NOTES

FRUCTOSE-1,6-DIPHOSPHATASE FROM *PSEUDOMONAS SACCHAROPHILA*

DEXTER D. FOSSITT AND I. A. BERNSTEIN

Departments of Biological Chemistry and Dermatology and the Institute of Industrial Health, The University of Michigan, Ann Arbor, Michigan

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During a study of the pathways for biosynthesis of ribose and deoxyribose in *Pseudomonas saccharophila* (Doudoroff, Enzymologia **9**:59, 1940; Fossitt and Bernstein, J. Bacteriol. *in press*), a phosphatase was found which would convert fructose-1,6-diphosphate to inorganic phosphate and fructose-6-phosphate. The studies reported here present the purification and properties of the enzyme.

The organisms were grown on a liquid mineral medium described by Wiame and Doudoroff (J. Bacteriol. **62**:187, 1951) with sodium gluconate as the carbon source. Extracts were prepared from the cells by the procedure of Weimberg and Doudoroff (J. Biol. Chem. **217**:607, 1955). Protein fractionation of the extract was carried out by adding a saturated solution of ammonium sulfate (adjusted to pH 7.5 with NH_4OH) to the extract. Protein precipitates were obtained from 0 to 25, 25 to 40, and 40 to 60% saturation with ammonium sulfate. The "40 to 60%" fraction was placed on a G-25 Sephadex column (3 by 40 cm) which had previously been equilibrated with 0.005 M tris(hydroxymethyl)aminomethane (tris)-HCl (pH 8.0). The column was eluted with 0.005 M tris-HCl (pH 8.0), and 10-ml fractions were collected. Portions of the fractions were assayed for protein (optical density at 280 mμ) and for inorganic salts (precipitation with 1% $BaCl_2$ in 0.5 N HNO_3). The protein fraction obtained (salt concentration approximately 0.005 M) was placed on a diethylaminoethyl cellulose column (2 by 16 cm), which had previously been equilibrated with 0.005 M tris-HCl (pH 8.0). Gradient elution was carried out with 0.005 M tris-HCl (pH 8.0) in a 500-ml mixing flask and 1 M tris-HCl (pH 8.0) in a reservoir (Fig. 1). The purified enzyme showed a requirement for cysteine and magnesium ions. Data from a typical purification procedure are shown in Table 1.

For a study of the stoichiometry of the reaction, a solution of 8 ml (0.3 units) of the purified phosphatase, 3.8 ml of 0.5 M tris-HCl (pH 7.5), and 0.1 ml of magnesium fructose-1,6-diphos-

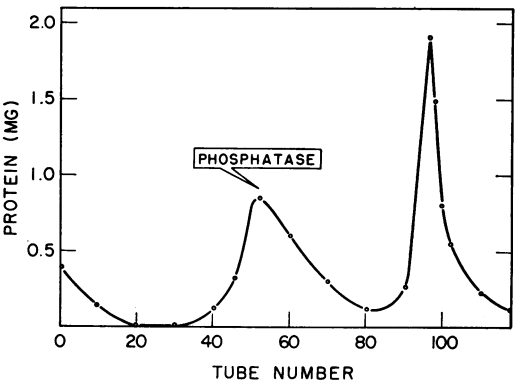

FIG. 1. *Chromatography of fructose-1,6-diphosphatase from Pseudomonas saccharophila on a diethylaminoethyl cellulose column. A 5-ml amount of eluant was collected in each tube. Details of the procedure are described in the text.*

TABLE 1. *Purification of fructose-1,6-diphosphatase from Pseudomonas saccharophila**

Fraction	Amount	Protein	Specific activity
	units		
Crude extract	(13.2)	9,720	(0.0013)
$(NH_4)_2SO_4$ 40 to 60% ppt	13.0	2,180	0.0061
Sephadex	10.3	850	0.012
Diethylaminoethyl cellulose	9.7	37.3	0.491

* Standard conditions of assay and preparation of fractions as described in text. Parentheses indicate values which were estimated since the assay system was not accurate for crude extracts.

