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URIDINE DIPHOSPHATE GLUCOSE:α-1,4-GLUCAN α-4-GLUCOSYLTRANSFERASE IN HEART
TWO FORMS OF THE ENZYME, INTERCONVERSION REACTIONS AND PROPERTIES

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SUMMARY

In the present paper methods are described for isolating UDP-glucose–glycogen transferase in partially purified form from rat and rabbit heart. The preparation of transferase kinase from rabbit heart free of transferase is also described. Using γ-^{32}P-labeled ATP it is shown that about 12 nmoles of ^{32}P is incorporated per unit *I* form converted to *D* or about 1 μmole of ^{32}P per 20 000 μg of transferase assuming a specific activity of 5 units/mg for the transferase. When ^{32}P-labeled transferase was incubated with a crude heart extract containing phosphatase, there was a coincident loss of radioactivity from the protein together with a conversion of the enzyme from the *D* to *I* form.

Partial acid hydrolysis and high-voltage paper electrophoresis of the ^{32}P-labeled rabbit heart and muscle transferases indicated that the maps were similar but clearly distinguishable.

INTRODUCTION

Because of the responsiveness of the heart to insulin and epinephrine, it has been of interest to examine the UDP-glucose:α-1,4-glucan α-4-glucosyltransferase (EC 2.4.1.11) enzyme system in heart. In accord with the work of others, it is shown that the *I* and *D* forms of transferase are present in rat as well as in rabbit heart. In the present paper, directions for preparing transferase *I* kinase from rabbit heart separated from transferase, and transferase separated from the kinase are detailed. The apparent K_m for ATP of the transferase *I* kinase from rabbit heart is shown to be $4.2 \cdot 10^{-5}$ M. In the kinase-catalyzed conversion ^{32}P from γ-^{32}P-labeled ATP is incorporated into serine phosphate of the transferase to the extent of 12 nmoles ^{32}P incorporated per unit *I* converted to *D*. Radioactive fingerprint studies show that the phosphorylated site of transferase from rabbit heart clearly differs from that of transferase from rabbit skeletal muscle.

* Part of these studies were taken from a thesis of N. E. BROWN to be submitted for the M.S. degree in Biochemistry, University of Minnesota.

In the opposite conversion reaction the phospho or *D* form is converted to the dephospho or *I* form by a phosphatase accompanied by a decrease of radioactivity in the protein. Some properties of the transferase from rat heart are given and differences with the analogous properties of the enzyme from skeletal muscle pointed out.

In another paper[1], experiments demonstrating the hormonal control of transferase *in vivo* and nonhormonal control of transferase by glycogen in the isolated perfused heart *in vitro* are presented.

MATERIALS AND METHODS

Transferase activity was determined by the incorporation of the uniformly ^{14}C-labeled glucose moiety of UDP-glucose into glycogen as described by VILLAR-PALASI *et al.*[2]. To tubes with 0.1 ml of transferase assay mixture containing 6.7 mM UDP-[^{14}C]glucose (specific activity 12 000 counts/min per μmole), 1 mg rabbit liver glycogen, and 50 mM Tris–HCl (pH 7.8), 0.05 ml of enzyme was added. Transferase *I* activity was determined in the absence of glucose 6-phosphate, total transferase activity in the presence of 10 mM glucose 6-phosphate. Tubes were incubated for 15 min (unless otherwise specifically indicated) at 30°, and the reaction stopped by adding 1.0 ml of trichloroacetic acid (6%) containing 1 mg/ml glycogen and 2 mg/ml LiBr. The precipitated protein was removed by centrifugation, and from an aliquot of the supernatant, the glycogen was precipitated with 2 vol. of 95% ethanol. The radioactive glycogen was washed twice with 66% ethanol, and the radioactivity determined in a liquid scintillation counter, Packard Model 3003, using the dioxane–cellosolve scintillation mixture described by BRUNO AND CHRISTIAN[3], allowing the glycogen to settle for at least 1 h (4°) before counting.

Transferase *I* kinase was determined by measuring the conversion of transferase *I* to *D* upon incubation with ATP and $MgCl_2$ basically as described by HUIJING AND LARNER[4]. To a solution containing transferase, ATP, and $MgCl_2$ at the concentrations indicated below (Fig. 2), kinase enzyme was added. The kinase reaction was terminated after 5–7 min by adding EDTA (pH 7) to a final concn. of 20 mM, and the transferase *I* activity that remained after this incubation was determined. For this purpose, aliquots of the kinase reaction mixture were transferred to tubes containing the transferase assay mixture and the transferase was assayed as described above.

