AMINO ACID SEQUENCE OF THE HEME a SUBUNIT OF BOVINE HEART CYTOCHROME OXIDASE AND SEQUENCE HOMOLOGY WITH HEMOGLOBIN

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SUMMARY: The amino acid sequence of the 11.6 K dalton heme a subunit of bovine heart cytochrome oxidase has been completed and is presented here. The sequence investigation has established the positions in the protein of all the possible heme ligands, namely cysteine, methionine, histidine and lysine residues. However, the isolation conditions may have caused the heme a to migrate from its original site or the heme is caged by peptides as pointed out in Reference 6. The sequence of the heme a subunit and the β-chain of hemoglobin shows homology. It is possible that these two proteins have arisen from a common ancestor in the distant past.

There are several laboratories involved in the studies of subunits of cytochrome oxidase (1-4) but few on the amino acid sequence investigation. Our laboratories have jointly engaged in the latter and have reported the partial amino acid sequence of the "copper-subunit" of the oxidase (5). In the present investigation, the complete amino acid sequence of the "11.6 K dalton" subunit which contains heme a is reported. Certain aspects of the structure-function and evolutionary relationship of this protein with other hemoproteins are discussed.

MATERIALS AND METHODS

Bovine cytochrome oxidase and the 11.6 K heme subunit were isolated as reported previously (6). The isolated heme a subunit was carboxymethylated as described by Crestfield et al. (7). The succinyl derivative of the carboxymethylated subunit was prepared by reaction with succinic anhydride according to Klotz (8). Tryptic digestion as well as CNBr cleavage of the protein derivative was carried out and the peptides were purified by paper chromatographic methods. The procedures for the determination of the amino acid composition (9), sequence determination (10), NH_2 and COOH-terminal amino acid

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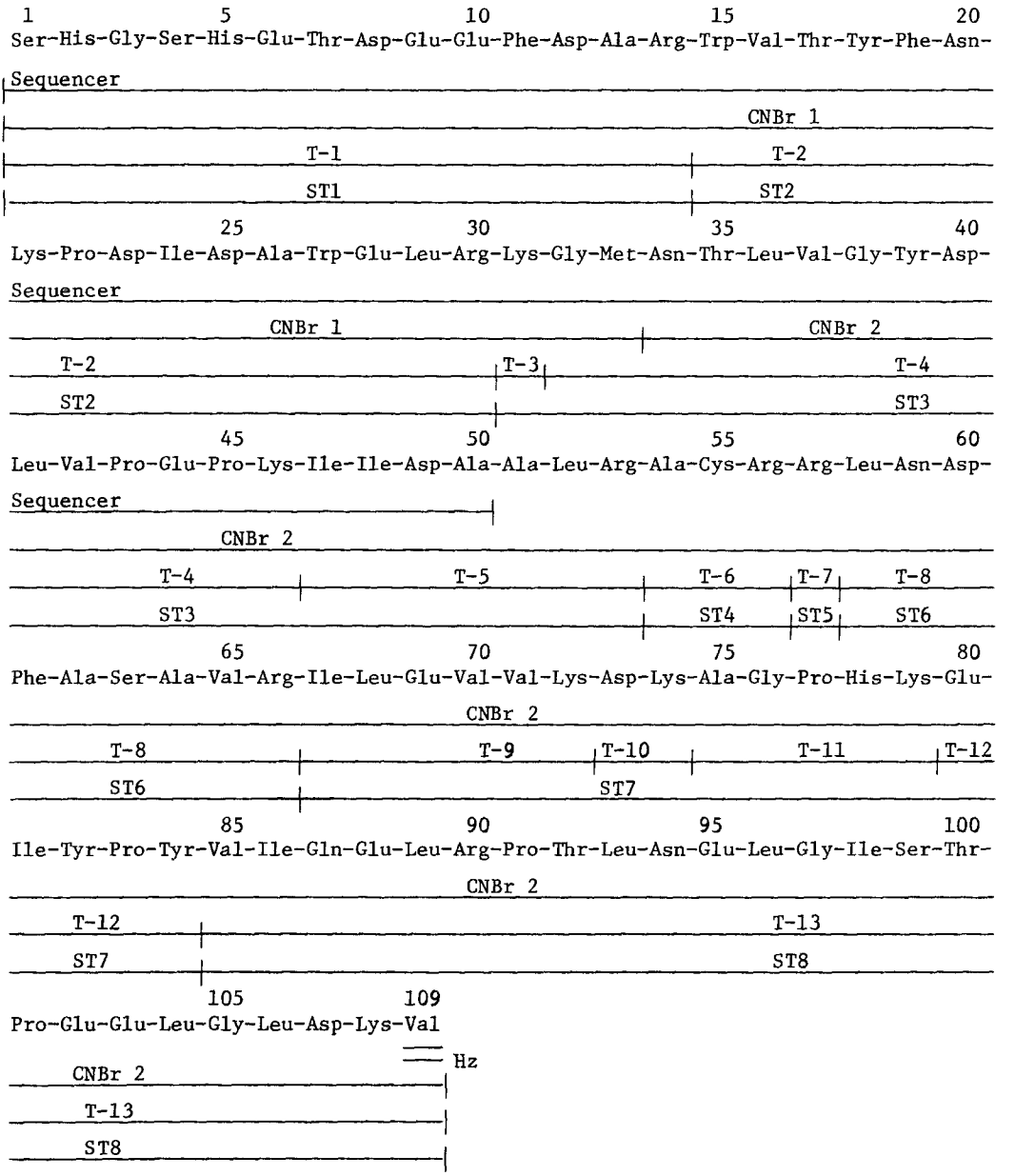

