*THE HOMOLOGY BETWEEN CYTOCHROME B_5, HEMOGLOBIN, AND MYOGLOBIN**

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The detailed structural information on hemoglobin and myoglobin and the extensive data on the amino acid sequence of cytochrome *c* from many species has been utilized to explore the structural and functional relationship among these heme proteins.[1-4] The recent determination of the primary structure of cytochrome b_5 provides an opportunity to extend this comparative study of heme protein molecular architecture.[5] In several respects, the single peptide chain of cytochrome b_5 resembles those of the globins. Their common structural unit is a polypeptide chain of relatively low molecular weight which binds one iron protoporphyrin IX group. Furthermore, these heme proteins can be reversibly dissociated into free heme and apoprotein and, in each case, imidazole residues are involved in the heme binding, resulting in a stabilization of the characteristic conformation of the polypeptide chain. On the other hand, cytochrome b_5 is clearly distinguished from the hemoglobins by its chemical and catalytic properties.[6] Particularly significant is the inability of cytochrome b_5 to bind oxygen and a number of other heme ligands. The question arises, therefore, whether the basis for the similarities and differences between the types of heme proteins is reflected in their primary structures.

The amino acid sequence of calf liver microsomal cytochrome b_5 is shown in Figure 1. By applying micromethods this has been accomplished with less than 2.5 μmoles, approximately 30 mg of the heme protein. The details of the sequence studies will be described elsewhere. Trypsin hydrolysis of the apocytochrome yields the 11 expected peptides. Each of these peptides was isolated in a homogeneous form by Dowex-1 and Dowex-50 column chromatography. Edman degradation, pepsin, and carboxypeptidase A and B were then used to establish the amino acid sequence of each peptide. Chymotrypsin cleavage occurs, as expected, at the aromatic amino acid residues. In addition a single histidine and several leucine and asparagine bonds were cleaved. Both short and long chymotryptic digests gave essentially the same peptide elution profile except that no cleavage occurred at leucine 38, phenylalanine 50, and threonine 57, during a limited chymotryptic digestion. The chymotryptic peptides provided sufficient overlaps to determine the order of tryptic peptides. The amino acid composition of arginine-containing peptides isolated from the tryptic digests of acetylated apoprotein provided an additional proof for the validity of the proposed order of the tryptic peptides. At the present time only the sequence of several amino acids in peptide T-2 and the position of two or three amide groups in T-1 peptide are unknown. In contrast to the apocytochrome, which is readily hydrolyzed by proteolytic enzymes, the heme protein is quite resistant to tryptic digestion and completely resistant to several other proteases. Thus tryptic hydrolysis of cytochrome b_5 yields only three small peptides, T-10 from the amino terminus and T-11 and T-9 from the carboxyl terminus. The resulting heme peptide with N-terminal alanine and carboxyl terminal arginine is unaltered in either its spectral or catalytic properties.[5]

