Catcheside, D. E. A. The extraction, partial purification and assay of anthranilic synthetase from Neurospora crassa.

Anthanilic synthetase may be extracted from chilled ground mycelium or from powdered lyaphylized mycelium with ice cold 0.05M pH 6.8 iminazole-HCl buffer. Nucleic acid and unwonted protein ore precipitated by 0.05M $MnCl_2$. Anthranilic synthetase is precipitated by ammonium sulfate between 35% and 50% saturation. In a typical preparation the precipitate contained 15% of the extracted protein and 75% of the enzyme. The activity contained in ammonium sulfate precipitates stored at -15°C remains undiminished for several months. In solution anthranilic synthetase is most stable at pH 6.8.

Continuous spectrophotometric and fluorimetric assays of anthranilic synthetase have been used, the former for kinetic determinations and for the early stages of purification and the latter as a routine assay following ammonium sulfate fractionation. Both assay systems were employed at 24°C and utilized a standard mixture containing the following concentrations of components: Tris buffer 0.15M, L-glutamine 7.5mM, L-tyrosine 1.5mM, EDTA 1.5mM, MgC12 9mM. The pH was adjusted to 8.1 with HCl. Tyrosine was added to inhibit chorismate mutase activity.

Fluorescence assay: A single-sided fluorimeter with quartz optics was used. The excitation source was a mercury arc filtered through 2 mm Chance OX1 and OX7 uv glass filters. An Ilford No. 622 "bright spectrum" filter was interposed in the fluorescence beam. The sensitivity of the instrument was adjusted to give full scale deflection with a solution of 10^{-5}M anthra-

nilic acid in pH 8.1 Tris buffer. The assay was conducted in a quartz cuvette containing 1 ml of the standard assay mixture, 0.05 ml of a 1.5M sodium chorismote solution and water and sample to a total volume of 1.5 ml.

A crude extract of tryp-4 A35, grown four days at 25°C in Vogel's medium supplemented with 20mg/l indole and 20g/l sucrose, was found to produce 0.15µM anthronilic acid/hr/mg of protein.

Spectrophotometric assay: The change in optical density at 32.2 kc/cm due to the utilization of chorismic acid and the production of anthranilic acid was followed in a recording spectrophotometer. The assay was conducted in a 1 cm path length quartz cuvette containing 2 ml of the standard assay mixture, 0.05 ml of a 10mM sodium chorismate solution and water and sample to a total volume of 3 ml. The production of 0.1 µM anthranilic acid produces on optical density change of 0.3. The spectrophotometric assay is unsatisfactory for quantitative determinations of activity prior to ammonium sulfate fractionation. Determinations of anthronilic synthetase moy be conducted directly on w-dissolved ammonium sulfate precipitates. Ammonium sulfate is non-inhibitory and at this stage of purification the presence of a small amount is desirable to prevent protein precipitation at 34°C and a consequent spurious optical density rise.

The most highly purified samples of anthranilic synthetase produced in this laboratory contain 4S and 10S components. The behavior of anthranilic synthetase activity on molecular sieves indicates that anthronilic synthetose is the 10S component. Anthranilic synthetose seems, therefore, to be a single protein composed of tryp-1 and tryp-2 specified subunits. As it becomes available, pure anthronilic synthetase will be tested for indole-3-glycerol phosphate synthetase activity to determine whether or not this activity, also specified by tryp-1, is located on the same active unit. ■ ■ ■ Department of Microbiology, Birmingham University, Birmingham 15, England.