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STEREOSPECIFICITY OF HYDROGEN TRANSFER IN THE SACCHAROPINE DEHYDROGENASE REACTION

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Summary

The stereospecificity of hydrogen transfer in the synthesis of saccharopine from α-ketoglutarate and L-lysine catalyzed by saccharopine dehydrogenase (N^5-(1,3-dicarboxypropyl)-L-lysine: NAD oxidoreductase (L-lysine-forming), EC 1.5.1.7) was examined by using [4A-^{3}H]- and [4B-^{3}H]NADH. The enzyme showed the A-stereospecificity. The NMR analysis of the saccharopine prepared with [4A-^{2}H]NADH revealed that the label was incorporated into the C-2 of the glutaryl moiety.

The kinetic mechanism of the yeast saccharopine dehydrogenase (N^5-(1,3-decarboxypropyl)-L-lysine: NAD oxidoreductase (L-lysine-forming), EC 1.5.1.7) reaction requires that all substrates must be present before the reaction can take place. The enzyme was shown to bind the substrates in an obligatory order, with the oxidized and reduced coenzymes adding first and leaving last. In the direction of saccharopine synthesis, the second substrate adding is α-ketoglutarate [1]. The binding study also confirmed these findings [2]. From this and other kinetic evidence [3,4], it is reasonable to assume that the oxidation-reduction by pyridine nucleotide coenzymes takes place on a Schiff base between lysine and α-ketoglutarate formed at the active site. However, the detailed mechanism of hydrogen transfer reaction remains to be elucidated. In the present investigation, the stereospecificity of hydrogen transfer and the site to which the hydrogen of NADH is transferred were examined.

When L-lysine and α-ketoglutarate were incubated with yeast saccharopine dehydrogenase in the presence of either [4A-^{3}H]- or [4B-^{3}H]NADH, the label was incorporated into saccharopine only with [4A-^{3}H]NADH. The

transfer of the label was quantitative and essentially no label remained in NAD^+ (Table I). The results clearly indicate that the *pro-R* hydrogen at the C-4 position of the dihydronicotinamide ring of the coenzyme is exclusively transferred without exchange with protons of the medium.

In order to confirm further the stereospecificity of hydrogen transfer and to locate the position of isotope incorporation, saccharopine was synthesized from lysine, α-ketoglutarate and [4A-^{2}H]NADH, and the NMR spectrum

TABLE I

STEREOSPECIFICITY OF HYDROGEN TRANSFER FROM COENZYME TO SACCHAROPINE

[4A-^{3}H]NADH was prepared by the reduction of NAD^+ with [1-^{3}H]ethanol (New England Nuclear) and yeast alcohol dehydrogenase; and [4B-^{3}H]NADH with DL-[2-^{3}H]glutamate (New England Nuclear) and bovine liver glutamate dehydrogenase. The reduced coenzyme was purified by chromatography on DEAE-cellulose (bicarbonate form). The synthesis of saccharopine was carried out by incubating 60 μmol of L-lysine, 15 μmol of α-ketoglutarate, a purified preparation of yeast saccharopine dehydrogenase [5] and 5 μmol of either [4A-^{3}H]- or [4B-^{3}H]NADH in 5 ml of 0.08 M NH_4HCO_3. Saccharopine and NAD^+ were separated by chromatography on Dowex 1X8 (acetate form). 0.1 M acetic acid and 0.1 M sodium acetate were used to elute saccharopine and NAD^+, respectively. Concentration of saccharopine in the effluent was determined by the amount of NADH formed on incubation with saccharopine dehydrogenase and excess NAD^+, and that of NAD^+ from its molar absorbance (ϵ = 18.0 cm^{-1}) at 260 nm. The radioactivity measurements were made in an Aloka model LSC-903 liquid scintillation spectrometer, using a toluene scintillation fluid.

