10. I. Omura and R. Sato, J. Biol. Chem., 239, 2370 (1964).
11. I. Omura and R. Sato, J. Biol. Chem., 239, 2379 (1964).
12. R. Pickering, Arzmeim.-Forsch., 27, 1992 (1977).
13. D. Platt, K. Förster, and L. Förster, Mech. Ageing Dev., 7, 183 (1978).
14. S. Reitman and S. Frenkel, J. Clin. Pathol., 28, 56 (1957).
15. Y. Shigeta, Gastroent. Jpn., 12, 435 (1977).

PRIMARY STRUCTURE OF HISTIDINE DECARBOXYLASE

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It is impossible to understand the mechanism of action of enzymes without a detailed study of their structural organization. It was for this reason, therefore, that we decided to investigate the primary structure of the enzyme histidine decarboxylase (HD) of Micrococcus sp. n., responsible for the catalytic decarboxylation of L-histidine, with the formation of the physiologically active substance, histamine [7].

The aim of this investigation was to summarize the results of a study of the primary structure of HD from Micrococcus sp. n. and the compare its primary structure with that of the HD from another bacterium, Lactobacillus 30a, as established by a group of American investigators [9, 12].

HD of Micrococcus sp. n. has mol. wt. of about 100 kD and consists of 3α- and 3β-polypeptide chains, differing in their molecular weight and amino-acid composition [10]. One difference between bacterial HD and pyridoxal-dependent HD of animal tissues is that the role of coenzyme in the former is played by the pyruvate residue, the carbonyl group of which takes part in the catalytic act like the carbonyl group of pyridoxal phosphate.

EXPERIMENTAL METHOD

Pyruvate residues in the bacterial HD molecule are covalently bound with N-terminal amino acids of α-chains, and determination of the N-terminal sequence of amino acids in this chain therefore required the use of methods unblocking the α-amino group of the N-terminal amino acid: conversion of the pyruvate residue into alanine by reductive amination or its removal by the reaction with o-phenylenediamine [1]. Analysis of the N- and C-terminal amino acid sequences and also of amino-acid sequences in the various tryptic peptides of the α-chain (maleylated and nonmaleylated) was insufficient to establish its primary structure [8, 11]. Further structural analysis was undertaken of large fragments of the α-chain obtained by chemical cleavage of the protein molecules: cleavage beyond tryptophan residues (with iodosobenzoic acid), cleavage at cysteine residues (with nitrothiocyanobenzoic acid), and restricted acid hydrolysis (with acetic acid) [3-5]. On the basis of the results of these investigations, the primary structure of the α-chain could be identified and it could be shown to consist of 225 amino-acid residues.

The β-chain of HD consists of 79 amino-acid residues and differs from the α-chain is not containing proline, histidine, tryptophan, and cysteine. The primary structure of the β-chain was established from the results of analysis of amino-acid sequences: of tryptic peptides of maleylated and nonmaleylated protein, and of the N- and C-terminal regions of the chain [2, 11].

EXPERIMENTAL RESULTS

The identified primary structures of the α- and β-chains together form the complete primary structure of HD of Micrococcus sp. n. (see Scheme 1).

