Chapter 16

MICROBIAL PRODUCTION OF OROTIC ACID
(VITAMIN B<sub>13</sub>)

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ABBREVIATIONS

ATC, aspartate transcarbamylase; AU, 6-azauracil; CPS, carbamyl phosphate synthetase; CTP, cytidine-trisphosphate. DHO, dihydroorotase; DHOdeh, dihydroorotate dehydrogenase; OA, orotic acid; OMP, orotidine 5'-monophosphate; OMPdec, OMP decarboxylase; OPRT, orotate phosphoribosyltransferase; OR, orotidine; PRPP, phosphoribosylpyrophosphate; pyr, pyrimidine; UMP, uridine-monophosphate; ura, uracil

1 INTRODUCTION

Orotic acid was first discovered in cow’s milk in 1905 and was later found to accumulate as an intermediate in the biosynthetic pathway of pyrimidine nucleotides in a variety of mutants of micro-organisms. On the other hand, a similar substance—which was a growth factor for rats, chickens and lactic acid bacteria—had been isolated from distillers’ dried solubles and had been known as vitamin B<sub>13</sub>. It was proved in 1953 that orotic acid and vitamin B<sub>13</sub> are identical. Orotic acid is generally formed in mammals, so it is not a vitamin in a strict sense.

Several reviews have been published on the production of orotic acid (Furuya, 1976; Enei, 1984; Kuninaka, 1986) and the biosynthesis of pyrimidine compounds (O’Donovan & Neuhard, 1970; Shiio, 1972).

In this chapter, recent aspects of the microbial production of orotic acid by the de novo pathway will be described.

2 HISTORICAL

Orotic acid has a complex history. It was isolated from the whey of cow’s milk (Biscaro & Belloni, 1905) and subsequently found to be present in the milk of...
various mammals. It was not until 1930 that the chemical structure of orotic acid was perfectly known (Bachstez, 1930). Further investigations on the compound were carried out late in the 1940s. Mitchell and co-workers (1947, 1948) observed that one of the pyrimidine-requiring mutants of *Neurospora* utilized orotic acid instead of the pyrimidines, while the others, which did not utilize orotic acid, accumulated large quantities of orotic acid in a culture medium. Afterwards, isotopic experiments led to the conclusion that orotic acid was the important intermediate in the biosynthetic pathway of pyrimidine nucleotides.

On the other hand, a growth factor for rats and chickens was discovered from distillers' dried solubles (DDS) and named vitamin B₁₃ (Novak & Hauge, 1948). Moreover, Wright *et al.* (1950) observed that DDS was also effective in promoting the growth of *Lactobacillus bulgaricus* and orotic acid could substitute for DDS. Subsequently, evidence has been given that vitamin B₁₃ was identical to orotic acid (Manna & Hauge, 1953).

The accumulation of orotic acid by mutants of several species of bacteria was reported, but the amounts accumulated were very low. In 1961, the accumulation of orotic acid by a uracil-requiring mutant of *Micrococcus glutamicus* (synon. *Corynebacterium glutamicum*) (a glutamic acid-producing microorganism), was reported (Tanaka *et al.*, 1961; Kinoshita & Tanaka, 1963; Konishita *et al.*, 1963). This was the first report aimed at the industrial production of orotic acid by fermentation procedure. Studies on the microbial production of orotic acid have mainly been reported by Japanese researchers.

The industrial production of orotic acid by fermentation started in the middle of the 1970s in Japan.

3 CHEMISTRY AND ASSAY

3.1 Chemistry

Chemical structure and properties of orotic acid (1,2,3,6-tetrahydro-2,6-dioxo-4-pyrimidinecarboxylic acid; uracil-6-carboxylic acid) and orotidine (3-β-D-ribofuranosylorotic acid, 6-carboxyuridine) are shown in Fig. 1 and Table 1. Data are quoted from the *Merck Index* (10th edn, 1983). Potassium, sodium,