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Abstract

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Wampler, D. E. and J. L. Fairley. An assay for argininosuccinate synthetase in Neurospora.

During an investigation of arginine synthesis in N. crassa, it become necessary for us to assay for argininosuccinate synthetase. A method bored on the disappearance of citrulline from reaction mixtures has been described (Newmeyer 1962 J. Gen. Microbiol. 28: 215), but little information was presented concerning conditions for optical activity. The present report describes a modified, more sensitive procedure developed in this laboratory.

Culture conditions and the method of preparing acetone powders have appeared elsewhere (Fairley and Wampler 1964 Arch. Biochem. Biaphyr. 106: 153). The acetone powder is added to 0.01 M phosphate buffer pH 7.4 (1 g/9 ml), homogenized in a Servall Omnimixer and centrifuged for 15 min at 23,000 x g. The residue is resuspended in a small volume of buffer and recentrifuged. The combined supernatant liquids (containing IO-15 mg protein/ml) are added to a column of Sephadex G-25 (a 2.2 x 25 cm column is adequate for a 15-ml sample) which has been equilibrated with 0.01 M phosphate buffer, pH 7.4. The type of buffer is not critical and any pH between 7.0 and 8.2 is satisfactory. Protein is eluted with the same buffer and tubes containing more than 2 mg protein per ml, as measured in the Biuret reaction, are saved. Preparations which are not parsed through Sephadex (or dialyzed for several hours) do not exhibit activity which is linear with enzyme concentration.

The Sephodex-treated enzyme is assyed in a Z-ml reaction mixture which contains: 38 μmoles potassium aspartate, pH 8.0; 22 μmoles $MgSO_4$; 1.5 μmoles L-citrulline; 2.0 μmoles ATP; 20 μmoles 3-phosphoglyceric acid and 100 μmoles Tris-chloride buffer, pH 8.0. In this crude stage at least 0.5 mg of protein must be used for appreciable reaction to occur. The mixture is incubated at 37°C for 10 minutes and the reaction is stopped with 1 ml of 1 N perchloric acid. Three ml of water is added (final volume 6 ml) and the mixture is centrifuged. A one-ml sample of the supernatant is assayed for citrulline by the method of Gerhord and Pardee (1962 J. Biol. Chem. 237: 891). The reaction can be run with 0.5 μmoles citrulline and arrayed without diluting (final volume 3 ml) but these conditions result in slightly lower apparent activity.

The reaction is linear with respect to protein concentration within the range used and with respect to time up to 10 minutes (Fig. 1). One of the reagents, ATP, is both a substrate and an inhibitor for the reaction as shown in Fig. 1. Fig. 2 shorn the

Fig. 1

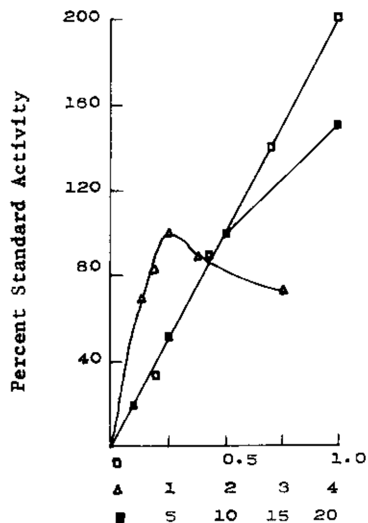
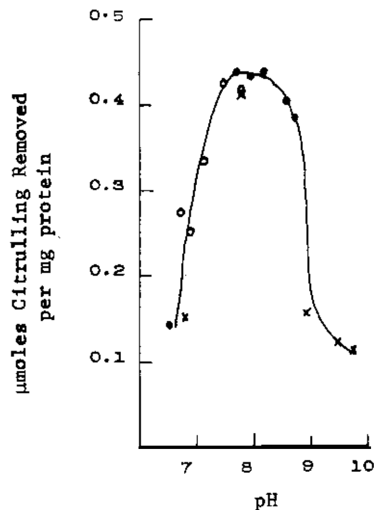


Fig. 2



relationship between activity and pH, using three buffers. There is a fairly broad pH optimum centered near pH 7.8.

The enzyme is quite stable, retaining at least 90% of its original activity after storage at 3°C for three days. It can also be dialyzed for up to 16 hours without detectable loss of activity.

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