Proc. Nat. Acad. Sci. USA
Vol. 69, No. 6, pp. 1380–1383, June 1972

Glutamate Dehydrogenase: Amino-Acid Sequence of the Bovine Enzyme and Comparison with That from Chicken Liver

KENNETH MOON, DENNIS PISZKIEWICZ*, AND EMIL L. SMITH

Department of Biological Chemistry, School of Medicine, and the Molecular Biology Institute, University of California, Los Angeles, Calif. 90024

Contributed by Emil L. Smith, March 31, 1972

ABSTRACT **Further investigation of the amino-acid sequence of bovine glutamate dehydrogenase (EC 1.4.1.3) has led to the correction of an earlier tentative sequence. The presently known sequence contains 500 residues in the subunit single peptide chain. The sequence of the homologous enzyme from chicken liver indicates that it contains 503 residues. Of the presently established 485 residues in the chicken dehydrogenase, only 26 residues differ from those in the bovine enzyme. The chicken dehydrogenase also differs in having an additional three residues at the amino terminus. The amino-terminal residue of the chicken enzyme was found to be cysteic acid.**

In a previous communication (1), a tentative amino-acid sequence was proposed for bovine liver glutamate dehydrogenase (EC 1.4.1.3). Although the sequence of the major portion of the subunit polypeptide chain had been determined (2–8), two gaps in the squence had not been overlapped and, in a number of locations, some sequence work had not been completed. We now wish to report essentially the complete sequence of the bovine enzyme, including some revisions of the earlier tentative sequence. Concurrently, we have investigated the sequence of the glutamate dehydrogenase of chicken liver with the possibility in mind that it might be somewhat easier to obtain complementary information that would span the gaps and, by homology, aid in completing the sequence of the bovine enzyme. In this communication, we report briefly on our findings; the detailed studies will be reported elsewhere.

RESULTS AND DISCUSSION

The enzymically active monomeric form of bovine liver glutamate dehydrogenase consists of six identical subunit polypeptide chains, each with a molecular weight of about 56,000 (1). Present information on the amino-acid sequence of the subunit of the bovine enzyme is shown as the continuous sequence in Fig. 1. Where a continuous line is given under this sequence, the chicken enzyme is identical to the bovine glutamate dehydrogenase. Tryptic and cyanogen bromide peptides from the chicken enzyme were obtained by conventional methods and aligned partially by homology. Residues that differ in the chicken dehydrogenase are explicitly shown. In the present paper all residue numbers will be from the sequence shown in Fig. 1; these differ somewhat, as explained below, from the previous sequence.

Present evidence indicates that there are 500 residues in the bovine enzyme, six less than the earlier tentative sequence (1). Landon *et al.* (2) isolated a tryptic peptide, T22, that gave an analysis indicating a content of 13 residues. It is now known that this is in fact a heptapeptide, representing residues 148 through 154. A 50% recovery, on acid hydrolysis, of the amino-terminal phenylalanyl residue of the peptide led to the earlier erroneous conclusion that there was one residue of phenylalanine and two residues of each of the other amino acids. Indeed, it had been noted in a number of our studies (8) that low recoveries of the amino-terminal residues of some pure peptides were encountered, presumably because of oxidative loss on handling or storage. Peptide T22 was reisolated from a tryptic digestion of the performic acid-oxidized bovine dehydrogenase. Again, this gave a relatively low recovery of the phenylalanyl residue: Phe, 0.65(1); Thr, 1.00(1); $MetSO_2$, 1.00(1); Glu, 1.12(1); Leu, 1.00(1); Ala, 1.00(1); Lys, 1.00(1). The complete sequence of this peptide was determined by sequential Edman degradation, as shown in Fig. 2 for residues 148 through 154. Further evidence that position 149 was occupied by a threonyl residue was provided from the overlapping peptide CNBr3-17 (5), representing residues 147 through 150. This was a tryptic peptide derived from peptide CNBr3. Based on composition only, the threonyl residue had originally been placed as preceding Arg-147 (5). One step of the Edman procedure revealed the amino-terminal residue to be arginine. The threonyl residue is precluded from occupying position 148, as it is occupied by a phenylalanyl residue, and thus must be placed in position 149.