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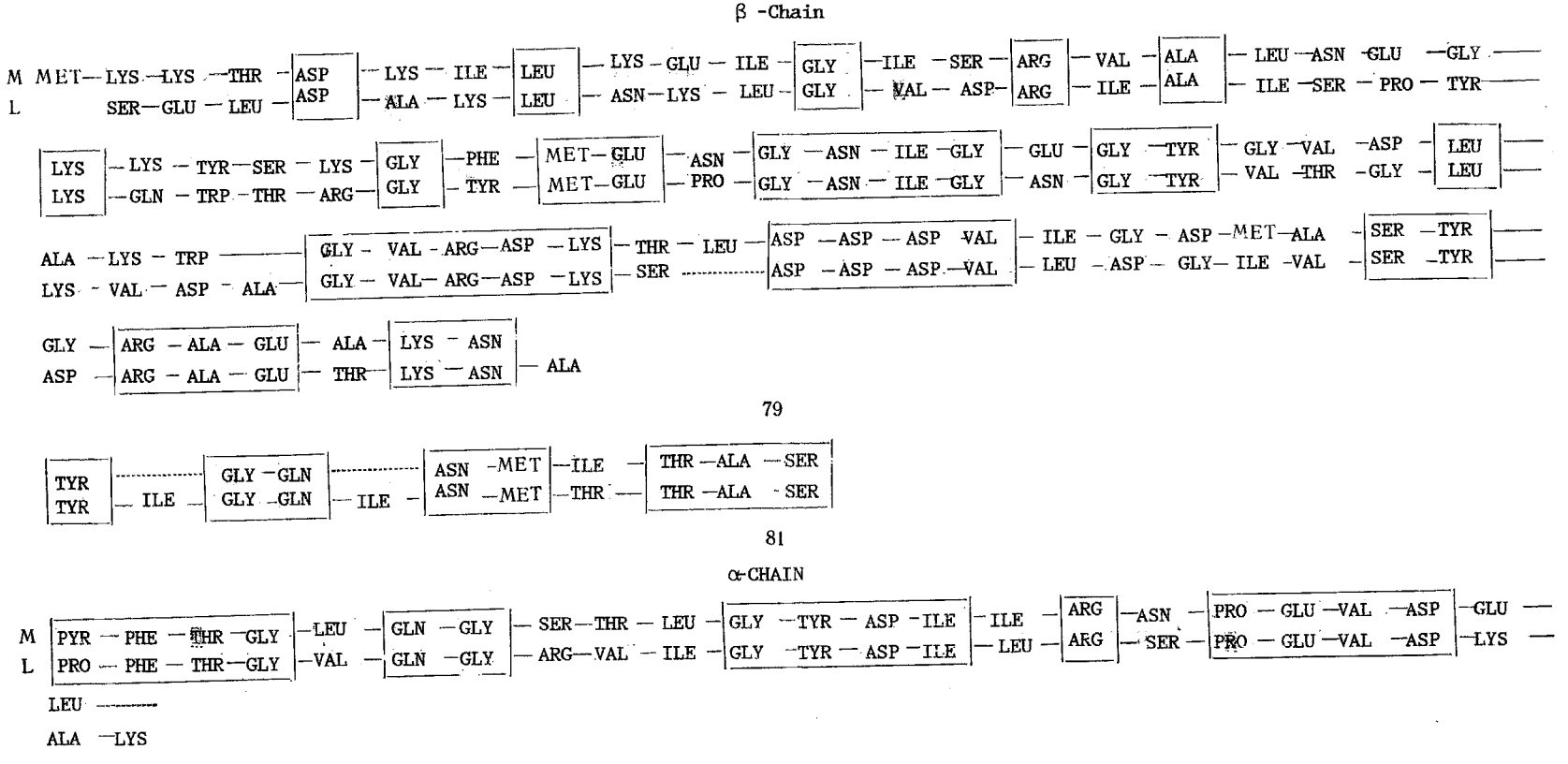
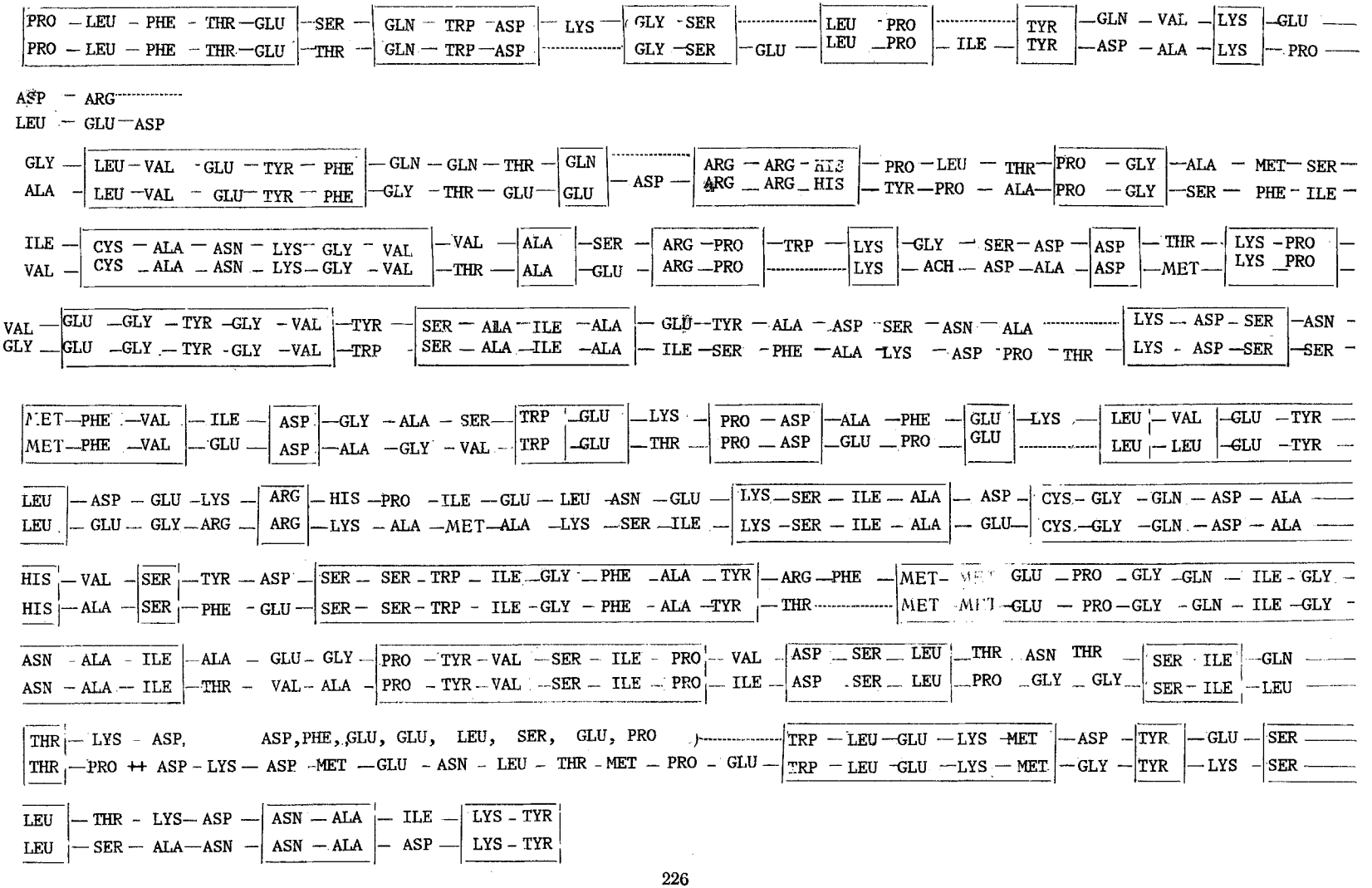
SCHEME 1