Fig. 1. The amino acid sequence of the heme a subunit of bovine heart cytochrome oxidase. The symbols CNBr-, T-, and ST- represent peptides obtained from cleavage of the carboxymethylcysteinyl-derivative of the protein by cynogenbromide, trpsin, and succinylation, respectively.

analyses (11, 12), methods used for the determination of the Pth[1] derivatives of amino acids (13) have been described previously (14, 15).

[1]Abbreviations: Hz, hydrazinolysis; Pth, 3-phenyl-2-thiohydantoin.

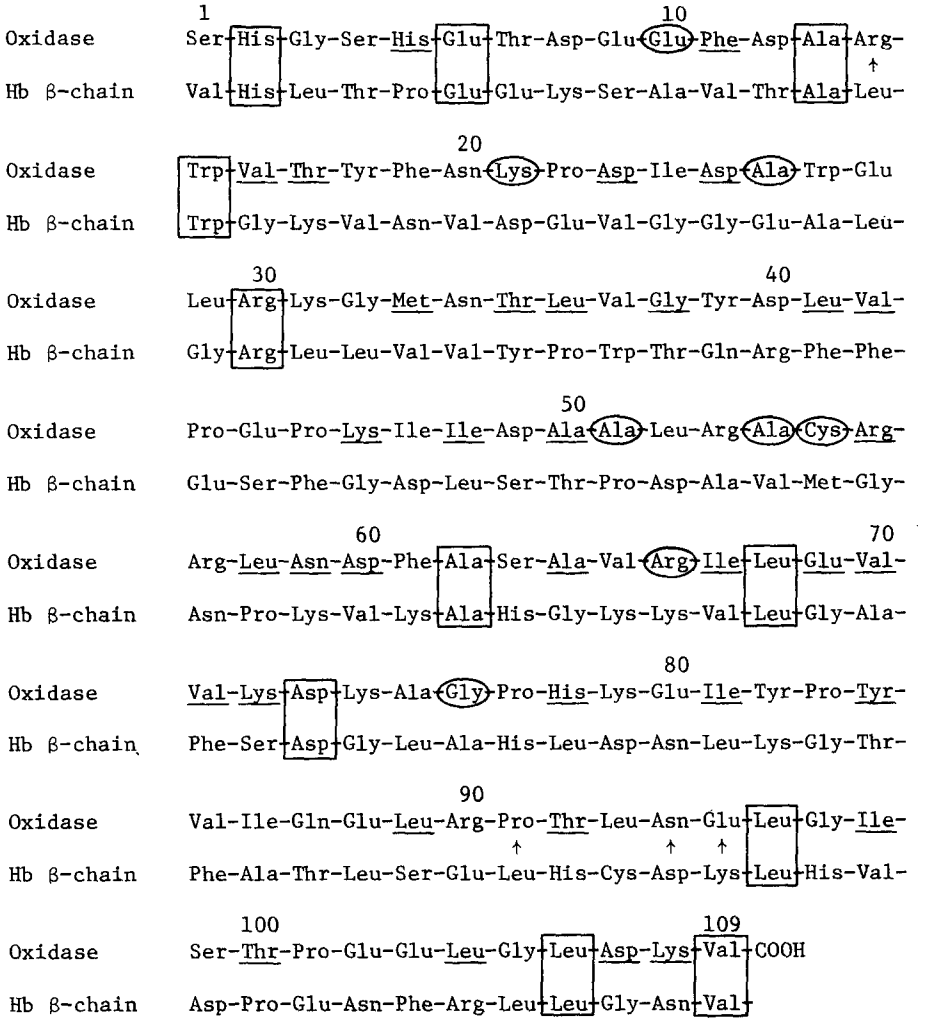

Fig. 2. Comparison of the amino acid sequence of the 11.6 K bovine heart cytochrome oxidase and β-subunit of hemoglobin. Identical sequences between the 11.6 K heme a subunit bovine heart cytochrome oxidase and the β-subunit of hemoglobin are squared off. Circled residues indicate sequences of the heme subunit which are identical with the β-chain of other animal species (16). Underlined residues are those with only one base difference per codon. ↑ indicates identical amino acid replacements observed in the abnormal β-chain of human hemoglobin (17).

RESULTS

The methods used and the results obtained for the sequence determination of the heme a subunit will be discussed elsewhere. Only a brief discussion of the methods that were used to determine the amino acid sequence of the pro-

tein will be given here. The protein was carboxymethylated and an aliquot was cleaved by trypsin and the tryptic peptides were isolated by paper chromatography. All of the lysine and arginine residues were cleaved except for the lysine residue 21 and the arginine residue 90 as shown in Fig. 1. The essential tryptic peptides were sequenced. Another aliquot was run in the Beckman 890 Protein Sequencer and the first 50 amino acids from the NH_2-terminus of the protein were determined as shown in Fig. 1. Another aliquot of the protein derivative was cleaved with cyanogen bromide and two fragments were obtained and isolated to a pure state. Only portions of the CNBr peptides were sequenced. A final aliquot was succinylated to block the ε-NH_2 groups of the lysine residues and then cleaved with trypsin. These fragments are indicated in Fig. 1 by the symbol ST and many were sequenced after purification by paper chromatography.

The sequence of the bovine heart cytochrome oxidase heme *a* subunit is compared (*cf*. Fig. 2) especially with the sequence of the β-chain of human hemoglobin as well as hemoglobins from other animal species summarized by Fasman (16).