The change of a leucine in the bovine enzyme for a methionine in the chicken enzyme at residue 302 proved to be useful since it permitted the isolation, from the cyanogen bromide cleavage products of the chicken enzyme, of a peptide that spanned one of the gaps. Studies on this peptide showed that two consecutive tryptic peptides in the bovine enzyme had been incorrectly placed in the polypeptide chain.

The carboxyl-terminal sequence of the CNBr peptide, residues 303 through 366, was determined as -Asn-Ile-Met. Previously, it had been indicated in the bovine enzyme that Glx was present instead of an asparaginyl residue (1). This was based on the composition of a large maleylated tryptic peptide, TOM 18+19 (4). It will be indicated below that the sequence in this region of the bovine enzyme is the same as in the chicken enzyme. The cyanogen bromide peptide (residues 303–366) was hydrolyzed with chymotrypsin and the resultant peptides were isolated. In the carboxyl-

* Present address: University of California, Irvine, Calif. 92664.

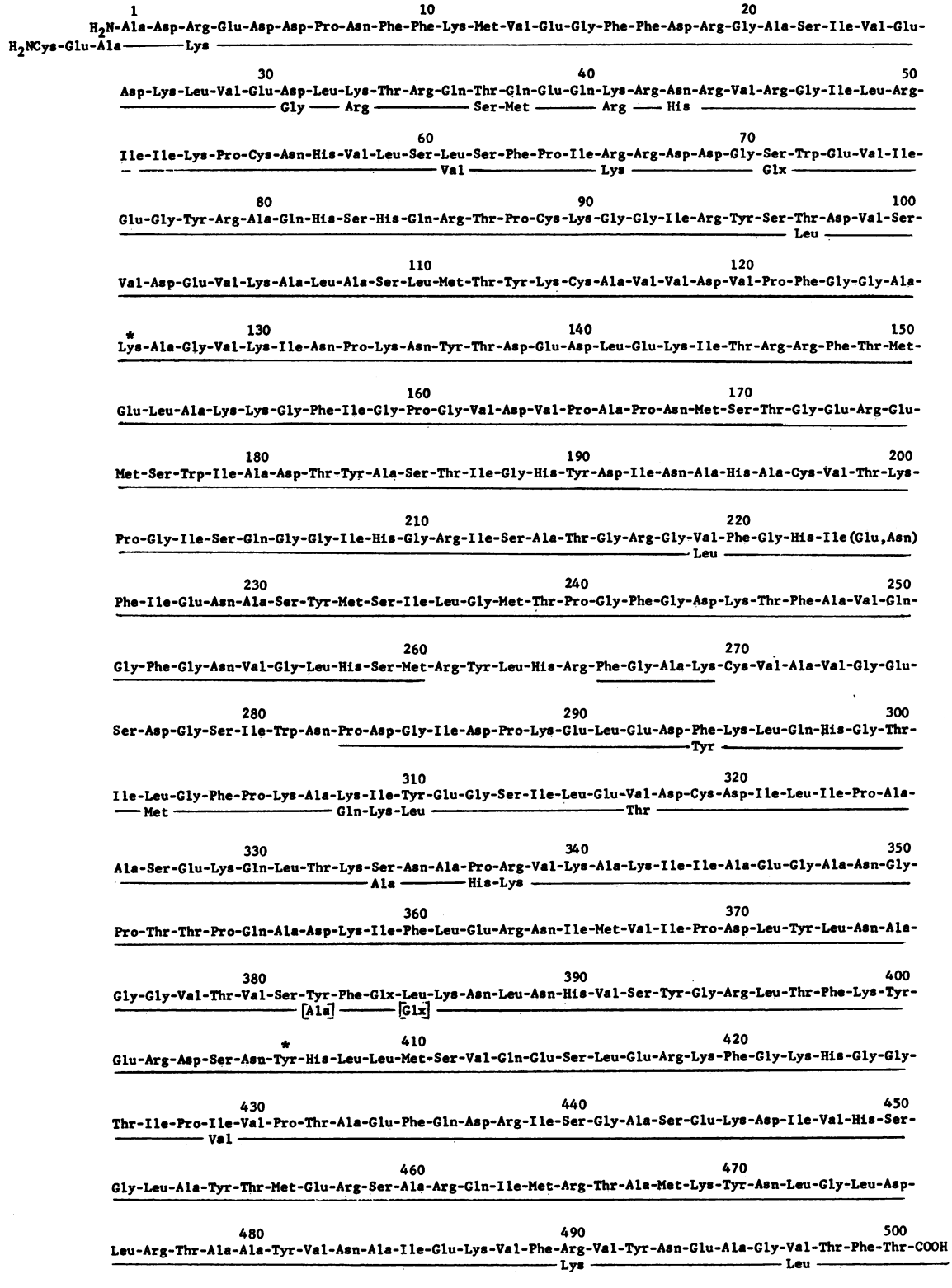

FIG. 1. The amino-acid sequence of bovine liver glutamate dehydrogenase. Residues that differ in the chicken liver enzyme, which possesses three additional residues at the amino-terminus, are shown below the bovine sequence. The amino-terminal residue of the chicken enzyme was found as cysteic acid but is shown as cysteine since it is not known whether the oxidation occurred before the isolation of the enzyme. Where no differences in sequence were found, a continuous line is shown. Gaps indicate regions of the sequence of the chicken enzyme for which pure peptides have not yet been isolated. Residues 382 and 385 have been replaced but precise placements of the two different residues, identified by amino-acid composition, have not been ascertained.