Transferase phosphatase activity was measured by the conversion of transferase *D* to *I*, measuring the increase in transferase *I* activity upon incubating a heart extract containing the phosphatase with transferase *D*. For the radioactive experiments, ^{32}P-labeled heart transferase *D* was used as substrate to determine ^{32}P release as well as conversion of *D* to *I* activity. The phosphatase conversion was carried out in 50 mM Tris–HCl, 50 mM mercaptoethanol (pH 7.3–7.4). After varying times, 0.05-ml aliquots were removed and pipetted into 0.1-ml aliquots of standard transferase assay reaction mixture containing 50 mM KF and assayed as above. The KF was included to prevent phosphatase conversion during the transferase assay. The ^{32}P released by phosphatase action was determined by counting the decreased radioactivity in the protein after precipitation with trichloroacetic acid at a final concn. of 5%. After centrifugation, the collected protein was washed five times with 6% trichloroacetic acid containing 50 mM nonradioactive P_i. The protein was then

dissolved by adding 0.5 ml 1 M NaOH, plated on planchets and counted in the Nuclear Chicago gas-flow counter Model D47.

Partial acid hydrolysis was done in 5.7 M HCl at 100° as described previously[5]. After hydrolysis, tubes were evaporated to dryness in a desiccator. Water was added to the samples and they were reevaporated to dryness several times to remove HCl. Samples were then dissolved in a small amount of water, applied to paper and separated by high-voltage electrophoresis according to NAUGHTON *et al.*[6].

Protein concentrations were determined by the biuret method after precipitation with trichloroacetic acid and washing of the precipitates with absolute alcohol. γ-^{32}P-labeled ATP was prepared by the method described previously[5]. For the stoichiometry experiments (Table II) γ-^{32}P-labeled ATP was prepared by the enzymic exchange method of GLYNN AND CHAPPELL[7]. Salivary α-amylase was prepared according to the method detailed by BERNFELD[8], using the procedure through Step 4 (2nd $(NH_4)_2SO_4$ precipitation).

Rabbit liver or oyster glycogen was obtained from Nutritional Biochemical Company. Before use, it was purified by passage of 8–10% solutions over a mixed-bed ion-exchange resin (Amberlite MB-3), reprecipitated with alcohol, and air dried.

RESULTS

Transferase activity in rat heart

In the 12 000 × *g* supernatants of rat hearts homogenized in EDTA, KF (see ref. 1), the transferase activity was markedly stimulated by glucose 6-phosphate. The activity in the absence of glucose 6-phosphate was only 10–20% of the transferase activity in the presence of this sugar phosphate. The total transferase activity ranged from 15 to 25 nmoles glucose incorporated into glycogen from UDPG per min per mg of protein in the 12 000 × *g* supernatant, or from 0.8 to 1.2 μmoles/min per g wet weight of tissue.

Purification of rat heart transferase

As shown in Table I rat heart transferase (*D* form) was purified after 3 steps approx. 250-fold with greater than 50% recovery. Frozen rats hearts were homogenized in 10 vol. (w/v) cold 50 mM KF, 10 mM EDTA (pH 7.0) and the homogenate was centrifuged for 10 min at 12 000 × *g* at 4°. To the supernatant, oyster glycogen

TABLE I

PURIFICATION OF RAT HEART UDP-GLUCOSE–α-GLUCAN GLUCOSYLTRANSFERASE

Fraction	*Vol. (ml)*	*Protein (mg/ml)*	*Specific activity (mU*/ mg protein)*	*Recovery (%)*
12 000 × *g* Supernatant	23	4.6	19	100
15% Alcohol precipitate	10	2.9	51	74
0.25 M NaCl peak	8	0.03	4700	56

* U = μmoles [^{14}C]glucose (from UDP-glucose) incorporated per min.