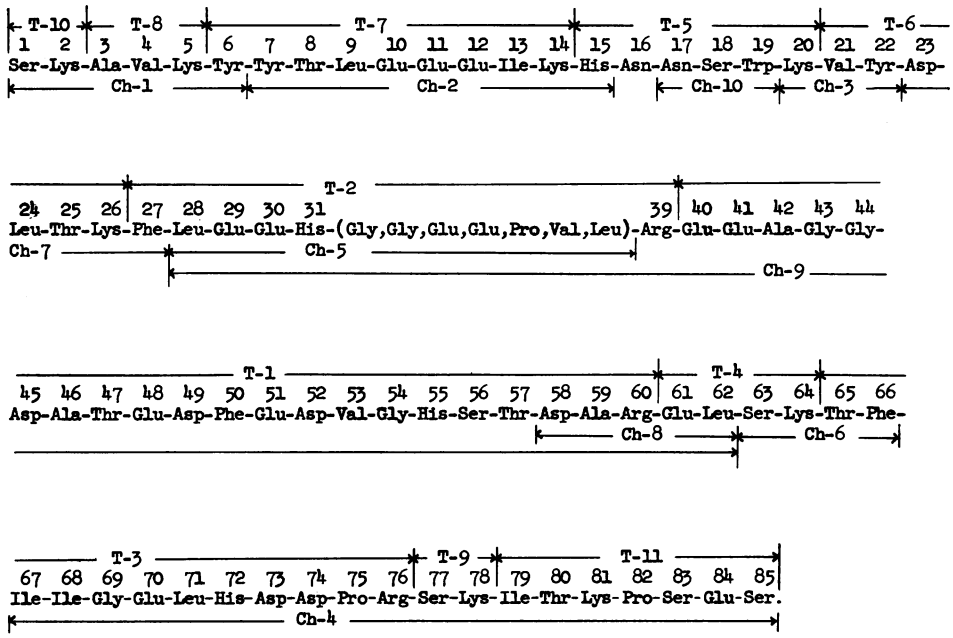

FIG. 1.—Amino acid sequence of calf liver microsomal cytochrome b_5. The tryptic and chymotryptic peptides are indicated by *T* and *Ch*.

Cytochrome *c*, the only other cytochrome with a known primary structure, bears no recognizable resemblance to cytochrome b_5. This is particularly significant for the 25 invariant amino acid residues which have been detected by the studies of Margoliash, Smith, and others on numerous cytochrome *c* preparations.[4, 7] Similarly, the structures of heme peptides of a number of bacterial cytochromes of the *c* type, including *Rhodospirillum rubrum* cytochrome c_2,[8] *Chromatium* cytochrome *cc'*,[9] and *Pseudomonas fluorescens* cytochrome-551,[10] have no complementary peptide segments in the cytochrome b_5 structure.

Several structural features of cytochrome b_5 are similar to sequences found in the α and β chains of hemoglobin (Table 1*A*). Most striking is the sequence 63–75 in cytochrome b_5 and the sequence of the α and β chains of hemoglobin and myoglobin containing the proximal heme-linking histidine. In this comparison the variations in this sequence are no more numerous or structurally drastic in the cytochrome b_5 sequence than among those of the α and β chains and of myoglobin. Remarkably a second, but more brief, sequence (Table 1*B*) also involves the distal heme link, histidine 63, of hemoglobin. This segment is comparable to the sequence 52–57. The existence of nine invariant amino acid residues in the hemoglobin and myoglobin chains suggests one further comparison (Table 2). Six of these residues are collected in the area of the two heme-linking histidyl residues. These have their counterpart in the cytochrome b_5 sequence. This comparison requires a large number of amino acid deletions from the cytochrome structure. Of the 74 residues involved in the globin sequence the comparable cytochrome b_5 segment has only 34 amino acid residues. Significantly, of the 34 residues, 19 are identical to hemoglobin β chain and with two or three exceptions the remainder represent similar structural substitutions such as aspartic for glutamic, leucine for

TABLE 1

COMPARISON OF CYTOCHROME b_5 SEQUENCES 63–76 AND 52–57, THE HEMOGLOBIN PROXIMAL AND DISTAL HISTIDINE SEQUENCES

					A										
	63	64	65	66		67	68	69	70	71	72	73	74	75	76
Cytochrome b_5	-Ser-	Lys-	Thr-	Phe-		-Ile-	Ile-	Gly-	Glu-	Leu-	His-	Asp-	Asp-	Pro-	Arg-
Hemoglobin (horse)															
											92				
β	-Lys-	Gly-	Thr-	Phe-	Ala-	Ala-	Leu-	Ser-	Glu-	Leu-	His-	CySH-	-Asp-	Lys-	
											92				
α	-Pro-	Gly-	Ala-	Leu-	Ser-	Asp-	Leu-	Ser-	Asp-	Leu-	His-	Ala-	-His-	Lys-	
Myoglobin (whale)	-Glu-	Ala-	Glu-	Leu-	Lys-	Pro-	Leu-	Ala-	Gln-	Ser-	His-	Ala-	-Thr-	Lys-	

			B					
	52	53		54	55	56	57	
Cytochrome b_5	-Asp-	Val-		-Gly-	His-	Ser-	Thr-	
Hemoglobin (human)								
					58			
α	-Glu-	Val-	Lys-	Gly-	His-	Gly-	Lys-	
					63			
β	-Lys-	Val-	Lys-	Ala-	His-	Gly-	Lys-	
					64			
Myoglobin (whale)	-Asp-	Leu-	Lys-	Lys-	His-	Gly-	Val-	

