Short Communication

The Equilibrium of the Reaction Catalyzed by Sucrose Phosphate Synthase

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ABSTRACT

The equilibrium constant for the reaction catalyzed by sucrose phosphate synthase was reported 25 years ago to be 3250 at pH 7.5. It has been redetermined and found to be about 2 in the direction of synthesis and 6 to 10 when measured in the opposite direction.

The reversible reaction catalyzed by the enzyme sucrose-P synthase is as follows: UDP-α-d-glucose + d-fructose 6-P ⇌ sucrose-P + UDP. That reaction is ubiquitous in green plants and of fundamental metabolic importance. Its equilibrium constant was reported to be 3250 at pH 7.5 (5) while that for the similar reaction by which sucrose is synthesized, UDP-α-d-glucose + d-fructose ⇌ sucrose + UDP, was determined to be 5 (2). The striking difference in those equilibria was questioned long ago since it seems to be without thermodynamic justification (6). In fact, an apparent equilibrium constant less than 20 can be estimated from data given by the author in the "Methods" section of his paper (5). Nonetheless, the reported value of 3250 has been repeated in various textbooks over the years without comment and, as far as I can learn, has never been examined further. The determination of the equilibrium constant is the subject of this paper. It appears not to differ significantly from that for the synthesis of sucrose.

MATERIALS AND METHODS

Enzyme. Sucrose-P synthase isolated from wheat germ was purchased from Sigma. It is prepared by a proprietary process, hence the details are not known to me. The preparation is stored and shipped by Sigma in solution in a buffer which contains 60% glycerol and 1 M (NH₄)₂SO₄. It was stored in the laboratory at −15°C, and portions, when needed, were dialyzed in the cold overnight against two volumes of 0.25 M Tris-HCl, 1 mM EDTA, 10 mM mercaptoethanol buffer (pH 7.5). After dialysis, it maintained its activity for at least 10 days at 5°C.

Reagents. Uridine 5'-diphosphate α-d-glucose labeled with ¹⁴C in the α-d-glucose moiety (150 μCi/μmol) was synthesized enzymically (7) from UTP and ¹⁴C-labeled α-d-glucose 1-P obtained from Amersham. ¹⁴C-Labeled sucrose-P α-d-glucopyranosyl [1→2]β-d-fructofuranosyl 6-P was produced from UDP-α-[¹⁴C]glucose and d-fructose 6-P in the course of the experiments. It was consequently labeled in the α-d-glucosyl moiety. All other reagents were purchased from the usual commercial sources.

Isolation and Measurement of Reaction Components. Compounds were isolated from reaction mixtures by electrophoresis on paper at pH 2.7 (0.1 M ammonium formate) and their radioactivity was determined as described previously (1). In some cases, sucrose-P concentration was measured by the phenol/sulfuric acid method with sucrose as a standard (4).

Estimation of Reaction Equilibria. A typical reaction mixture contained in a total volume of 23 μl: 0.015 μmol UDP-α-d-glucose [¹³C] (about 0.02 μCi), 0.02 μmol d-fructose 6-P (Na), 0.1 μmol MgCl₂ and 15 μl sucrose-P synthase (0.1 unit; 0.28 mg protein) in the Tris-HCl, EDTA, mercaptoethanol dialysis buffer (pH 7.5). Mixtures were incubated in sealed thin wall glass capillaries at 37°C for 40 min and were applied directly to the electrophoresis paper moistened with buffer on the apparatus.

RESULTS AND DISCUSSION

To calculate the equilibrium constant in the forward direction (toward the synthesis of sucrose phosphate) the concentrations of reactants and products at the end of the reaction period was estimated as follows:

\[
\frac{[\text{UDP-α-d-glucose}]}{\text{Total } ^{14}\text{C}}} = \frac{[\text{UDP-α-d-glucose}]}{[\text{UDP-α-d-glucose}]} \times \frac{[\text{UDP-α-d-glucose}]}{[\text{UDP-α-d-glucose}]},
\]

\[
\frac{[\text{sucrose-P}]}{\text{Total } ^{14}\text{C}}} = \frac{[\text{sucrose-P}]}{[\text{sucrose-P}]} \times \frac{[\text{sucrose-P}]}{[\text{sucrose-P}]},
\]

\[
[\text{UDP}] = [\text{sucrose-P}]
\]

\[
[\text{d-fructose 6-P}] = [\text{d-fructose 6-P}], - [\text{sucrose-P}]
\]

In five experiments equilibrium values of 1.8, 1.8, 1.8, 1.92, and 1.5 were calculated for an average apparent \( K_{eq} = 1.8 \).

The only radioactive compounds produced by this enzyme preparation under these conditions were sucrose-P and UDP-α-d-glucose. Increased time of incubation and/or further addition of enzyme did not change the proportions of reactants and products upon termination of the reaction. Upon extended incubation some hydrolysis of UDP to UMP was noted.

Some of the sucrose-P synthesized enzymically in these determinations was combined and isolated again by paper electrophoresis at pH 2.7. It was used to determine the equilibrium constant of the reaction in the reverse direction (toward the synthesis of UDP-α-d-glucose and d-fructose 6-P). Because of the small amounts of material available it was difficult to obtain reliable precision. Equilibrium values of 6, 8, and 13 were calculated in three experiments.

The difference in the equilibrium values I have determined from those reported 25 years ago may be due to impurities in the sucrose-P substrate used at that time. It had been synthesized enzymically, purified on an ion exchange resin, and precipitated.
as the calcium salt. Although CaCl₂ added to my reaction mixtures had no effect on equilibrium concentrations, it was found that the reverse reaction was considerably reduced in extent when sucrose P synthesized enzymically had been obtained by elution from paper chromatograms instead of from electrophoresis papers. Using sucrose P prepared from paper chromatograms, apparent equilibrium constants as high as 50 to 100 were obtained.

The difference in equilibrium constants measured in the forward and reverse directions is not unprecedented in the biochemical literature (3, 8, 9). In a particularly relevant publication, for example, Pontis and Salerno (8) showed that in the reaction catalyzed by sucrose synthetase from wheat seeds there are protein factors that inhibit sucrose synthetase cleavage activity without affecting the synthesis of sucrose.

LITERATURE CITED
1. Barber GA 1985 The synthesis of guanosine 5'-diphosphate D-glucose by enzyme extracts of mung beans (Phaseolus aureus) and other higher plants.