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# PRESENCE OF A SINGLE ENZYME CATALYZING THE PYROPHOSPHOROLYSIS OF UDP-GLUCOSE AND UDP-GALACTOSE IN *BIFIDOBACTERIUM BIFIDUM**

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## Summary

The enzyme preparation catalyzing the pyrophosphorolyses of UDP-glucose and UDPgalactose almost at the same rate was purified about 900-fold from *Bifidobacterium bifidum* grown on glucose medium. The two activities were always associated with each other, and their activity ratio was always constant throughout the purification steps. The final preparation was revealed homogeneous by polyacrylamide gel electrophoresis in the presence and absence of sodium dodecyl sulfate. There was no significant difference in thermal stabilities of the two activities. From these results it was concluded that the UDPglucose and UDPgalactose pyrophosphorylase activities in *B. bifidum* are catalyzed by a single enzyme protein.

Uridine diphosphate galactose (UDPGal) pyrophosphorylase (EC 2.7.7.10) activity provides an alternative pathway of galactose metabolism as follows:

$$\text{Gal-1-}P + \text{UTP} \rightleftharpoons \text{UDPGal} + PP_i \quad (1)$$

$$\text{UDPGal} \rightleftharpoons \text{UDPGlc} \quad (2)$$

$$\text{UDPGlc} + PP_i \rightleftharpoons \text{UTP} + \text{Glc-1-}P \quad (3)$$

$$\text{Gal-1-}P \rightleftharpoons \text{Glc-1-}P \quad \text{Sum}$$

Since the pyrophosphorylase for Reaction 3 (EC 2.7.7.9) and the epimerase for Reaction 2 (EC 5.1.3.2) are widely distributed, it is of interest to characterize UDPGal pyrophosphorylase for Reaction 1. UDPGal pyrophosphorylase activity was discovered by Kalcker et al. [1] in yeast and by

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Isselbacher [2] in mammalian tissues. Nevertheless, they used crude preparation and the possibility of the apparent reactions by hexose-1-*P* uridylyltransferase (EC 2.7.7.12) and UDPglucose (UDPGlc) pyrophosphorylase was not excluded. UDPGal pyrophosphorylase has been demonstrated in crude extract of cultured human skin fibroblasts [3] and human liver [4], but at very low level compared with UDPGlc pyrophosphorylase activity.

We found extremely high UDPGal pyrophosphorylase activity in *Bifidobacterium bifidum* (a predominant bacterium in the stools of breast-fed infants) and reported some properties of the partially purified enzyme [5,6]. Recently we succeeded in purifying the enzyme to homogeneity. *B. bifidum* UDPGal pyrophosphorylase activity was unable to be separated from UDPGlc pyrophosphorylase activity and their activity ratio remained constant throughout the purification. In this report we describe a procedure for the purification of the enzyme and some evidence that both pyrophosphorolysis activities are catalyzed by a single enzyme protein.

*Bifidobacterium bifidum* strain, growth medium and culture conditions were the same as those described previously [5,6]. Glucose-grown cells were used in this experiment. UDPGlc and UDPGal pyrophosphorylase activities were assayed mainly in the direction of their pyrophosphorolyses by the procedures described previously [5,6] except that the concentration of UDPGlc or UDPGal was changed to 8 mM. One unit of the enzyme was defined as the amount which liberates 1 μmol UTP per min at 37°C. All operations for purification were carried out at 0—5°C and the buffer used in these procedures was potassium phosphate containing 20% glycerol.

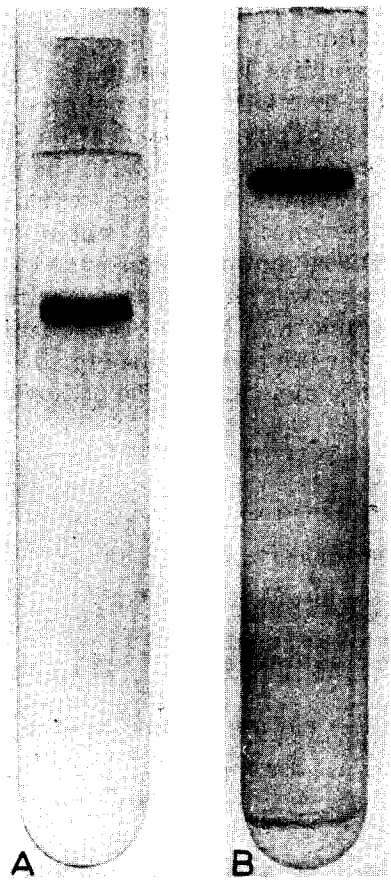
A crude extract, prepared from *B. bifidum* (400 l culture medium) by sonication, was fractionated with solid $(NH_4)_2SO_4$. UDPGlc and UDPGal pyrophosphorylase activities were found in the fraction precipitated between 0.35 and 0.55 $(NH_4)_2SO_4$ saturation. The precipitate was dissolved in 0.01 M buffer (pH 7.5) and dialyzed against the same buffer. The dialyzed solution was applied to a DEAE-cellulose column (5 × 60 cm) previously equilibrated with 0.01 M buffer (pH 7.5). The activities were eluted with the buffer containing 0.35 M NaCl. The active fractions were concentrated and dialyzed against 0.01 M buffer (pH 6.5). The dialyzed enzyme solution was again charged on a DEAE-cellulose column (1.6 × 18 cm) and developed with 0.01 M buffer (pH 6.5). The activities were eluted with the buffer containing 0.2 M NaCl. The active fractions were concentrated and applied on a Sephadex G-150 column (2.6 × 120 cm). The column was eluted with 0.01 M buffer (pH 6.5). The enzyme was then applied to a hydroxyapatite column (1.4 × 3 cm) equilibrated with 5 mM buffer (pH 6.5). UDPGlc and UDPGal pyrophosphorylase activities were still found in the same eluate of 35 mM buffer. The purification procedures are summarized in Table I.

