STEREOCHEMISTRY OF THE OXIDATION AT THE α CARBON OF BUTYRYL-CoA AND OF THE ENZYMIC HYDROGEN EXCHANGE

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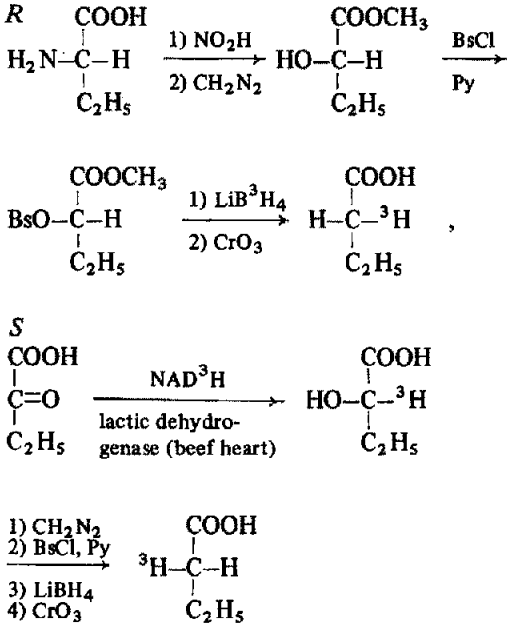
In our preceding paper [1] we showed that the Pro-R hydrogen at the β position of buryryl-CoA is removed during enzymic oxidation by acyl-CoA dehydrogenase extracted from pork liver. In this communication we report our results with regard to the α hydrogens during oxidation and during the exchange observed in the presence of enzyme.

R and S butyric acids 2-^{3}H were prepared according to the outline:



Bs = benzenesulfonyl

The two butyric acids -2-^{3}H purified by preparative glc. were obtained with a chemical purity > 99%. The specific activity for the R-acid was 2.71×10^7 cpm/mmole and for the S-acid was 9.15×10^6 cpm/mmole.

The synthesis of butyryl-*S*-CoA derivative and the enzymic oxidation were carried out as in our preceding paper [1]. Our results are reported in the following table:

Table 1

	Reaction (%)	Total radioactivity (cpm)	Recovered water	Incorporation (%)
R-butyryl-^{3}H-2-CoA	100	19,400	12,600	65
	100	18,800	14,200	75
	0	6,100	4,000	65
	0	6,100	100	2
S-butyryl-^{3}H-2-CoA	100	1,500	200	15
	100	2,700	340	13
	0 *	1,500	190	10
R-butyryl-^{3}H-3-CoA	0 *	5,100	100	2

* Without electron transfer and acceptor agents.
** Without enzyme.

These results prove that it is the R-butyryl-2-^{3}H-CoA which loses tritium during enzymic oxidation. The departure from the theoretical values for incorporation of tritium in water is probably due to incomplete optical purity of the butyric-2-^{3}H acids. In fact

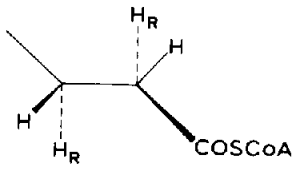


Fig. 1.

it is known that nitrous acid deamination of α-amino acids to give α-bromo acids occurs with an optical yield of about 75% [2]. The α-hydroxybutyric acid obtained in this work by nitrous acid deamination had an optical purity of about 75%. The enzymic reduction of α-ketobutyric acid [3] gives L-α-hydroxybutyric acid (as shown by the oxidation reaction of the butyryl-2-^{3}H-CoA obtained from this hydroxyacid) with a large optical purity such that the percentage of tritium lost during oxidation is equal to half the amount of racemisation at the time of conversion of the α-hydroxybutyric acid to butyric acid. This is in agreement with the value found in a similar case [4].

These results, together with those published earlier [1] show that the oxidation of butyryl-CoA by acyl-CoA dehydrogenase involves the removal of the R hydrogens in both the α and β positions *. Therefore the hydrogens removed are in a trans relationship, the butyryl group being in the anti conformation, and thus gives *trans*-crotonyl-CoA as has been suggested previously [5].

When the two samples R and S-butyryl-2-^{3}H-*S*-CoA are incubated under the standard conditions, but without the enzyme, the radioactivity of the water is only slightly above background noise, thus demonstrating a very small amount of exchange. On the other hand, when incubated with the enzyme, for the same period as required for 100% oxidation, but in the absence of electron transfer and acceptor agents, the α pro-R hydrogen is incorporated in the water at a rate only slightly different from that observed under standard oxidation conditions. Our present experimental conditions do not allow detection of a rate difference between oxidation and exchange.

On the other hand, R-butyryl-3-^{3}H-CoA under the same conditions loses only a very small amount of tritium.

Thus for the case of butyryl-CoA dehydrogenase, there is a notable difference in the rates of exchange at the α and β positions, larger than the difference noted for succinodehydrogenase [6].

* Professor D.Arigoni and Drysdale informed us kindly of their results in accordance with ours.

** The oxidation experiments were done on 0.3 μmole of butyryl-SCoA instead of 6.3 μmole as written.

References

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