Pyrimidine Biosynthesis in *Serratia marcescens*: Polypeptide Interactions of Three Nonsequential Enzymes

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Orotidine-5'-monophosphate pyrophosphorylase (OMPppase, E.C. 2.4.2.10) and orotidylate decarboxylase (OMPdecase, E.C. 4.1.1.23) were purified from *Serratia marcescens* HY. These enzymes required physical association for maximal catalytic activities and formed a fragile complex with dihydroorotase (DHOase, E.C. 3.5.2.3). OMPppase reversibly lost 50% of its activity upon separation from DHOase. The kinetic characteristics of OMPppase were modified by this separation. In the presence of DHOase, the $K_m$s for PRPP and orotate were stoichiometric: $2.3 \times 10^{-6}$ M and $2.6 \times 10^{-6}$ M, respectively. Following separation, the $K_m$s were significantly different: $1.3 \times 10^{-6}$ M for PRPP and $4.1 \times 10^{-6}$ M for orotate. OMPppase and OMPdecase could be reversibly separated by acrylamide gel electrophoresis, but the separation was accompanied by a loss of catalytic efficiency for both enzymes. DHOase readily associated into multiple molecular forms and could not be purified. The DHOase–OMPppase–OMPdecase interactions demonstrate that a weakly aggregated, multifunctional enzyme complex participates in the biosynthesis of pyrimidine nucleotides in *S. marcescens*. This unique association of nonsequential biosynthetic enzymes may represent a larger complex which provides a channeling or regulatory unit.

KEY WORDS: *Serratia marcescens*; pyrimidine biosynthesis; enzyme aggregation; regulation; bacteria.

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INTRODUCTION

Orotate is converted to uridine-5'-monophosphate (UMP) by a two-step reaction sequence involving a reversible condensation with 5'-phosphoribosyl-1-pyrophosphate (PRPP), catalyzed by orotidine-5'-monophosphate (OMP) pyrophosphorylase (OMPppase), and the irreversible decarboxylation of OMP by orotidylate decarboxylase (OMPdecase). The sequence was first described in yeast by Lieberman et al. (1954, 1955). Umezu et al. (1971) have been able to physically separate the two enzymes in fungi and characterize the highly purified enzyme preparations. In mammalian cells, these two enzymes appeared to be a dependent aggregate (Jones, 1972). Appel has purified a homogeneous protein catalyzing both activities from cow brain (Appel, 1968) and the enzymes have been copurified 500-fold in calf thymus (Kasbekar et al., 1964). The two enzyme activities have been separated by starch gel electrophoresis, although OMPppase activity was often lost. Jones has suggested that a single protein complex of at least two nonidentical subunits was responsible for these sequential catalytic activities (Jones, 1971, 1972). Furthermore, it has been observed that these two enzymes may aggregate in several oligomeric forms (Brown et al., 1975; Shoaf and Jones, 1973; Grobner and Kelley, 1975). It is possible that the two enzyme activities reside in a single protein in mammalian cells; however, the evidence suggests that they are a tightly associated aggregate (Jones, 1971, 1972).

The bacterial enzymes catalyzing these reactions (Beckwith et al., 1962; Nagano et al., 1966; O'Donovan and Neuhard, 1970) have not been well characterized, although OMPppase and OMPdecase appeared to form a complex in Micrococcus glutamicus (Nagano et al., 1966) and Serratia marcescens (Wild and Belser, 1977). OMPppase activity has been shown to be rate-limiting on the conversion of a free pyrimidine base to phosphorylated nucleotides (Fausto, 1969). The equilibrium constant for the OMPppase reaction is 0.1, and although highly specific it is unfavorable for biosynthesis. By coupling OMPppase with OMPdecase, pyrimidine biosynthesis readily proceeds (Reichard, 1959).

The accompanying article (Wild and Belser, 1977) has suggested that OMPppase activity is modulated by the availability of the pyrC gene product (DHOase) both in vivo and in vitro and that a DHOase–OMPppase–OMPdecase complex might effect pyrimidine biosynthesis in S. marcescens. Studies presented in this article examine the enzymatic characteristics of OMPppase and OMPdecase and their interaction with DHOase. These enzymes have been purified from S. marcescens HY and form a coupled system. We believe that in Serratia pyrimidine biosynthesis involves a series of polypeptide interactions that determine the catalytic efficiency of the enzymes of the pathway as well as contributing to the overall control of nucleotide formation.