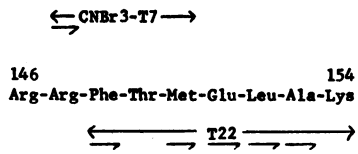

FIG. 2. Amino-acid sequence of T22. *Arrows* indicate residues identified by the Edman degradation procedure.

terminal hexapeptide, residues 361 through 366, Leu-Glu-Arg-Asn-Ile-Met, residues 361 through 363 clearly were derived from peptide T40+41 (3), and the remaining three residues were derived from the carboxyl end of the intact CNBr peptide, as given above. This finding clearly demonstrated that a span of 29 residues, representing peptides T42+43A and T44 (2) (Fig. 3), that had previously been placed in this region of the sequence (4) of the bovine enzyme, did not belong in this part of the molecule†.

The above result on the chicken enzyme was verified for the bovine enzyme by the following procedure. A mixture of peptides CNBr2 (residues 13–111) and CNBr10 (residues 260–366) (5) was prepared from a cyanogen bromide digest of the bovine enzyme. A unique -Asn-Gly- sequence (residues 349 and 350) in peptide CNBr10 was expected to be susceptible to cleavage by hydroxylamine (9). The mixture of peptides CNBr2 and CNBr10 was treated with hydroxylamine by essentially the procedure of Bornstein and Balian (10). From the reaction mixture, a peptide comprising residues 350 through 366 was isolated; this proved to have the same composition and sequence as obtained from the studies on the chicken enzyme.

It should be noted that Landon *et al.* (8) had isolated a thermolysin peptide, CNBr10-L12, having the sequence, Leu-Glu-Arg-Asn. This peptide could not be fitted into the original tentative scheme (1) unless it was assumed that an allotypical replacement of an asparaginyl for an isoleucyl residue had occurred (8). This clearly is not the case and peptide CNBr10-L12 represents residues 361 through 364.