Through these procedures, the two activities were always associated with each other. The enzyme preparation catalyzing the pyrophosphorolysis of UDPGlc and UDPGal was purified about 900-fold. The activity ratio of UDPGal pyrophosphorolysis to UDPGlc pyrophosphorolysis remained almost constant (about 1 : 1) at the standard assay system. The final preparation (hydroxyapatite fraction) was revealed homogeneous by polyacrylamide gel electrophoresis in the presence and absence of SDS (Fig. 1A,B). These results strong-

TABLE I

PURIFICATION OF UDP-Gal and UDP-Glc PYROPHOSPHORYLASE FROM *B. BIFIDUM*

| Step | Protein (mg) | UDPGal activity | | UDPGlc activity | | Ratio of UDPGal / UDPGlc |
|---|---|---|---|---|---|---|
| | | Total (units) | Specific (units/mg) | Total (units) | Specific (units/mg) | |
| 1. Cell-free extract | 42 000 | 797 | 0.019 | 707 | 0.017 | 1.13 |
| 2. $(NH_4)_2SO_4$ (0.35—0.55) | 25 600 | 555 | 0.022 | 486 | 0.019 | 1.16 |
| 3. 1st DEAE-cellulose (pH 7.5) | 599 | 108 | 0.180 | 101 | 0.168 | 1.07 |
| 4. 2nd DEAE-cellulose (pH 6.5) | 45.6 | 42.1 | 0.923 | 38.3 | 0.840 | 1.10 |
| 5. Sephadex G-150 | 5.34 | 20.0 | 3.73 | 18.9 | 3.53 | 1.06 |
| 6. Hydroxyapatite | 0.780 | 13.3 | 17.0 | 11.9 | 15.2 | 1.12 |



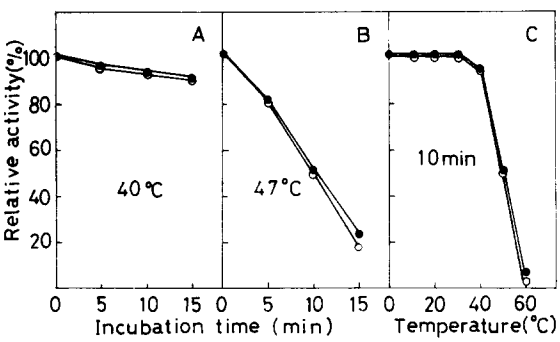




Fig. 1. Electrophoretic patterns of pyrophosphorylase on polyacrylamide gels. (A) Electrophoresis was carried out according to the method of Davis [7]; 20 μg protein was applied. Electrophoresis was conducted at 2 mA for 90 min at pH 8.6 (Tris/glycine). (B) SDS electrophoresis was carried out by the method of Weber et al. [8]; 20 μg protein was applied. Electrophoresis was conducted at 8 mA for 4 h at pH 7.0 in sodium phosphate. Migration is from top to bottom.

Fig. 2. Thermal stability of UDPGlc and UDPGal pyrophosphorolysis activities. Enzyme solution in 0.01 M potassium phosphate buffer (pH 6.5) was incubated at 40° C (A) and 47° C (B). After periods indicated, the activities were assayed. (C) After incubation for 10 min at each temperature indicated the activities were assayed. ○; UDPGlc pyrophosphorolysis activity, ●; UDPGal pyrophosphorolysis activity.

ly suggest that both pyrophosphorolyses for UDPGlc and UDPGal were catalyzed by a single enzyme protein.

When the enzyme solution was heated at 40°C and 47°C, the activities for UDPGlc and UDPGal reduced almost equally (Fig. 2A,B). When the solution was incubated at different temperatures for 10 min, the two activities were also lost at the same rate (Fig. 2C). These results also support that the UDPGal pyrophosphorylase is identical to the UDPGlc pyrophosphorylase. We conclude here that the UDPGlc and UDPGal pyrophosphorylase activities in *B. bifidum* are catalyzed by a single enzyme protein.

Chacko et al. [3] reported the existence of UDPGal pyrophosphorolysis activity in the crude extracts of human skin fibroblasts and concluded that UDPGal pyrophosphorylase was present in addition to UDPGlc pyrophosphorylase based on their differential thermal stabilities. Hansen et al. [4,9,10], on the other hand, found that crystalline human liver UDPGlc pyrophosphorylase which was homogeneous on the criteria of physicochemical and immunological methods catalyzed the pyrophosphorolysis of UDPGal at the rate of 1—10% of UDPGlc, but the two activities showed different heat inactivation rates. However we could not find such a difference in thermal stabilities of *B. bifidum* enzyme. The activity ratio of UDPGal pyrophosphorolysis to UDPGlc pyrophosphorolysis in *B. bifidum* enzyme was much higher than that in the other enzymes. From these facts this enzyme in *B. bifidum* is thought to be a new type of UDPGlc pyrophosphorylase. The presence of this type of pyrophosphorylase strongly suggests the existence of an alternative pathway of galactose metabolism in *B. bifidum*.

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