was added to a final concn. of 1 mg/ml. While the solution was stirred and cooled in an ice–salt bath, 100% alcohol (−20°) was added slowly to a concentration of 15%. The mixture was stirred for 10 min and centrifuged at −10° for 15 min at 6000 × *g*. The precipitate was taken up in 50 mM Tris–HCl, 5 mM EDTA, 50 mM mercaptoethanol buffer (pH 7.8). Insoluble protein was removed by centrifugation for 10 min at 12 000 × *g* at 4°. The clear supernatant was applied to a DEAE-cellulose column (height 150 mm, diameter 12 mm), equilibrated with 50 mM Tris–HCl, 5 mM EDTA and 50 mM mercaptoethanol. Due to the high ionic strength of the buffer, a large part of the applied protein does not bind to the DEAE-cellulose. However, all the transferase activity is retained on the column. Subsequent stepwise elution with the buffer containing 100 and 250 mM NaCl yielded two protein peaks. As in the case for the skeletal muscle transferase[9] only the 250 mM NaCl peak contained the transferase activity, the phosphorylase being eluted in the 100 mM NaCl peak.

Preparation of ^{32}P rabbit heart transferase

To prepare ^{32}P-labeled heart transferase, rabbit heart transferase free of phosphorylase was phosphorylated with [γ-^{32}P]ATP in the presence of transferase *I* kinase.

Five male rabbits were sacrificed after seconal anesthesia. The hearts were quickly removed, washed in cold isotonic saline, blotted and weighed. To 40 g of heart, 240 ml of 250 mM sucrose containing 50 mM Tris–HCl (pH 7.5) was added and the heart homogenized 1.5 min in a Waring Blendor at 4°. The homogenate was centrifuged for 30 min at 17 000 × *g* to remove cellular debris. The supernatant fluid was filtered through glass wool and the pH adjusted to 7.7 with 1 M Tris base; then centrifuged at 150 000 × *g* for 3 h. The pellets so obtained were stored at −100° until used.

18 pellets were resuspended in 100 ml of 50 mM Tris–HCl, 5 mM EDTA, 50 mM mercaptoethanol buffer (pH 7.8) and incubated for 60 min at 30°. The incubated enzyme was then centrifuged 60 min at 50 000 × *g* in the Spinco Model L-2 using the 50T rotor. The supernatant fluid (50 ml) was applied to a DEAE-cellulose column (30 ml bed volume). The column was first washed with 2 bed volumes of Tris–EDTA–mercaptoethanol buffer (pH 7.8) and then washed with 15 bed volumes of Tris–EDTA–mercaptoethanol buffer (pH 7.8) containing 100 mM NaCl to completely remove the phosphorylase. The transferase enzyme was eluted with the same buffer containing 350 mM NaCl. The higher concentration of NaCl (350 mM rather than 250 mM) was used to insure elution of the transferase *I* kinase together with transferase, 20 mg of rabbit liver glycogen was added to the pooled peak fraction, which was then chilled and the enzyme precipitated by adding 95% ethanol to a final concentration of 30% at −10°. After 5 min, the precipitated enzyme was recovered by centrifugation for 15 min at 27 000 × *g*. The enzyme was taken up in 0.50 ml of Tris–EDTA–mercaptoethanol buffer containing 50 mM KF (pH 7.8).

To 0.3 ml of the enzyme, [γ-^{32}P]ATP (1.5 μmoles) and Mg^{2+} (3 μmoles) were added in a total volume of 1 ml. The phosphorylation was allowed to proceed at 30° for 15 min, after which the enzyme was precipitated with ethanol (30%) at −10° as above and the precipitate collected by centrifugation for 15 min. In order to remove the glycogen, the precipitated enzyme was taken up in 0.5 ml of 20 mM Tris, 2 mM EDTA, 50 mM mercaptoethanol containing 40 mM KF (pH 6.9) and the solution

transferred into a dialysis sac. Salivary α-amylase (26 μg) was added, and the enzyme dialyzed against 2 l of the same buffer for 20 h at 4°. To remove retained polysaccharide the ^{32}P-labeled transferase was applied to a Sephadex G-200 column (50 ml bed volume), eluted with the same buffer and the peak fractions containing the radioactivity pooled and used for the phosphate release and other experiments described below. The transferase enzyme prepared from heart was free of detectable phosphorylase and other [γ-^{32}P]ATP-incorporating proteins including phosphorylase kinase. The transferase kinase which was present did not under these conditions itself significantly incorporate radioactivity from [γ-^{32}P]ATP.