phate were incubated at 37 C. At time intervals, samples of the incubation mixture were acidified, centrifuged, neutralized to pH 7.0 with dilute NaOH, and assayed for glucose-6-phosphate and fructose-6-phosphate (Racker and Schroeder, Arch. Biochem. Biophys. **74**:326, 1958), inorganic phosphate (Fiske and SubbaRow, J. Biol. Chem. **66**:375, 1925), and fructose-1,6-diphosphate (Racker, J. Biol. Chem. **167**:843, 1947). The results are shown in Table 2, and indicate that for each mole of fructose-1,6-diphosphate which disappeared, 1 mole of inorganic phosphate and 1 mole of fructose-6-phosphate were formed.

Various sugar phosphates were tested as substrates for the purified phosphatase. Glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, ribose-5-phosphate, and dihydroxyacetone-phosphate were inactive as substrates. Of the substrates tested, only fructose-1,6-diphosphate was dephosphorylated by this enzyme.

TABLE 2. *Stoichiometry of phosphatase reaction*

Compound	Initial	Final
	μmoles	*μmoles*
Fructose-1,6-diphosphate...	10.0	0.94
Inorganic phosphate.....	0.0	9.36
Fructose-6-phosphate*.....	0.0	7.76
Glucose-6-phosphate*.....	0.0	1.52

* Enzyme preparations contained hexose phosphate isomerase activity. Standard assay procedures were as described in the text.

SAFRANINE O: RELIABLE SELECTIVE DYE FOR CHARACTERIZATION OF *BRUCELLA SUIS*

M. MOREIRA-JACOB

National Laboratory of Veterinary Research, Lisbon, Portugal

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Among the tests used for the taxonomic division of *Brucella* organisms, the dye-inhibition test is generally regarded as fundamental because, apart from offering valuable results, it is the only Huddlesonian test capable of differentiating *per se* the three basic species of the genus: *B. melitensis*, *B. abortus*, and *B. suis*. In its original version, the test included four dyes: thionine, basic fuchsin, methyl violet, and pyronine (Huddleson, Am. J. Public Health **21**:491, 1931). Later, Huddleson (*Brucellosis in Man and Animals*, rev. ed., Harvard University Press, Cambridge, Mass., 1943, p. 49) discontinued the use of the last two substances. Both versions of the test have been recommended and followed, but several investigators of repute in this field have given preference to the sole use of the first two anilines (Renoux, Carrère, and Quatrefages, Ann. Inst. Pasteur **82**:277, 1952; Hulse, World Health Organization, Expert Panel on Brucellosis, Rept. no. 78, 1952; Spink, *The Nature of Brucellosis*, University of Minnesota Press, Minneapolis, 1956, p. 39; Parnas and Kosakowna, Arch. Inst. Pasteur Tunis **35**:179, 1958; Alton, Brit. Vet. J. **115**:96, 1959; Castañeda, Bull. World Health Organ. **24**:73, 1961). In fact, I have tried methyl violet and pyronine in more than 90 strains of the different species, and none of the dyes has shown enough selectivity to deserve being included as a determinative bacteriostatic for *Brucella* speciation.

Meanwhile, the search for a substitute seems to be well justified by the following points: (i) a substitute would reinforce the results of the other conventional methods of identification; (ii) it might help reduce the number of so-called "intermediate" or aberrant strains so frequently found in the genus *Brucella*; and (iii) it would help in the identification of *B. suis*, which is serologically indistinguishable from *B. abortus*. Moreover, it would be particularly helpful in identifying the two *B. suis* variants: H_2S-negative and thionine-resistant, respectively (*B. suis* types II and III of Huddleson). With this aim in view, it was decided to give a trial to safranine O. An aniline of the quinone-imide group, it yielded promising results to Cameron and Meyer (Am. J. Vet. Res. **13**:10, 1952) and later to Pickett, Nelson, and Liberman (J. Bacteriol. **66**:210, 1953). By a series of preliminary experiments, it was inferred that the limits of selective concentration of safranine (not corrected for impurities) were