β -Chain
M MET—LYS—LYS—THR—ASP/ASP—LYS—ILE—LEU/LEU—LYS—GLU—ILE—GLY/GLY—ILE—SER—ARG/ARG—VAL—ALA/ALA—LEU—ASN—GLU—GLY
L SER—GLU—LEU—ALA—LYS—ASN—LYS—LEU—VAL—ASP—ILE—SER—PRO—TYR
LYS/LYS—LYS—TYR—SER—LYS—GLY/GLY—PHE—MET—GLU/MET—GLU—ASN/PRO—GLY—ASN—ILE—GLY/GLY—ASN—ILE—GLY—GLU—GLY—TYR/GLY—TYR—GLY—VAL—ASP—LEU/LEU
GLN—TRP—THR—ARG
ALA—LYS—TRP
LYS—VAL—ASP—ALA—GLY—VAL—ARG—ASP—LYS/GLY—VAL—ARG—ASP—LYS—THR—LEU—SER
VAL—THR—GLY
ASP—ASP—ASP—VAL/ASP—ASP—ASP—VAL—ILE—GLY—ASP—MET—ALA—SER—TYR/SER—TYR
LEU—ASP—GLY—ILE—VAL
GLY—ARG—ALA—GLU/ARG—ALA—GLU—ALA—LYS—ASN/LYS—ASN—ALA
ASP
THR
79
TYR/TYR—ILE—GLY—GLN/GLY—GLN—ILE—ASN—MET/ASN—MET—ILE—THR—THR—ALA—SER/THR—ALA—SER
81
α-CHAIN
M PYR—PHE—THR—GLY/PRO—PHE—THR—GLY—LEU—VAL—GLN—GLY/GLN—GLY—SER—THR—LEU—ARG—VAL—ILE—GLY—TYR—ASP—ILE/GLY—TYR—ASP—ILE—ILE—LEU—ARG/ARG—ASN—SER—PRO—GLU—VAL—ASP/PRO—GLU—VAL—ASP—GLU—LYS
L LEU
ALA—LYS