Properties of rat heart transferase

The preparation that was obtained consisted almost entirely of the *D* form (6% *I*). The K_m for UDP-glucose determined under conditions previously outlined[9,10], in the presence of glucose 6-phosphate (10 mM) was $9 \cdot 10^{-4}$ M. The K_a for activation by glucose 6-*P* was $2 \cdot 10^{-4}$ M. The K_m for UDP-glucose is three times higher than the K_m for UDP-glucose of rat, rabbit and dog skeletal muscle transferase (*D* form)[9,10]. The K_m for UDP-glucose of the rat skeletal muscle transferase was redetermined at the same time as the K_m for UDP-glucose of the transferase from rat heart. This value for the skeletal muscle enzyme was found to agree well with the published values of the K_m's for UDP-glucose for the rat, rabbit and dog skeletal muscle transferase (*D* forms), indicating that the difference between the K_m's for UDP-glucose of the enzymes from rat skeletal muscle and heart is significant[2].

The heart transferase also differed from the skeletal muscle enzyme in that it was less completely precipitated at pH 5.1–5.8 in the presence of 1 mg/ml of added oyster glycogen than was the muscle enzyme.

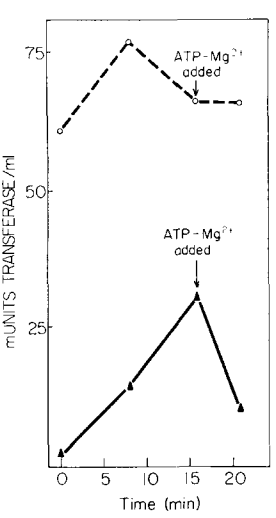
Interconversion reactions of rabbit heart transferase

When transferase *I* and total activity was determined in extracts of rabbit heart (Fig. 1) the initial percent *I* activity was low (4.4%) similar to rat heart. Upon incubation of extracts at 30° the percent *I* activity increased considerably (19.8% at 8 min, 46.2% at 16 min) as the enzyme became converted from the *D* to *I* form. 5 min after addition of 10 mM ATP and 8 mM Mg^{2+} in the presence of 0.1 mM 3′,5′-cyclic adenylate, the percent *I* had decreased toward the initial value (16.1%).

These two interconversion reactions of rabbit heart transferase are completely analogous to those of skeletal muscle transferase described by FRIEDMAN AND LARNER[12]. They are also similar to the two interconversion reactions noted by SØVIK *et al.*[14] as well as by F. HUIJING AND J. LARNER (unpublished observations) in extracts of rat heart. Since the two interconversion reactions of the *D* and *I* forms of transferase in skeletal muscle occur by way of a phosphorylation–dephosphorylation reaction sequence, it became of interest to study the mechanism of the interconversion reactions of heart with [γ-^{32}P]ATP.

Rabbit heart transferase I kinase

When rabbit heart transferase was prepared by DEAE-cellulose column chromatography, transferase and transferase *I* kinase were both eluted free of phosphorylase in the 350 mM NaCl column fraction. If transferase was eluted with 250 mM NaCl, transferase *I* kinase was not detected or was present to a much smaller extent.



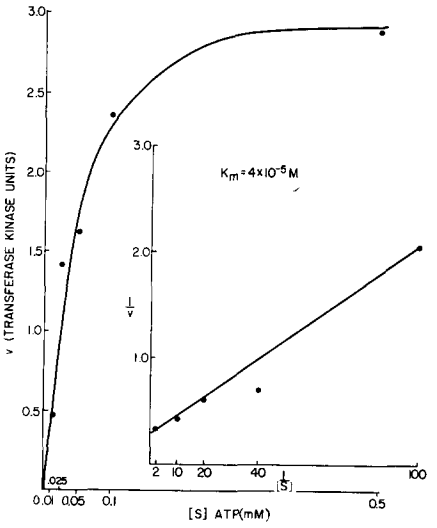


Fig. 1. The heart was removed from a fed male rabbit anesthetized with seconal, washed in ice-cold isotonic saline and homogenized with 7 vol. (w/v) of 250 mM sucrose, 50 mM Tris–HCl (pH 7.5) in the Waring blendor for 2 min. The homogenate was centrifuged for 30 min at 17 000 × *g* at 3°, and the supernatant incubated at 30° for the indicated times. At 16 min, to the experimental tube, ATP (10 mM), $MgCl_2$ (8 mM) and 3′,5′-cyclic adenylate (0.1 mM), all final concentrations, were added. At the indicated times, 0.05-ml aliquots were removed, added to 0.1-ml aliquots of the standard assay mixture (± glucose 6-phosphate), and incubated for 10 min at 30° in order to assay transferase. ▲—▲, transferase *I* activity; ○— — —○, total transferase activity (*D* + *I*).