Peptic peptide, P52 (1), had provided definitive evidence for the overlap of tryptic peptides T42+43A and T44 (Fig. 3). It was known that these last two tryptic peptides had to be derived from either peptide CNBr10 or peptide CNBr2 (8), and since it was demonstrated that this sequence is not part of CNBr10 it must have been derived from the region between residues 50 and 80, i.e., from CNBr2 and in the other gap of the earlier tentative sequence (1). It was also known, from a study of the tryptic peptides derived from the maleylated bovine enzyme (4), that residues 51 and 80 must each be preceded by an arginyl residue.

Evidence that residues 79 and 80 are linked was provided by a chemical cleavage at tryptophan-72. A mixture of peptides CNBr2 and CNBr10 from the bovine enzyme was treated with a bromine adduct of 2-(2-nitrophenylsulfenyl)-3-methylindole under the conditions described by Burnett and Eylar (11). After chromatography of the mixture on Sephadex G-50, a peptide was isolated that was shown to comprise residues 73 through 111, thus establishing a definitive overlap.

The evidence for the overlap of residues 50 and 51 in the bovine enzyme is not so compelling inasmuch as it relies on a peptic peptide, comprising residues 50 through 60, with an overlap of only one amino-acid residue, namely, Arg-50. However, no peptides have been found in any of our earlier or present studies on the bovine or the chicken enzymes that are not accommodated by the sequence shown in Fig. 1.

Homology relationships of the bovine and chicken enzymes

The essential lysyl residue of glutamate dehydrogenase, residue 126 (previously numbered residue 97), in both the bovine (6) and chicken liver enzymes react with pyridoxal 5′-phosphate with resulting inactivation of the enzyme. As indicated in Fig. 1, the amino-acid sequence around the essential lysyl residue from residues 98 through 218 is identical in both enzymes. This is also the case for a long stretch of the carboxyl-terminal region including the sequence around tyrosine-406 (previously numbered 412), which upon nitration modified susceptibility of the enzyme to GTP as an allosteric inhibitor (12). In fact the overall homology of the two enzymes throughout the entire sequence is very considerable indeed.

From tryptic and cyanogen bromide digests of the chicken dehydrogenase we have accounted for some 485 residues out of a total of 503, assuming that there are no deletions or additions in those peptides of the chicken enzyme which, as yet, have not been isolated in pure form. Surprisingly, there are only 26 known changes in the sequence of the chicken enzyme when compared with the bovine enzyme for the 500 residues possessed in common by both enzymes.

A striking difference between the two enzymes occurs at the amino-terminus where the chicken enzyme has three additional residues. Thus, glutamate dehydrogenase is one of a number of examples (see ref. 13 for a recent review) of homologous proteins from different species in which additional residues occur at either the amino- and/or the carboxyl-ends of the polypeptide chains.

It is also noteworthy that the amino-terminal residue of the chicken enzyme was always found to be present in oxidized form as cysteic acid, presumably because of oxidation either in the nascent peptide chain, after the protein was folded, or during the isolation procedures. All other cysteine residues were found as carboxymethylcysteine residues after carboxymethylation under reducing conditions. The presence of an amino-terminal cysteic acid residue had no detectable in-

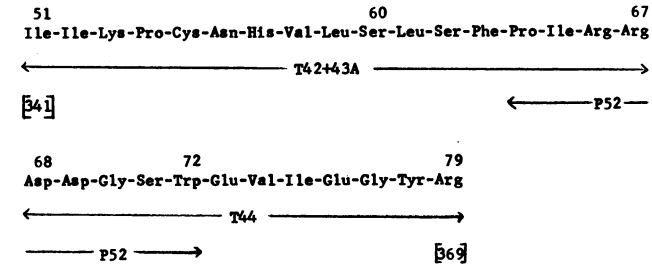

FIG. 3. Amino-acid sequence of peptides T42+43A and T44 previously indicated as residues 341 through 369, as shown in brackets below the sequence, have now been found to represent residues 51 through 79.

