

An assay for argininosuccinate synthetase in Neurospora

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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 became necessary for us to assay for argininosuccinate synthetase. A method based on the disappearance of citrulline from reaction

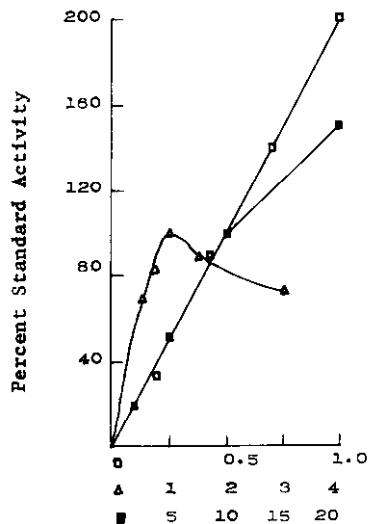
mixtures has been described (Newmeyer 1962 J. Gen. Microbiol. 28:215), but little information was presented concerning conditions for optical activity. The present report describes a modified, more sensitive procedure developed in this laboratory.

Culture conditions and the method of preparing acetone powders have appeared elsewhere (Fairley and Wampler 1964 Arch. Biochem. Biophys. 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 re-centrifuged. The combined supernatant liquids (containing 10-15 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 passed through Sephadex (or dialyzed for several hours) do not exhibit activity which is linear with enzyme concentration.

The Sephadex-treated enzyme is assayed in a 2-ml reaction mixture which contains: 38 μ moles potassium aspartate, pH 8.0; 22 μ moles $MgSO_4$; 1.5 μ moles L-citrulline; 2.0 μ moles ATP; 20 μ moles 3-phosphoglyceric acid and 100 μ moles 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 Gerhard and Pardee (1962 J. Biol. Chem. 237: 891). The reaction can be run with 0.5 μ moles citrulline and assayed without diluting (final volume 3 ml) but these conditions result in slightly lower apparent activity.

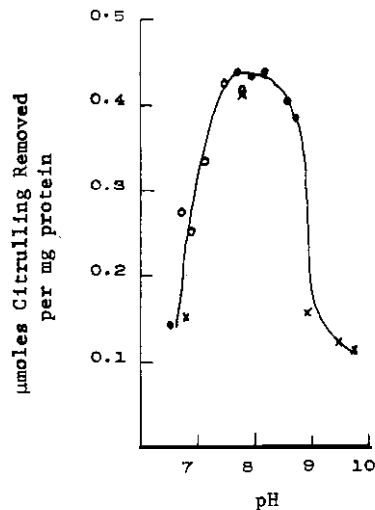
The reaction is linear with respect to protein concentration within the range used and with respect to time up to 10 minutes (Fig. 1). One of the reagents, ATP, is both a substrate and an inhibitor for the reaction as shown in Fig. 1. Fig. 2 shows the

Fig. 1



- Enzyme concentration in mg protein per ml
- ▲ ATP concentration in M x 10³
- Time in minutes

Fig. 2



- Potassium Phosphate
- × Glycine-NaOH
- Tris-chloride

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. ■ ■ ■ Department of Biochemistry, Michigan State University, East Lansing Michigan. (Resent address of DEW; Dartmouth Medical School, Hanover, New Hampshire).