Fig. 2. Apparent K_m for ATP of rabbit heart transferase *I* kinase. Rabbit heart transferase (0.012 unit transferase *I*) (eluted from the DEAE column with 250 mM NaCl) and rabbit heart transferase *I* kinase (21 units), both prepared as described in the text, were incubated in 45 mM Tris–HCl, 8 mM $MgCl_2$, 4.5 mM EDTA, 40 mM KF and concentrations of ATP which varied from 0.01 mM to 0.5 mM in total volumes of 55 μl at pH 8.2. 30-μl aliquots were then removed after 5 min of incubation at 30° and pipetted into equal volumes of 50 mM EDTA (pH 8.2) to terminate kinase action. 30-μl aliquots were then removed from the kinase-inhibited reaction mixtures, added to 60-μl aliquots of standard transferase assay mixture and then incubated for 12 min at 30° to determine transferase activity.

To completely remove the remaining detectable transferase *I* kinase, the 250 mM NaCl DEAE-cellulose column fraction was reprecipitated at −10° in the presence of oyster glycogen (1 mg/ml) with 95% ethanol added to a final concn. of 15%. Under these conditions, the transferase was precipitated without detectable kinase. To prepare transferase *I* kinase free of detectable transferase, the 150 000 × *g* supernatant already low in transferase was used. 1 mg of oyster glycogen was added per ml of supernatant and the pH lowered to 4.9 by dropwise addition of 1 M acetic acid with stirring. The mixture was allowed to stand at 0° for 5 min and the preciptaited enzyme collected by centrifugation at 27 000 × *g* for 15 min. The precipitate was suspended in 1/15 the original volume of 50 mM Tris–HCl, 5 mM EDTA (pH 7.8) and either used immediately or stored frozen in small aliquots at −60°.

If the transferase *I* kinase so prepared was found to contain detectable transferase activity, the latter was removed by reprecipitation with 95% ethanol at −10° in the presence of 1 mg/ml of added oyster glycogen as above, collecting the fraction

between 15 and 30% ethanol, which contained the kinase without detectable transferase.

When transferase *I* kinase (21 units per 55-μl assay tube)[11] free of detectable transferase was incubated with rabbit heart transferase (0.012 unit per 55-μl assay tube) (containing 1.6 units of transferase *I* kinase per 55-μl assay tube as a contaminant) in the presence of Mg^{2+} and varying ATP concentrations at pH 8.2, an apparent K_m for ATP of $4.2 \cdot 10^{-5}$ M was determined (Fig. 2). This value is similar to the apparent K_m for ATP of $7 \cdot 10^{-5}$ M determined previously for rabbit muscle transferase *I* kinase[12]. The sensitivity of rat heart transferase *I* kinase to 3',5'-cyclic adenylate is documented in another paper[1].

Stoichiometry of ^{32}P incorporation

The ratio ^{32}P incorporated per unit of *I* form converted to *D* was determined. As shown in Table II, using rabbit heart transferase *I* (0.0272 unit) and transferase *I* kinase (5.4 units) neither of which separately incorporated appreciable radioactivity into protein, a mean value of 12 nmoles ^{32}P was incorporated into transferase per unit transferase *I* converted to *D*. This value is 2.5–6 times greater than the 2–5 nmoles per unit transferase converted previously determined in the rabbit skeletal muscle system[12]. When the stoichiometry of the interconversion reaction of the rabbit skeletal muscle transferase enzyme system was redetermined under the same conditions as were used for the quantitation of transferase interconversion in rabbit heart, a mean value of 9.5 nmoles of ^{32}P incorporated per unit *I* form converted to *D* was obtained. The previous experiments are considered to be less accurate than the present ones, since previously two separate parallel reaction mixtures were employed: one for the

TABLE II

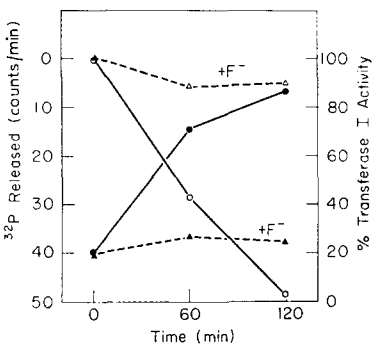
QUANTITATIVE RELATIONSHIP OF ^{32}P INCORPORATION TO CONVERSION OF *I* TO *D* IN RABBIT HEART

The reaction mixture contained [γ-^{32}P]ATP, 0.4 μmole; $MgCl_2$, 3 μmoles; transferase *I*, 0.0272 unit; and transferase *I* kinase, 5.4 units in a total volume of 1 ml, pH 8.2.

