STEREOCHEMISTRY OF THE EXCHANGE CATALYZED BY SUCCINIC DEHYDROGENASE

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Received April 23, 1964

It has previously been demonstrated (1) that succinic dehydrogenase catalyzes proton exchange of essentially one of the protons of both L-chlorosuccinate, a new substrate (2,3), and of succinate, albeit in the latter case the data was not quantitatively definitive. These results and the trans nature (2,4) of the succinic dehydrogenase catalyzed reaction implied a stereospecific location of the exchangeable proton of both substrates. Accordingly, this communication deals with location of the exchangeable proton of both succinate and L-chlorosuccinate.

Monodeuterio-succinic acid obtained by exchange was identified as L(+)-monodeuterio-succinic acid (I) by its optical rotatory dispersion curve (5). Table I gives details of the exchange experiments with succinic acid. It is to be noted that both the 4 hour and 6 hour samples give specific rotation.values on the basis of the calculated monodeuterio-succinic acid content, that are in agreement with the values reported for authentic L(+)-monodeuterio-succinic acid(6). Table II gives the distribution of deuterium, as obtained by negative ion mass spectrometry (7), in the 4 hour sample referred to in Table I. It is readily apparent that the previous conclusion (1) that exchange proceeds by substitution of essentially one proton of succinic acid is corroborated. It may be also mentioned that were ex-

Table I

Deuterated Succinic Acids Obtained by Exchange[a]

Sample	Atom % Deuterium	Specific Rotation[b] 294	278	270	250	244	238
4 hour	4.7	5.1	7.8	9.6	17.5	28.8	41
6 hour	6.4	4.4	6.2	7.5	11.0	18.8	29
L(+)-[c]	16.6	6.5	9.1	11.0	20.6	27.0	37

[a] Individual reaction mixtures, 400 ml. total volume, 50% D_2O, 0.0635M succinate, 0.0548M fumarate, 0.045M phosphate, pH 7.3, 40 ml. Slater enzyme preparation, (Ref. 2), were incubated at 38°. Reactions were stopped by addition of 12 N sulfuric acid and the acid mixture isolated by ether extraction. The acids were separated by column chromatography and the isolated succinic acid further purified by sublimation.

[b] Specific rotations in methanol at the indicated wave lengths in mu were calculated on the basis of the monodeuterio-succinic acid content calculated from the deuterium analysis.

[c] Authentic L(+)-monodeuterio-succinic acid; specific rotations interpolated from those reported by Englard (6).

Table II

Deuterium Distribution in Exchanged Succinic Acid[a]

Mass No.	Normal Acid[b]	Exchanged Acid[c]	Excess
117	82.0	82	---
118	3.6	14.9	11.3
119	0.8	3.1	2.3

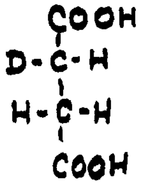
[a] Relative heights of parent peaks obtained by negative ion mass spectrometry according to the method of Phares, Long and Carson (Ref. 7).

[b] Distribution in normal acid according to Phares et al (7).

[c] The 4 hour sample from Table I.

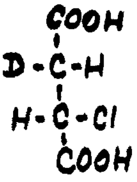
change to occur by substitution of two of the four protons of a succinic acid molecule, it would be expected on the basis of the trans dehydrogenation of succinic acid (2,4) that meso-dideuterio-succinic acid would result.

Monodeuterio L-chlorosuccinic acid, obtained by exchange, yielded, on reduction with hydrogen and palladium, monodeuterio-succinic acid with a rotatory dispersion curve similar to that of L-monodeuterio-succinic acid, albeit of lower specific rotation. An almost identical curve was obtained with monodeuterio-succinic acid made by reduction of normal L-chlorosuccinic acid with deuterium and palladium, so that it may be provisionally concluded that mono-deuterio-L-chlorosuccinic acid, obtained by exchange, has the threo configuration, II.

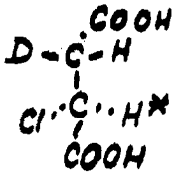


I

Ia



II



IIa

Table III gives details of these experiments and comparison of specific rotations of the monodeuterio-succinic acid obtained by (H_2, Pd) from exchanged L-chlorosuccinic acid and of the monodeuterio-succinic acid obtained (D_2,Pd) from normal L-chlorosuccinic acid with that of authentic L(+)-monodeuterio-succinic acid indicates that extensive racemization on reduction has occurred in both instances. In the case of reduction of normal L-chlorosuccinic acid with D_2,Pd, it is clear that hydrogenolysis of the chloro group has proceeded with the expected (8) retention of configuration but with extensive racemization at the site of the hydrogenolysis, as also expected (9). On the other hand, the monodeuterio-L-chlorosuccinic acid obtained by exchange has apparently undergone racemization at an adjacent carbon atom on hydrogenolysis with H_2,Pd. In this latter instance it is possible preferential exchange of hydrogen with inversion occurs since it has been noted that the α-hydrogen of lactic acid exchanges with inversion of configuration (10).

Table III

Deuterated Succinic Acids from L-chlorosuccinic Acids

Sample	Atom % Deuterium	Reductant	Specific Rotation[a]				
			280	270	260	250	240
from exchanged L-chlorosuccinic acid[b]	7.27	H_2,Pd[c]	0.6	1.2	2.0	3.7	6.0
from authentic L-chlorosuccinic acid	16.61	D_2,Pd[c]	1.2	1.6	2.5	4.3	7.4
L(+)-monodeuterio-succinic acid	16.66	----	3.8	5.4	8.2	13.4	22.6

[a] Ten mgs. per ml. in water.

[b] A reaction mixture, 500 ml. total volume, 0.040M L-chlorosuccinate, 0.007M chlorofumarate, 0.05 M phosphate, pH 7.3, containing 40 ml. of a Slater preparation, (Ref. 2), was incubated under anaerobic conditions at 37° for 4 hours. The reaction was stopped by the addition of 12 N sulfuric acid to pH 1.5 and the acids extracted with ether. After drying and evaporation of the ether extract, deuterated L-chlorosuccinic acid was obtained by crystallization of the crude residue from ethyl acetate.

[c] A solution of 2.5 millimoles of 2-chlorosuccinic acid in 10 ml. of water was hydrogenolyzed for two hours in the presence of 0.1 g. of 10% palladium-charcoal catalyst at an initial pressure of 60 pounds. The succinic acids were isolated and purified by crystallization after removal of catalyst by filtration.

[d] Specific rotations interpolated from those reported by Englard(6).

The conformation of the respective exchanged acids in the enzyme-substrate complex may be depicted (2) as Ia and IIa, the unexchanged hydrogen is transferred to enzyme as a hydride ion according to our previously depicted mechanism (1) and it may be suggested since D-chlorosuccinic acid, an inhibitor (2,3), does not undergo exchange (1) that transfer of the starred hydrogen to enzyme is the initiating step in exchange and dehydrogenation.

It is of interest to note that on proceeding from natural isocitric acid to succinic acid via Krebs' Cycle reactions in D_2O, L(+)-monodeuterio-succinic acid results (6). With this datum and present and previous (11) stereochemical information the stereochemical course of the Krebs' Cycle may be completely depicted.

Acknowledgements.- Support of this work by Grant No. GM-06245 from the National Institute of General Medical Science, U.S. Public Health Service, is gratefully acknowledged. We wish to thank Messrs. Grote and Thiel of the Bendix Corporation and Drs. Sprinson and Beychok for the rotatory dispersion curves, and Dr. E.F. Phares for the mass spectrometer data.

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