribenzoate showed that, as in rat, the *I* form was more sensitive than the *D* form to this inhibitor.

UDPG-α-glucan transglucosylase (transglucosylase)[1] has been prepared from the skeletal muscle of a number of different species (e.g., Villar-Palasi and Larner, 1958; Hauk and Brown, 1959; Robbins *et al.*, 1959; Trivelloni, 1960; Takeuchi and Glener, 1960; Hess and Pearse, 1961a,b; Traut, 1962; Kornfeld and Brown, 1962; Rosell-Perez and Larner, 1962, 1963, 1964).

Rosell-Perez and Larner (1961a) and Rosell-Perez *et al.* (1962) demonstrated that two forms of this enzyme could be prepared from rat muscle and differentiated kinetically. Rosell-Perez and Larner (1962) also showed that the form of the enzyme that was prepared from the skeletal muscle of frog and toadfish exhibited differences from that of rat with respect to kinetic behavior toward UDPG with and without glucose-6-P and Mg^{++}. The form of the enzyme from toadfish was highly dependent on glucose-6-P for activity, whereas the enzyme from frog was absolutely dependent. Recently, these authors (Rosell-Perez and Larner, 1963, 1964) isolated, purified, and characterized kinetically both forms, the independent or *I* form and

* This work was supported in part by a grant (A-2366) from the National Institute of Arthritis and Metabolic Diseases of the United States Public Health Service and by a Public Health Service research career program award (AM-K6-985) from the National Institutes of Health.

† Present address: Catedra de Bioquimica, Facultad de Farmacia, Universidad de Barcelona, Pedralbes-Barcelona, Spain.

[1] Abbreviations used in this work: UDPG, uridine diphosphoglucose; Tris, tris(hydroxymethyl)aminomethane; glucose-6-P, glucose 6-phosphate; transglucosylase, UDPG-α-glucan transglucosylase; TPN, nicotinamide adenine dinucleotide phosphate; DEAE-cellulose, diethylami-_oethyl-cellulose.