Time (min)	*Conversion* I to D (%)*	*D activity formed (μunits/ml)*	*^{32}P incorporated (nmoles/ml)*	*^{32}P incorporated** per unit enzyme converted (natomes ^{32}P/unit I converted to D)*
5	12	3.4	0.0418	12.3
10	16	4.4	0.0447	10.0
30	34	9.3	0.117	12.6
60	55	14.9	0.184	12.3

* Assayed as described in the text.

** 75-μl aliquots were spotted on squares of filter paper and inactivated by immediately immersing them in 10% trichloroacetic acid. Papers were washed 2 times in 5% trichloroacetic acid at room temperature, then once in 5% trichloroacetic acid at 90° for 15 min. This was followed by 2 more washes in 5% trichloroacetic acid at room temperature and successive washes in 50% ethanol–50% ether, and ether. Papers were dried under a heat lamp and counted in the liquid scintillation spectrometer (Packard Model 3003) in a mixture consisting of 5% (w/v) 2,5-diphenyloxazole in toluene.



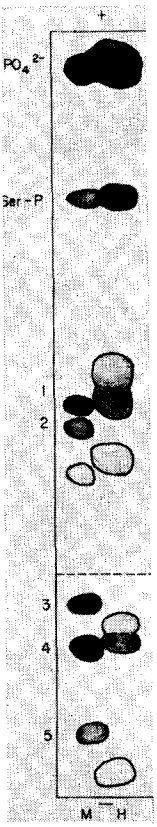


Fig. 3. Phosphatase-catalyzed *D* to *I* conversion reaction. △— — —△, ^{32}P release in the presence of KF, left-hand ordinate; ▲— — —▲, *D* to *I* conversion in presence of KF, right-hand ordinate: ○—○, ^{32}P release in absence of KF, left-hand ordinate; ●—●, *D* to *I* conversion reaction in absence of KF, right-hand ordinate. 0.28 ml ^{32}P-labeled rabbit heart transferase, prepared as described in the text (0.11 unit/ml), was incubated with 0.1 ml unlabeled muscle transferase *D* (14.7 units/ml) prepared as described by VILLAR-PALASI *et al.*[2] together with 0.6 ml rabbit heart extract as a source of phosphatase in the presence and absence of 100 mM KF in a total volume of 1 ml. The heart extract was prepared by homogenizing a rabbit heart with 9 vol. (w/v) of Tris–EDTA–mercaptoethanol buffer (pH 7.8) and centrifuging at 12 000 × *g*. The reaction mixtures were incubated at 30° for 120 min, and at the times indicated 0.05-ml aliquots were removed, analyzed for transferase and 0.1-ml samples were precipitated with trichloroacetic acid, washed, plated and counted for ^{32}P (see MATERIALS AND METHODS).

Fig. 4. Ionogram (pH 3.5, 60 V/cm, 45 min) of acid hydrolysate (5.7 M HCl, temp., 100°, 30 min) of ^{32}P-labeled rabbit muscle (M) and heart (H) transferase; Ser-P, serine phosphate. Bands 1 through 5 from muscle transferase were as denoted in the text and previously[5].

radioactivity incorporation measurements, and the second, which was nonradioactive, for the enzyme activity measurements. In the present experiments both measurements were made using aliquots of a single reaction mixture employing [γ-^{32}P]ATP (ref. 7), rather than [β-^{32}P, γ-^{32}P]ATP as was used previously. From the present experiments, it can be calculated that about 1 μmole ^{32}P was incorporated into 20 000 μg of transferase protein, assuming a specific activity of transferase of 5 units/mg protein (Table I).

