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Mechanistic similarities in the reactions catalyzed by dioldehydrase and methylmalonyl-CoA mutase*

Dioldehydrase, in the presence of dimethylbenzimidazolylcobamide coenzyme (DBCC), catalyzes the conversion of 1,2-propanediol to propionaldehyde. This reaction involves the transfer of a hydrogen from C-1 of propanediol to C-2. This hydrogen transfer proceeds, as do all hydrogen transfers, in rearrangements involving DBCC, without exchange with the hydrogen of the solvent. It has now been shown that the hydrogen transfer catalyzed by dioldehydrase, is not an intramolecular process, but a two-step process in which the coenzyme functions as hydrogen carrier[1,2]. The hydrogen atoms at C-5′ of the adenosyl moiety of DBCC are involved in this transfer. This conclusion is based on the following observations: (1) When 1,2-[1-^{3}H]propanediol is converted to propionaldehyde in the presence of dioldehydrase and DBCC, tritium is incorporated into the DBCC. The tritium is incorporated exclusively into the C-5′ position of the adenosyl moiety of the coenzyme (P. A. FREY AND R. H. ABELES, unpublished results). (2) When 1,2-propanediol is converted to propionaldehyde in the presence of dioldehydrase and DBCC, synthetically tritiated at the C-5′ position of the adenosyl moiety, all of the tritium of DBCC is transferred to propionaldehyde.

If the mechanism of action of methylmalonyl-CoA mutase (methylmalonyl-CoA CoA-carbonylmutase, EC 5.4.99.2) is similar to that of dioldehydrase, it would be expected that the conversion of methylmalonyl-CoA to succinyl-CoA, in the presence of DBCC, tritiated at the C-5′ of the adenosyl moiety ([^{3}H]DBCC), should lead to the formation of tritiated succinic acid. This experiment was carried out under the following conditions: 0.02 μmole [^{3}H]DBCC (426 000 disint./min), 35.8 μmoles DL-methylmalonyl-CoA and 134 units** of methylmalonyl-CoA mutase[4] from *Propionibacterium shermanii*, 52 W, (5–10% purity) were incubated in 0.1 M Tris–sulfate buffer, pH 7.4, in a total volume of 12 ml for 10 min at 37°. Succinic acid (508 μmoles) carrier was then added and the reaction mixture was heated for 2 min at 100° to hydrolyze succinyl-CoA. Succinic acid was isolated by continuous extraction with ether. It was then recrystallized from water to constant specific activity. The specific activity of the succinic acid was 188 disint./min per μmole. A control experiment without enzyme, in which succinyl-CoA was added instead of methylmalonyl-CoA, was carried out under the same conditions. Succinic acid isolated from the control experiment contained 2 disint./min per μmole.

Similar experiments were carried out with methylmalonyl-CoA mutase from mammalian sources[5]. Transfer of tritium from tritiated DBCC to succinyl-CoA was also obtained in this case.

Abbreviation: DBCC, dimethylbenzimidazolylcobamide coenzyme.

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** Methylmalonyl-CoA mutase was assayed spectrophotometrically as follows: Tris–sulfate, pH 7.4, 300 μmoles; DPNH, 0.3 μmole; methylmalonyl-CoA, 0.4 μmole; sodium acetoacetate, 80 μmoles; DBCC, 0.02 μmole; succinyl-CoA–acetoacetate transferase[3], 145 units; β-hydroxyacyl-CoA dehydrogenase (Calbiochem), 0.02 mg; and methylmalonyl-CoA mutase in a total volume of 1.0 ml. The reaction was carried out at 30°. The rate of formation of succinyl-CoA is measured by the rate of oxidation of DPNH. One unit is defined as the amount of enzyme which catalyzes the formation of 1 μmole of succinyl-CoA per min.

These experiments establish that in the reaction catalyzed by methylmalonyl-CoA mutase, hydrogen is transferred from the C-5′ position of the coenzyme to the substrate, as in the reaction catalyzed by dioldehydrase. This similarity between the two reactions indicates that they proceed by similar mechanisms. The same conclusions were reached by RETEY AND ARIGONI[6] based upon experiments in which tritium transfer to succinate was shown with coenzyme in which tritium had been introduced by dioldehydrase and 1,2-[1-^{3}H]propanediol.

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Bestimmung der Kynurenin Aminotransferase

Nach einer Ganzkörperbestrahlung werden die Tryptophanmetabolite Kynurensäure und Xanthurensäure von Mäusen vermehrt ausgeschieden[1]. In Verbindung mit enzymatischen Messungen nach Einwirkung ionisierender Strahlen[2] sollte daher die Aktivität der Kynurenin Aminotransferase (L-Kynurenin:Oxoglutarat Aminotransferase, EC 2.6.1.7) gemessen werden. Dabei ergab sich, daß die bisher in der Literatur beschriebenen Methoden zur Messung dieser enzymatischen Aktivität[3,4] aus verschiedenartigen Gründen unbefriedigend waren. So erschien die Inkubation mit 3-Hydroxykynurenin[4] für eine Untersuchung mit größeren Testreihen zu kostspielig.

KNOX[3] hatte die Kynurenin Aminotransferase bestimmt, indem er nach der Inkubation die Absorption des enteiweißten Testansatzes bei 310, 330 und 365 mμ maß. Aus diesen Werten wurde dann mit Hilfe der molaren Extinktionskoeffizienten der Anthranilsäure, Kynurensäure und des Kynurenins bei den drei genannten Wellenlängen der Gehalt dieser Metabolite ermittelt. Aus der Menge an gebildeter Kynurensäure wurde auf die Aktivität der Kynurenin Aminotransferase geschlossen. Bei unseren Untersuchungen lieferte diese Methode vor allem wegen der hohen Konzentra-