Short Communications and Preliminary Notes

AN INSTANCE OF PARTIALLY ASYMMETRIC ENZYMATIC SYNTHESIS

by

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It is well known that certain hydrolytic enzymes show imperfect optical specificity, in that both antipodes of a metabolite may be acted upon, although often at very different rates[1]. It is usually assumed, however, that enzymes concerned with synthetic processes show a high degree of optical specificity and to the author's knowledge there is no published information of a partially asymmetric synthesis by a purified enzyme.

We have recently observed that the acetylmethylcarbinol (acetoin, AMC) synthesized from pyruvate and acetaldehyde, or acetaldehyde alone, by highly purified preparations of the α-carboxylase of wheat germ is a mixture of optical isomers, predominantly the (+) form. The enzyme used in these experiments was an 11,000-fold purified preparation, which was homogeneous in the Tiselius apparatus[2,3]. The fact that the same enzyme protein catalyzed both the decarboxylation of α-keto acids and the synthesis of AMC from pyruvate and acetaldehyde or from acetaldehyde alone was established as follows. The ratio of synthetic to decarboxylase activities remains constant throughout purification. AMC synthesis and decarboxylase action have identical p_H optima, Mg^{++}, and diphosphothiamin requirements. Partial inactivation by ageing, acid, heat treatment or *p*-chloromercuribenzoate results in an identical decline of both types of enzymatic activities[2,3].

The identity and purity of the enzymatically produced AMC was established by periodate degradation[4], by oxidation to diacetyl[5], by WESTERFELD's colorimetric method[5], and by isolation of its crystalline semicarbazone, which gave the correct melting point and C, H, and N analysis.

In a typical experiment 4.2 mg of our most purified enzyme (Q_{CO_2} = 62,000) were incubated at p_H 6 with 0.25 *M* sodium pyruvate, 0.25 *M* acetaldehyde, 0.001 *M* $MgSO_4$, and 80 γ DPT in a total volume of 3.5 ml for 270 minutes at 30°. After deproteinization and fractional vacuum distillation at p_H 6 and 0°, about 3 ml of a solution were obtained, containing 11.7 mg/ml AMC. The optical rotation of this solution in a 2 dm tube was $\alpha = 0.87°$; $[\alpha]_D^{29} = 37°$. In other experiments, with acetaldehyde alone or with both acetaldehyde and pyruvate as substrates, the specific rotation ranged from 34° to 37°.

Published values of the optical rotation of AMC cover an extensive range. The author considers the most reliable values reported to be those of BERL AND BUEDING[6], who determined the rotation of the (—) AMC produced by enzymes from filarial nematodes, *Aerobacter aerogenes*[6], and pig heart[7]. These authors report a value of $[\alpha]_D = -84 \pm 2°$ for the AMC isolated from each of these systems. TANKO *et al.*[8] have reported similar values for (—) AMC accumulated by various animal tissues during pyruvate metabolism. On the basis of this figure and our observation of specific rotation of 37°, the AMC produced by wheat germ carboxylase consisted of 72% of the (+) and 28% of the (—) form.

When authentic (—) AMC, isolated from filariae by BUEDING, was incubated with the wheat germ enzyme under similar conditions, no racemization was observed: $[\alpha]_D^{27} = -81°$. Thus no racemase is present in the enzyme and neither does the isolation procedure give rise to racemization.

Although the enzyme preparations used in this study may not be completely homogeneous in terms of rigid criteria of the purity of proteins, in view of the evidence cited it appears most unlikely that more than one enzyme with acetoin-synthesizing activity was present, since if this were the case these enzymes would have to have identical properties and have identical decarboxylase: acetoin synthesizing ratios and would differ only in the optical activity of the AMC they produce. Furthermore, the optical activity of the AMC accumulated appears to be independent of the purity of the carboxylase preparation. As a matter of fact, a wide variety of crude plant meals (peas, soy

bean, lupine, lucerne) have been shown[8] to produce AMC from added pyruvate or acetaldehyde, *under the influence of carboxylase*, with an optical rotation of $[\alpha]_D^{20} = 37$ to 40°.

