Mutants of *Saccharomyces cerevisiae* with an Altered Regulation of Chorismate Mutase

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Mutants of *Saccharomyces cerevisiae* with an increased sensitivity to the tryptophan analogue DL-5-methyltryptophan (5MT) were isolated SCHÜRCH, MIOZZARI and HÜTTER, J. Bact. *117*, 1131–1140, 1974). A number of these 5MT sensitive mutants were characterized and assigned to one of the following 3 classes: class I, strains with altered activity and/or feedback sensitivity of anthranilate synthase; class II, strains with elevated uptake of 5MT; class III, mutants with altered regulation of the tryptophan-biosynthetic enzymes which do not exhibit increases in activity in the presence of 5MT. The object of the following study was to characterize 3 5MT sensitive mutants which do not belong to one of the above mentioned groups. The 3 mutants were shown to possess the following characteristics: They belong to the same complementation group and are considered allelic, although they show slightly different phenotypes. – The 5MT inhibition no longer occurs in the presence of tryptophan, anthranilic acid or indole. – Enzymatic analysis revealed changes in chorismate mutase: a) greatly increased activity of chorismate mutase; b) diminished feedback sensitivity of this enzyme to tyrosine; c) reduction in its ability to be activated by tryptophan. – Genetic studies indicate that this mutation is dominant and is localized in the chorismate mutase gene. – Two possibilities to explain the phenotype of the mutation are being studied. We are either dealing with a promotor mutation in the chorismate mutase structural gene, or the mutation affects the equilibrium between an inactive (or less active) and an active form of the enzyme.

Some Applications of an Immobilized Esterase

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The immobilized esterase described earlier (KONECNY and SLANICKA, Biochim. biophys. Acta *403*, 573, 1975; Path. Microbiol. *42*, 245, 1975) was used for the quantitative alkalimetric titration of cephalosporins, for the prepparation of larger quantities of deacetyl cephalosporins, and as an enzyme thermistor for their calorimetric assay. The highly active catalyst required for these applications was prepared by coupling the enzyme to zirconia-clad controlled-pore amino glass via glutaraldehyde, and had an activity of 200 units/g. The titrations and the preparative runs were carried out in 10–20 m*M* phosphate in a differential recirculation reactor (FORD, LAMBERT, COHEN and CHAMBERS, Enzyme Engin. *2*, 267, 1972) at pH 7 and pH 8 and 25 °C, the reaction time for complete conversion being about 60 and 30 min, respectively. In 80 successive runs at pH 8 the immobilized enzyme retained 70% of its initial activity, the rate of loss being highest at the beginning. In the analytical runs the titres agreed within 2% with the theory. The enzyme thermistor was the earlier version of the two devices described by MOSBACH and coworkers (MOSBACH and DANIELSSON, Biochim. biophys. Acta *364*, 140, 1974; MOSBACH, DANIELSSON, BORGERUD and SCOTT, ibid. *403*, 256, 1975). In current work the sluggish response of the system arizing from its high heat capacity was reduced to a few minutes by substituting the isothermal Tronac calorimeter for the thermistor.

Product Inhibition During the Rifamycin S Fermentation

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The production of rifamycin S by a mutant strain of *Nocardia mediterranei* in a complex medium was investigated. The formation of the antibiotic was associated with the growth of the organism. Growth and production occurred in two phases. After a first phase of rapid growth and production metabolic activity slowed down and continued at a reduced rate until the energy source in the medium was exhausted. Substrate limitation and product inhibition were examined as possible reasons for this behaviour. Improved mixing conditions reduced pellet formation but did not change the course of the fermentation. Thus, substrate limitation could be excluded as an explanation for the drop in growth and production rates. It was found that rifamycin S inhibits its own synthesis as well as the growth of the producing organism. By using an in vitro system the specific rifamycin S synthesizing activity (RS-activity) of mycelium from different stages of a fermentation could be measured. This RS-activity paralleled the production rates calculated from a fermentation. The values increased in the first part of a fermentation and then dropped rapidly. Addition of rifamycin S to a growing culture inhibited this initial increase in RS-activity and prevented in this way the organism from producing higher quantities of the antibiotic. The implications of these results for the development of an improved production process are discussed.

Some Properties of the Endotoxin Unit of *Bacillus thuringiensis*

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In a previous study it was shown that purified crystal suspensions of *Bacillus thuringiensis* var. *thuringiensis*, which were dissolved by non-proteolytic solvents such as NaOH, mercaptoethanol/urea or mercaptoethanol/guanidine hydrochloride, yielded peptides with a molecular weight $\geqslant$ 800,000. This material retained its full toxic activity. Further degradation by purified gut juice proteases resulted in a toxic component with a molecular weight of 100,000 accompanied by a non-toxic low molecular weight fraction $\leqslant$ 10,000. The molecule of MW 100,000 is referred to as the toxic unit. Using the same technique, endotoxin crystals belonging to the varieties *kurstaki, entomocidus, aizawai, morrisoni, tolworthi, darmstadtiensis* and *toumanoffi* were analyzed. It was demonstrated by gel filtration and by gel electrophoresis that the predominant protein component of each of the above mentioned varieties appeared in the molecular weight range of 100,000. The low molecular weight fragments were also present. According to our results, it may be presumed that the toxic units of the different varieties were very similar. In further experiments the high molecular weight component (MW $\geqslant$ 800,000) was heated to 100 °C for 1 h. Enzymatic degradation of the heat treated protein yielded only a low molecular weight component whereas the toxic unit was missing. Thus, the intact structure of this protein is essential for the formation of the toxic unit by gut juice proteases. Finally, the SH-groups of the toxic protein unit were carboxymethylated