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CELL FREE SYNTHESIS OF O-SUCCINYLBENZOIC ACID FROM ISO-CHORISMIC ACID, THE KEY REACTION IN VITAMIN K_2 (MENAQUINONE) BIOSYNTHESIS

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Summary: An enzyme preparation from E. coli catalyzes the formation of o-succinylbenzoic acid (1, OSB, i.e. 4-(2'-carboxyphenyl)-4-oxobutanoic acid) from α-ketoglutaric acid and iso-chorismic acid (2) but not from chorismic acid (3).

The formation of o-succinylbenzoic acid (OSB, 1) is the key reaction in vitamin K_2 (menaquinone) biosynthesis. This reaction links the shikimic acid pathway to the vitamin K_2 biosynthetic pathway.[1] A crude enzyme preparation from several bacterial strains has been obtained that catalyzes the synthesis of OSB (1) in the presence of chorismic acid (3), α-ketoglutaric acid and thiamin pyrophosphate [2,3].

[Scheme I]

In these experiments[2,3] a commercially available sample of barium chorismate (Sigma Chemical Company) was employed. HPLC analysis of this material in our laboratory showed, however, that it contained ca. 0.2 % iso-chorismic acid (2) which is a product of chorismic acid in Aerobacter aerogenes (i.e. Klebsiella pneumoniae) (62-1)[4]. The presence of iso-chorismic acid (2) in this sample might have accounted for the observed[2,3] synthesis of OSB (yield less than 0.2 %). The idea that iso-chorismic acid rather than chorismic acid would

be the starting material for OSB biosynthesis (Scheme I) has also been favoured by Haslam[5].

In order to test this hypothesis enzyme preparations were obtained in the following way: An extract prepared by sonication from E. coli K_{12} or E. coli AN 154 (kindly supplied by Dr. I.G. Young, Canberra, Australia) was centrifuged and the supernatant (48 000xg) treated with protamine sulfate in order to precipitate the ketoglutarate dehydrogenase[6]. The supernatant (48 000xg) of this solution was incubated (Table I) in the presence of chorismic acid, α-ketoglutaric acid and thiamin pyrophosphate.

Table 1. Cell-free conversion of iso-chorismic acid and α-ketoglutaric acid to OSB in the presence of a partially purified extract from E. coli AN 154

Incubation mixture	OSB (nmol)	OSB (%) (iso-chorismic acid = 100 %)	Relative activity (%)
a) Complete	8.31	88.5	100
minus α-ketoglutaric acid	b)n.d.	n.d.	n.d.
minus thiamin pyrophosphate	3.44	36.6	41.4
minus iso-chorismic acid	n.d.	n.d.	n.d.
Complete, heat-inactivated enzyme	n.d.	n.d.	n.d.
Complete, with c) chorismic acid but without iso-chorismic acid	n.d.	n.d.	n.d.

a) The complete incubation mixture contained protein (1.58 mg), iso-chorismic acid (9.39 nmol) (chorismic acid (12.9 nmol)), α-ketoglutaric acid (0.39 μmol), thiamin pyrophosphate (0.12 μmol), Tris-HCl (50 μmol, pH 9) in a final volume of 0.42 ml. Incubation was carried out for 60 min at 37°C. Final pH 8.25.

b) n.d. = not detectable by HPLC

c) purified by HPLC.

OSB synthesis was not observed when this incubation mixture was analyzed for OSB using HPLC (limit of detection 50 pmol). Subsequently a sample of *iso*-chorismic acid (**2**) was prepared and identified as described[4]. Incubation with this isomer (Table I) actually gave OSB in a yield of 88.5 % with reference to the *iso*-chorismic acid employed (Table I). The product OSB was isolated by HPLC. Labelled OSB was obtained when either ^{14}C-*iso*-chorismic acid or U-^{14}C ketoglutaric acid were used in the incubations. The synthesis of OSB was diminished when thiamin pyrophosphate was omitted from the incubation mixture. This is in accord with previous findings[3].

The OSB formed was identified in the following way: The radioactive OSB cochromatographed (silica gel, tlc) with authentic material in four different solvent systems without loss of specific activity. The enzymically formed OSB was also converted to its dimethylester (diazomethane) and to its spirodilactone by sublimation. The derivatives again cochromatographed with authentic samples without loss of specific activity. The product OSB was also converted enzymically to its mono coenzyme A ester[7] which was found to be identical (HPLC) with the ester obtained from authentic OSB.

The protein fraction catalyzing the synthesis of OSB was free of ketoglutarate dehydrogenase (see above). We conclude that the OSB synthase and the ketoglutarate dehydrogenase are distinct catalytic entities and that *iso*-chorismic acid rather than chorismic acid is the immediate precursor of OSB. We are aware, however, that at present the following problem remains unresolved: Mutants of E. coli (AN 154 and AN 191) were reported[8] to be blocked between chorismic acid and *iso*-chorismic acid but yet produced vitamin K_2[8]. This led to the conclusion that chorismic acid is the branch point for vitamin K_2 biosynthesis. These results are inconsistent with our observations.

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