Coenzyme	Radioactivity (dpm/μmol × 10^{-4})		
	Before reaction	After reaction	
	NADH	NAD^+	Saccharopine
[4A-^{3}H]NADH	4.28	0.20	4.10
[4B-^{3}H]NADH	1.30	1.25	0.11

of the resulting saccharopine was determined. Fig. 1 shows the spectra of saccharopine prepared with NADH and [4A-^{2}H]NADH. Both compounds gave an identical spectrum except the signals at 3.2 to 3.6 ppm. It was shown previously with N^5-(1-carboxyethyl)-L-lysine that the protons at C-2 of the lysine moiety and C-2 of the propionyl moiety gave overlapping peaks in this region, and the proton at the propionyl residue constituted the high field signal [7]. From this fact and by comparison with L-lysine spectrum (not shown), the high field signals in the 3.2—3.6 ppm regions may be assigned to the C-2 proton of the glutaryl moiety. Fig. 1B shows that this signal is completely absent in saccharopine prepared with [4A-^{2}H]NADH. Integration of the signals in this region also indicates a loss of signal corresponding to one proton. Thus, the NMR study shows that the *pro-R* hydrogen at C-4 of NADH is quantitatively transferred to C-2 of the glutaryl moiety of saccharopine.

The results obtained in this study are consistent with the idea that direct hydrogen transfer occurs from *pro-4R* position of NADH to the ketimine carbon of a Schiff base between the enzyme-bound lysine and α-ketoglutarate. The stereospecificities of a great many pyridine nucleotide dehydrogenases are now known (see, for example, Ref. 8). From available data, the stereospecificity of hydrogen transfer appears to be characteristic of the enzyme, not of the substrate or the type of reaction catalyzed. Among dehydrogenases catalyzing the C-N bond formation and cleavage, alanine dehydrogenase is

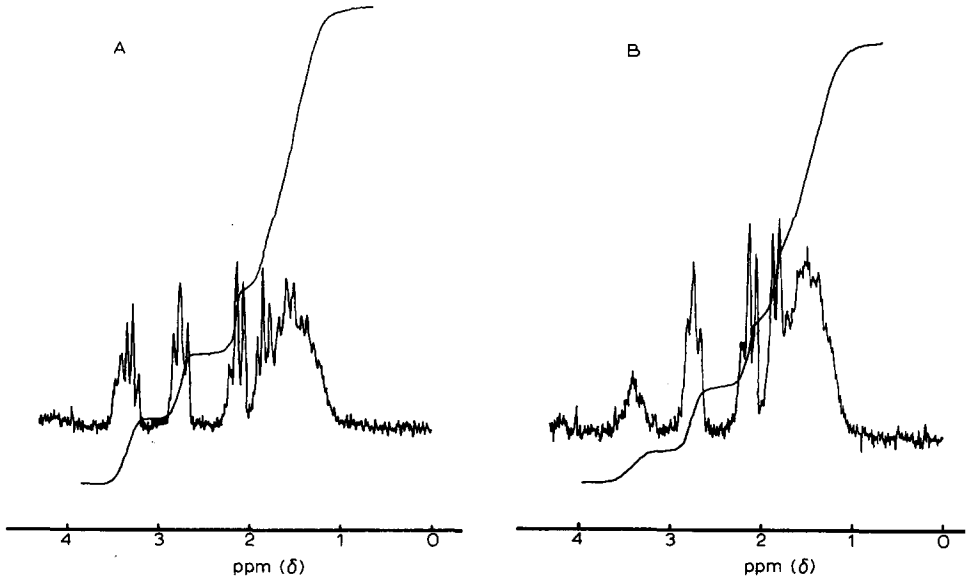

Fig. 1. The NMR spectra of saccharopine prepared with NADH (A) and with [4A-^{2}H]NADH (B). [4A-^{2}H]NADH was prepared by the reduction of NAD^+ with [$^2H_6$]ethanol (Merck, Sharp and Dohme, Canada; anhydrous, 99% isotopic purity) and the yeast alcohol dehydrogenase essentially as described by Dalziel [6]. Saccharopine synthesized by incubation of L-lysine, α-ketoglutarate, and NADH or [4A-^{2}H] NADH with saccharopine dehydrogenase was purified by chromatography on Dowex 1. The materials were recrystallized from H_2O. The NMR spectra were taken at 90 MHz in a Varian EM-390 spectrometer on samples dissolved in 0.18 M NaO^{2}H (Merck, Sharp and Dohme, Canada) in ^2H_2O (5%). The ambient temperature was 25° C.

shown to be A-stereospecific [9], while glutamate dehydrogenase [10] and leucine dehydrogenase [11] are B-specific. It is of interest to note that octopine dehydrogenase, which catalyzes the reaction analogous to saccharopine dehydrogenase, belongs to the B-class [12].

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