If it is admitted that AMC synthesis is catalyzed by a single enzyme in our preparations, namely α-carboxylase, two explanations may be offered for the above observations. It is possible that the enzyme contains several active centres, which produce (—) or (+) AMC, and that the optical activity of the product isolated is the net result of the action of all of these centres. The second possibility, which the author considers much more likely, is that the ratio of (+) and (—) AMC produced represents the probability of a second molecule of acetaldehyde reacting with a molecule of the activated enzyme-acetaldehyde complex from two opposite spatial directions. Fig. 1 is a schematic representation of the postulated course of AMC synthesis. In the first stage (B) pyruvate forms a 2-point combination with the active centre of the enzyme. The latter consists of a protein-Mg-DPT complex. The carbonyl group of the pyruvate approaches the free amino group of DPT, in a manner suggested by LANGENBECK[9]. The activation step would involve the formation of an intermediate complex, possibly a Schiff's base (C)[9] or a hydrogen-bonded structure (D), between the carbonyl and amino groups, and CO_2 would be split off. The resulting enzyme-acetaldehyde complex is in a reversible equilibrium with free acetaldehyde (E). Thus the complex (C or D) could form directly from acetaldehyde or, indirectly, from pyruvate by decarboxylation *in situ*. In the presence of a sufficient excess of acetaldehyde a second molecule of the latter could react with complex C, forming an unstable enzyme-AMC compound, which would rapidly and irreversibly break down to free AMC and free enzyme (F). According to this scheme it would be the spatial direction whereby the second molecule of acetaldehyde approaches the intermediate complex (C or D) which determines which antipode will be produced.

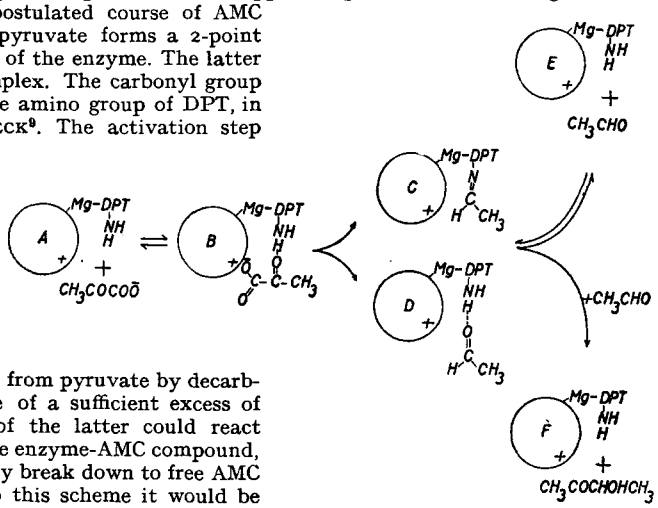

Fig. 1

The relative amounts of the two optical antipodes produced would represent the relative steric hindrances offered by the side-chains of the protein to the second molecule of aldehyde as it approaches the double bond in structures C or D during the addition of the second molecule of acetaldehyde from either one of two opposite directions. The possible mechanism of this addition (or condensation) reaction will be discussed elsewhere.

It should be mentioned that the production of AMC by carboxylase after decarboxylation of pyruvate *in situ* is in accord with the theories of DIRSCHERL, LANGENBECK and their co-workers[9,10].

If the contentions of this paper should be proven correct, and if in the plant kingdom AMC indeed arises generally as a result of carboxylase action[8], then the uniform optical rotation (34° to 40°) observed by TANKO *et al.* in a wide variety of plants indicates essentially the same active centre in the α-carboxylases of all of these plants, including wheat germ, although the individual apo-enzymes might differ sufficiently to be distinct immunological entities.

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