DISCUSSION

The brief report documents the complete amino acid sequence of the 11,600 dalton subunit of bovine heart cytochrome oxidase. The protein exists as the single polypeptide chain and contains 109 amino acids. It should be emphasized here that there are two distinct subunits which correspond to the heme *a* and heme a_3 of cytochrome oxidase. The two subunits have molecular weights of 11,600 and 40,000 daltons. Concerning the potential ligands, it is interesting to note that the heme *a* subunit contains all of the known heme ligands which are found in other heme proteins. These are: cysteine 55; methionine 33; lysine residues in positions 21, 31, 46, 72, 74, 79 and 108; and the histidine residues on positions 2, 5, and 78. The heme *a* is bound very tightly to the isolated subunit and even trifluoroacetic acid cannot readily remove the heme *a* but can be easily extracted by sulfenyl chloride.

The striking feature of the amino acid sequence of the oxidase subunit is that it shows about 20% homology with the β-subunit of hemoglobins which have been sequenced from various animal species (see Fig. 2). In addition, there are many amino acid replacements which involve one purine or pyrimidine change in the amino acid codon. The homology indicates a possible common ancestor for the two hemoproteins. It is interesting to note that at residues 14, 91, 94 and 95, abnormal hemoglobins (17) contain the same amino acid replacements as are observed when the heme *a* subunit and the β-chain of hemoglobin are compared. It also follows that there is a sequence homology of the present subunit with the α-subunit of hemoglobin and myoglobin (18). Efforts are underway to use computer programs to show that, indeed, the 11.6 K dalton heme *a* subunit of bovine heart cytochrome oxidase and the β-chain of hemoglobin have arisen from a common ancestor. However, visual inspection certainly indicates a homology and if one allows for the differences in the size of protoporphyrin IX and heme *a*, which are present in hemoglobin and the oxidase heme *a* subunit, respectively, the observed homology becomes even more remarkable. However, the definitive function of 11.6 K dalton subunit is not clearly defined although it is possible that it is a heme *a* rather than the heme a_3 subunit (*cf.* 6).

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