It is interesting to compare the primary structure of HD of Micrococcus sp. n. with the primary structure of HD of Lactobacillus 30a, for these enzymes differ mainly in their three-dimensional organization (quaternary structure). For instance, HD of Micrococcus sp. n. is a 3(α + β) trimer, where HD of Lactobacillus 30a is a 6(α + β) hexamer. Comparative analysis of the primary structures of these enzymes shows that they have considerable structural similarity (52%), evidence of their origin during evolution from a common ancestor. However, despite the considerable similarity of the primary structures of HD from different bacterial sources, differences between them in amino-acid sequences in many regions of the polypeptide chains lead to the formation of different quaternary structures also. Nevertheless, differences found in these enzymes, both in their primary structures and in their corresponding quaternary structures do not affect the basic function, i.e., catalytic decarboxylation of L-histidine. The existance of conservative regions of amino-acid sequences of the primary structures of these enzymes must also be noted, including regions with residues whose functional groups play a direct part in enzymic catalysis. These are the N-terminal region of the α-chain with the pyruvate residue (PYR-PHE-THR-GLY-) and the region with a cysteine residue (-ASP-CYS-GLY-SLN-ASP-) in a hydrophilic enrivonment [6].

LITERATURE CITED

1. A. E. Alekseeva and V. N. Prozorovskii, Biokhimiya, 41, No. 9, 1584 (1976).
2. A. E. Alekseeva, V. N. Prozorovskii, and O. G. Grebenshchikova, Biokhimiya, 41, No. 10, 1760 (1976).
3. A. E. Alekseeva, V. N. Prozorovskii, and O. G. Grebenshchikova, Biokhimiya, 50, No. 8, 1330 (1985).
4. A. E. Alekseeva, O. G. Grebenshchikova, and V. N. Prozorovskii, Biokhimiya, 51, No. 8, 1235 (1986).
5. O. G. Grebenshchikova and V. N. Prozorovskii, Biokhimiya, 51, No. 7, 1100 (1986).
6. N. A. Gonchar, O. G. Grebenshchikova, and V. N. Prozorovskii, Vopr. Med. Khimii, No. 6, 94 (1984).
7. S. R. Mardashev and L. A. Semina, Dokl. Akad. Nauk SSSR, 156, No. 2, 465 (1964).
8. V. N. Prozorovskii, A. E. Alekseeva, O. G. Grebenshchikova, and L. G. Rashkovetskii, Biokhimiya, 46, No. 2, 269 (1981).
9. Q. K. Huynh, P. A. Recsai, G. L. Vaalert, and E. Snell, J. Biol. Chem., 259, No. 5, 2833 (1984).
10. V. N. Prozorovskii (V. Prozorovski) and H. Jörnvall, Eur. J. Biochem., 42, 405 (1974).
11. V. N. Prozorovskii (V. Prozorovski) and H. Jörnvall, Eur. J. Biochem., 53, 169 (1975).
12. G. L. Vaalert, P. A. Recsai, J. L. Foz, and E. E. Snell, J. Biol. Chem., 257, 21770 (1982).