Rabbit heart transferase phosphatase

The heart transferase phosphatase was then studied. Using an experimental design similar to that previously employed with the muscle system[12] the conversion of *D* to *I* activity and ^{32}P released from the protein were simultaneously determined. The reaction was run in the presence and absence of KF which has been shown to completely inhibit the phosphatase in skeletal muscle[13]. In order to increase the activity of the transferase enzyme and in order to determine whether the heart phosphatase would utilize as substrate the muscle *D* form of transferase, the following experiment was run. ^{32}P-labeled heart transferase was added to unlabeled muscle transferase *D* (ref. 2), and the combined substrate containing no detectable phosphatase was incubated with a heart extract containing the phosphatase. As shown in Fig. 3, within limits of accuracy of the methods employed, there was a correspondence between conversion of the enzyme from the *D* to *I* form and the decrease in radioactivity in the protein. F^-, which inhibited completely the *D* to *I* conversion reaction, inhibited completely the release of phosphate from the protein. The experiment thus demonstrates that the heart phosphatase will utilize the muscle *D* form as a substrate, although there is no indication as to the relative rates of reaction of the heart phosphatase on the heart as compared to the muscle *D* forms of transferase.

Phosphorylated site of rabbit heart transferase

The enzyme labeled with ^{32}P was submitted to partial acid hydrolysis in 5.7 M HCl at 100° for 30 min. After high-voltage electrophoresis at pH 3.5 and radioautography, a characteristic fingerprint pattern of bands was observed (Fig. 4) which clearly shows that ^{32}P was incorporated into serine phosphate and labeled peptides consisting of three migrating toward the anode and three migrating toward the cathode. When directly compared with the pattern of five major bands from skeletal muscle transferase previously identified[5] muscle band 1 (Ile–Ser-P) and band 4 (composed of Ser-P–Val–Arg and Ile–Ser-P–Val–Arg) overlap corresponding bands of the heart transferase.

The other three bands of the skeletal muscle enzyme were similar in their electrophoretic migration with the four bands of the heart enzyme but clearly did not overlap. It is of interest to note that the 3 skeletal muscle bands which do not overlap at pH 3.5 have in common a structural feature; namely, a glutamic (or glutamine) residue which is not present in those bands which do overlap with the heart enzyme. A similar pattern of partial but not total overlap was observed when the electrophoresis was done at pH 6.5. A strict interpretation of the data must rest in an amino acid sequence analysis of the heart enzyme phosphorylated site. However, it seems that the sites in the muscle and heart enzymes although similar, are not identical.

DISCUSSION

Rat heart transferase was isolated and purified as the *D* form of the enzyme in high yield in a 3-step procedure. A K_m for UDP-glucose of $9 \cdot 10^{-4}$ and a K_a for glucose 6-phosphate of $2 \cdot 10^{-4}$ M were determined. The K_m for UDP-glucose is significantly higher than that of the *D* forms of the muscle enzymes of the species studied previously. The rat heart enzyme also differed in its behavior during fractionation from the muscle enzyme. In confirmation of the work of SØVIK *et al.*[14], rabbit

heart and rat heart have also been shown to contain the two interconversion systems for transforming the *D* and *I* forms of the enzyme and *vice versa*.

The chemical mechanism of the two interconversion reactions was studied in rabbit heart. Transferase *I* kinase was obtained free of transferase by precipitating the 150 000 × *g* supernatant at pH 4.9 in the presence of added glycogen. Traces of transferase still remaining were removed by reprecipitating the kinase with ethanol and collecting the 15–30% fraction. Transferase free of transferase *I* kinase was prepared by reprecipitating the 250 mM NaCl DEAE-cellulose column fraction with ethanol in the presence of glycogen collecting the 0–15% fraction. The apparent K_m of transferase *I* kinase for ATP was determined as $4.2 \cdot 10^{-5}$ M. In the transferase *I* kinase catalyzed *I* to *D* conversion reaction γ-^{32}P from labeled ATP was incorporated into serine phosphate of transferase to the extent of 12 nmoles per unit transferase converted. The serine phosphate was identified in a radioactive fingerprint pattern of the peptides produced after acid hydrolysis of the labeled enzyme which were separated by high-voltage electrophoresis. A direct comparison of the fingerprint patterns of rabbit muscle and heart transferases revealed that the phosphorylated sites of the enzymes were similar but clearly not identical. When ^{32}P-labeled heart transferase *D* together with unlabeled skeletal muscle *D* was converted back to the *I* form by the phosphatase present in an extract of rabbit heart, the rate of ^{32}P release corresponded with the rate of conversion of *D* to *I*. F^-, which completely inhibited the release of ^{32}P, also inhibited the *D* to *I* conversion. It can be concluded therefore that in heart, as well as in skeletal muscle, the chemical mechanism of the interconversion of the *D* and *I* forms of transferase is by a phosphorylation and dephosphorylation of the enzyme.

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