Hemoglobin and myoglobin data from Perutz, M. F., *J. Mol. Biol.*, **13**, 646 (1965).

isoleucine, etc. It is clear from the tertiary structure of myoglobin established by Kendrew and associates[2] that the deletions in the cytochrome sequence occur mainly in the helical or folded regions of the globin chains. The possibility exists, therefore, that a similar spatial orientation of the six invariant amino acid residues exists in the shorter complementary sequence of cytochrome b_5[5] with a reduced helix content. Furthermore, the three longest segments of amino acid deletions occur on the side of the heme involving the distal histidine, the oxygen binding site. A tighter folding in this area of cytochrome b_5 might bring this histidine nearer to the iron in either oxidation state and result in the observed inability to react with oxygen, carbon monoxide, and cyanide.

TABLE 2

COMPARISON OF CYTOCHROME b_5 SEQUENCE 40–76 AND HEMOGLOBIN PEPTIDE SEGMENT CONTAINING SIX INVARIANT RESIDUES

	40	41	42	43	44	45	46																		
Cytochrome b_5	-Glu-	Glu-	Ala	*Gly*	Gly	Asp	Ala																		
Hemoglobin (horse) β	-Glu-	Glu-	Glu-	Val-	*Gly*-	Gly-	Glu-	Ala-	Leu-	Gly-	Arg-	Leu-	Leu-	Val-	Val-	Tyr-	Pro-								
					24																				
	47	48		49	50	51																			
Cytochrome b_5	-*Thr*-	Glu-		-Asp-	*Phe*-	Glu-																			
β	-Trp-	*Thr*-	Gln-	Arg-	Phe-	*Phe*-	Asp-	Ser-	Phe-	Gly-	Asp-	Leu-	Ser-	Gly-	Pro-	Asp-	Ala-								
	38				42																				
			52			53		54	55	56															
Cytochrome b_5			-Asp-			-Val-		-Gly-	*His*-	Ser-															
β	-Val-	Met-	Gly-	Asp-	Pro-	Lys-	Val-	Lys-	Ala-	*His*-	Gly-	Lys-	Lys-	Val-	Leu-	His-	Ser-								
										63															
	57	58		59	60				61	62	63	64	65	66			67								
Cytochrome b_5	-Thr-	Asp-		-Ala-	Arg-				-Glu-	Leu-	Ser-	Lys-	Thr-	Phe-			-Ile-								
β	-Phe-	Gly-	Glu-	Gly-	Val-	His-	His-	Leu-	Asp-	Asp-	Leu-	Lys-	Gly-	Thr-	Phe-	Ala-	Ala-								
	68	69	70	71	72	73	74	75	76																
Cytochrome b_5	-*Ile*-	Gly-	Glu-	Leu-	*His*-	Asp-	Asp-	Pro-	Arg-																
β	-*Leu*-	Ser-	Glu-	Leu-	*His*-	Cys-	Asp-		-Lys-																

Hemoglobin from Smith, D. B., *Canad. J. Biochem.*, **42**, 755 (1964). Invariant residues, from Perutz, M. F., J. C. Kendrew, and H. C. Watson, *J. Mol. Biol.*, **13**, 669 (1965), are italicized.

This homology thus leads to the speculation that the heme binding site, and thus the tertiary structure of these areas of the cytochrome b_5 peptide, may bear some resemblance to those in the α and β chains of hemoglobin and in myoglobin. The distinguishing spectral and catalytic properties of cytochrome b_5 and its inability to bind either oxygen or carbon monoxide would be attributed to the subtle differences in the heme environment involving stable liganding of both the fifth and sixth iron coordination positions in the cytochrome. This initial speculation may well fall when structural data for cytochrome b_5, comparable to those which exist for hemoglobin and myoglobin, are obtained. It does provide additional reason for completing the sequence studies on cytochrome b_5 from several species which sample the phylogenic order, to test the extent to which the six amino acid residues remain invariant in this heme protein, and emphasizes the need for X-ray structural analysis of this protein. (A suitable crystal form of cytochrome b_5 has been obtained.) It also raises the question as to whether similar structural redundancy will be found in other cytochromes of the *b* type.

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