† The earlier evidence that peptide T42+43A was joined to peptide T41 was indicated by a tryptic peptide obtained from the maleylated enzyme, peptide TOM 18+19 (4). On reinvestigation it was found that peptide TOM 18+19 was an equimolar mixture of peptides TOM 18 and TOM 19 and not a single joined sequence.

fluence on the enzymic activity of the chicken glutamate dehydrogenase.

We are indebted to Miss Dorothy McNall for technical assistance. This work was supported by Grant GM-11061 of the National Institutes of General Medical Sciences of the United States Public Health Service.

1. Smith, E. L., Landon, M., Piszkiewicz, D., Brattin, W. J., Langley, T. J. & Melamed, M. D. (1970) "Bovine Liver Glutamate Dehydrogenase: Tentative Amino Acid Sequence," *Proc. Nat. Acad. Sci. USA* **67**, 724–730.
2. Landon, M., Melamed, M. D. & Smith, E. L. (1971) "Sequence of Bovine Liver Glutamate Dehydrogenase: I. Isolation of Tryptic Peptides from the Carboxymethylated Protein," *J. Biol. Chem.* **246**, 2360–2373.
3. Landon, M., Piszkiewicz, D. & Smith, E. L. (1971) "Sequence of Bovine Liver Glutamate Dehydrogenase: II. Sequence of Tryptic Peptides," *J. Biol. Chem.* **246**, 2374–2399.
4. Brattin, W. J. & Smith, E. L. (1971) "Sequence of Bovine Liver Glutamate Dehydrogenase: III. Tryptic Peptides from the Maleylated Protein," *J. Biol. Chem.* **246**, 2400–2418.
5. Langley, T. J. & Smith, E. L. (1971) "Sequence of Bovine Liver Glutamate Dehydrogenase. IV. Cyanogen Bromide Peptides," *J. Biol. Chem.* **246**, 3789–3801.
6. Piszkiewicz, D., Landon, M. & Smith, E. L. (1970) "Bovine Liver Glutamate Dehydrogenase: Sequence of a Hexapeptide Containing a Lysyl Residue Reactive with Pyridoxal 5'-Phosphate," *J. Biol. Chem.* **245**, 2622–2626.
7. Landon, M., Langley, T. J. & Smith, E. L. (1971) "Sequence of Bovine Liver Glutamate Dehydrogenase: V. Sequence of a 44-Residue Peptide Obtained by Cyanogen Bromide Cleavage," *J. Biol. Chem.* **246**, 3802–3806.
8. Landon, M., Langley, T. J. & Smith, E. L. (1971) "Sequence of Bovine Liver Glutamate Dehydrogenase: VI. Peptides from Tryptic and Thermolysin Hydrolysates of Two Large Cyanogen Bromide Fragments," *J. Biol. Chem.* **246**, 3807–3816.
9. Bornstein, P. (1970) "Structure of α1-CB8, a Large Cyanogen Bromide Produced Fragment from the α1 Chain of Rat Collagen. The Nature of a Hydroxylamine-Sensitive Bond and Composition of Tryptic Peptides," *Biochemistry* **9**, 2408–2421.
10. Bornstein, P. & Balian, G. (1970) "The Specific Nonenzymatic Cleavage of Bovine Ribonuclease with Hydroxylamine," *J. Biol. Chem.* **245**, 4854–4856.
11. Burnett, P. R. & Eylar, E. H. (1971) "Oxidation and Cleavage of the Single Tryptophan Residue of the A1 Protein from Bovine and Human Myelin," *J. Biol. Chem.* **246**, 3425–3430.
12. Piszkiewicz, D., Landon, M. & Smith, E. L. (1971) "Bovine Glutamate Dehydrogenase: Loss of Allosteric Inhibition by Guanosine Triphosphate and Nitration of Tyrosine-412," *J. Biol. Chem.* **246**, 1324–1329.
13. Smith, E. L. (1970) in "Evolution of Enzymes," *The Enzymes*, ed. Boyer, P. D. (Academic Press, New York), Vol. 1, pp. 267–339.