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Distribution of L-Alanine Dehydrogenase and L-Leucine Dehydrogenase in Bacteria

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It has been well known that L-glutamic dehydrogenase is extensively distributed in various organisms and plays an important role in the syntheses of amino acid and protein. Besides L-glutamic dehydrogenase, L-alanine dehydrogenase and L-leucine dehydrogenase were found in *Bacilli* by WIAME and PIÉRARD[1] and by STRUCK and SIZER[2], respectively. Moreover, ZINK and SANWAL[3] have reported on the distribution and substrate specificity of NAD-dependent L-leucine dehydrogenase in *Bacilli*. Recently, HERMIER[4] suggested the existence of L-valine dehydrogenase in *Bacillus subtilis*. Now, it seems reasonable to assume the existence of other amino acid dehydrogenases or some dehydrogenases which have broad substrate specificity. Distribution and physiological significance of amino acid dehydrogenases are very interesting problems. HONG *et al.*[5] reported on the distribution of L-alanine and L-glutamic dehydrogenases in *Bacilli*. We have also studied on the distribution of L-alanine and L-leucine dehydrogenases in several microorganisms, and some experimental results will be reported here.

Bacteria were cultivated on the ordinary pepton broth-agar plates for 18 hours at 37°, except for 42 hours at 24° in the cases of *Micrococcus citreus*, *Sarcina lutea*, *Acetobacter xylinum*, and *Serratia marcescens*. Cell-free extracts of these bacteria were made by sonic vibration or by grinding with quartz sand. Protein content was determined by the method described by LAYNE[6] or by the turbidimetry with trichloroacetic acid[7].

Enzyme activities were estimated by measuring the changes of optical density at 340 mμ ascribing the formation of NADH or NADPH.

Specific activities were expressed as the change in optical density/min/mg protein $\times 10^3$. These reactions were always performed at 25 to 30°.

Experimental results are presented in Table 1, in which all *Bacilli* examined are shown to have L-alanine dehydrogenase activity as previously reported by several investigators[2-5]. However, it was found that L-alanine dehydrogenase examined in this study was specific for not only NAD but also for NADP. It

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TABLE 1. L-Alanine and L-leucine dehydrogenases in several bacteria

	Specific activity of			
	L-alanine dehydrogenase		L-leucine dehydrogenase	
	NADP-specific	NAD-specific	NADP-specific	NAD-specific
Bacillus subtilis	26	37	20	30
B. subtilis-AS*	128	387	128	121
B. subtilis-HAS*	66	179	317	514
B. mesentericus	85	148	62	71
B. mesentericus-AS*	80	18**	11	7**
B. mycoides	51	29**	28	+
B. mycoides-AS*	67	21**	34	12
Acetobacter xylinum	48	64	35	52
Sarcina lutea	14	37	0	0
Micrococcus citreus	+	+	0	0
Staphylococcus aureus	+	+	0	0
Proteus vulgaris	0	0	0	0
Serratia marcescens	0	0	0	0
Pseudomonas aeruginosa	0	0	0	0
Escherichia coli	0	0	0	0

* AS, 80% ammonium sulfate ppt.; HAS, heated AS-fraction which was prepared by centrifugation after heating at 60° for 5 min. Both the fractions were used after dialysis more than 2 hr against running water.

** NAD (about 40% pure) were used in these experiments.

was unexpected that L-alanine dehydrogenase activity was not found in *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, and *Serratia marcescens*, because these bacteria could oxidize L-alanine rapidly[8]. On the contrary, although *M. citreus* and *Sarc. lutea* could oxidize L-alanine only weakly[8], it was found that both the bacteria had L-alanine dehydrogenase activity. On the relationship between amino acid oxidation and the dehydrogenation, it is not able to derive any conclusion from these data. L-Alanine and L-leucine dehydrogenase reactions may be functioning not only as catabolic reaction but also as anabolic one in living cells, because these reactions were reversible and the reverse reactions were proceeded as rapid as the forward reactions (Fig. 1).

As to L-leucine dehydrogenase, the enzyme has been found in *Bacilli*[2,3]. In this study, the enzyme was also ascertained in several *Bacilli* and *Acetobact. xylinum*, which required NADP or NAD. In the case of the heat-treated preparation of *B. subtillis*, L-leucine dehydrogenase activity was found to be higher than L-alanine dehydrogenase activity as seen in Table 1 and Fig. 1.

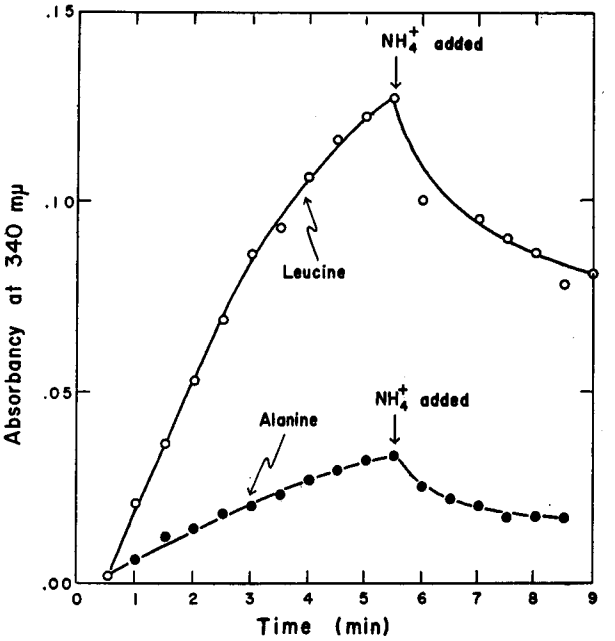

Fig. 1. Activity and reversibility of L-alanine and L-leucine dehydrogenases in HAS-fraction of *B. subtilis*. Reaction mixtures in 3 ml cuvettes contained 100 μmoles of glycine-NaOH buffer, pH 11.0, 50 μmoles of substrate, 0.6~0.8 μmole of NAD or NADP and cell-free extract. After 5.5 min, 0.1 ml of 2 M ammonium chloride was added.

It has been confirmed that L-leucine : pyruvate aminotransferase is not involved in leucine dehydrogenation reaction at least in *Acetobact. xylinum*.

Preparation of NADP-linked L-leucine dehydrogenase in *B. subtilis* and of L-alanine and L-leucine dehydrogenases in *Acetobact. xylinum* and investigations on their properties are in progress.

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