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Primary structure of the membranous segment of cytochrome b_5

(hydrophobic peptides/isolation/sequencer analysis/tryptophanyl cleavage/secondary structure)

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ABSTRACT The primary structure of the membranous segment of porcine liver microsomal cytochrome b_5 has been determined. This polypeptide is at the COOH terminus of the cytochrome molecule and consists of 43 amino acids. It is essential for the insertion of the cytochrome into the endoplasmic reticular membrane. Automated sequence analysis of tryptic and cyanogen bromide/anhydrous heptafluorobutyric acid peptides provided data from which the following unique amino acid sequence was deduced: Ile-Ala-Lys-Pro-Ser-Glu-Thr-Leu-Ile - Thr - Thr - Val - Glu - Ser - Asn - Ser - Ser - Trp - Trp - Thr - Asn - Trp - Val - Ile - Pro - Ala - Ile - Ser - Ala - Leu - Val - Val - Ser - Leu - Met - Tyr - His - Phe - Tyr - Thr - Ser - Glu - Asn. A prediction of α-helices, β-structures, and β-turns based on the sequence of this polypeptide is also presented.

Cytochrome b_5 is a membrane-bound hemoprotein found almost exclusively in the endoplasmic reticulum of hepatocytes, where it serves a key role in electron transport (1). While it has been shown to be the primary donor of reducing equivalents for the NADH-dependent Δ-9 stearyl CoA desaturase (2, 3), there is evidence suggesting that it participates in NADPH-linked microsomal cytochrome *P*-450-catalyzed reactions as well (4, 5). The native cytochrome b_5 consists of a single polypeptide chain folded into two functionally distinct and independent segments: a polar moiety containing the noncovalently bound heme group, and a membranous segment which anchors the protein in the endoplasmic reticulum. The intact cytochrome may be isolated using detergents, and under appropriate conditions may be reinstated into microsomal or synthetic vesicles with its catalytic properties unaltered (6). Treatment of microsomal membranes with trypsin releases only the heme-peptide, which is rapidly reduced by NADH-cytochrome b_5 reductase, but does not interact with microsomal membranes. This polar moiety consists of some 85 residues, depending on the species, derived from the NH_2-terminal portion of the molecule (1).

Although much is known about the chemical and physical properties of microsomal membranes, presently there is sparse information about the amino acid arrangements of peptides responsible for protein–membrane interactions. The importance of sequence information in understanding the structure-function relationship of proteins has been clearly demonstrated by studies on cytochrome *c* (7).

In previous studies, we have reported the amino acid sequence of the heme-binding peptide of cytochrome b_5 from six mammalian and one avian species (8, 9), as well as described the isolation and characterization of the native cytochrome molecule from several mammalian species (10–12). This communication reports documented primary structure of an entire membranous segment from any microsomal protein and proposes a secondary structure for this polypeptide based on the sequence data.

MATERIALS AND METHODS

Porcine cytochrome b_5 was isolated as described by Ozols (11). Cyanogen bromide (CNBr) was purchased from either Pierce Chemical Co. (Rockville, IL) or Eastman Organic Chemicals (Rochester). Formic acid (88%) was a product of Baker Adamson. Anhydrous heptafluorobutyric acid (F_7But), as well as other reagents for sequence analysis, were obtained from Beckman (Spinco division).

Heme-free apocytochrome was prepared by treatment of the cytochrome with cold acetone containing 0.2% HCl (vol/vol). The apoprotein was suspended in 8 M urea/10 mM Tris·HCl buffer at pH 8.1, and then diluted with 0.1 M ammonium bicarbonate, pH 8.1, to reduce urea concentration to 2 M. A 0.1% solution of trypsin (Worthington, L-1-tosylamido-2-phenyl-ethyl chloromethyl ketone-treated) in 1 mM HCl was prepared immediately prior to use and added to denatured apocytochrome solution to give an enzyme protein ratio of 1:40. Trypsin digestion was at 25° for 18 hr. The clear digest was lyophilized and dissolved by addition of 0.5 ml of 88% formic acid followed by 0.5 ml of glacial acetic acid. This solution was applied to a column of Sephadex G-75 (2.9 × 105 cm) that had been equilibrated with 9 M acetic acid. The gel filtration was at 20°, and fractions of 3 ml were collected at a flow rate of 13 ml/hr. Peptide fractions were identified by the ninhydrin reaction after alkaline hydrolysis (11). Peptide C-4 (residues 79–98) and the COOH-terminal peptide (residues 130–133) were isolated from a chymotryptic digest of cytochrome b_5 as described (1).

Dye-sensitized photooxidation of methionyl residues was performed as follows: 600 nmol of porcine apocytochrome b_5 was dissolved in 2.0 ml of 84% acetic acid and transferred to a Pyrex test tube (1 × 10 cm) equipped with a glass jacket. The temperature of the protein solution was maintained at 0° by circulating 95% ethanol through the glass enclosure. A 0.04% solution of methylene blue dissolved in 84% acetic acid was prepared immediately prior to use and added to the protein solution in the dark to give a dye:protein ratio of 1.5:1.0 (nmol/nmol). The reaction vessel was irradiated using a single 150 W tungsten light source at a distance of 30 cm for 8 hr, while O_2 was slowly bubbled into the protein solution. At the end of the irradiation period, the sample was freed from solvent using a vacuum assembly equipped with a cold trap in the line and redissolved in 10% acetic acid. The protein was separated from the dye by gel filtration on a column of Bio-Gel P-2 (0.9 × 40 cm) developed with the sample buffer.

The CNBr/F_7But cleavage of photooxidized apoprotein was

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Abbreviations: CNBr, cyanogen bromide; F_7But, anhydrous heptafluorobutyric acid.

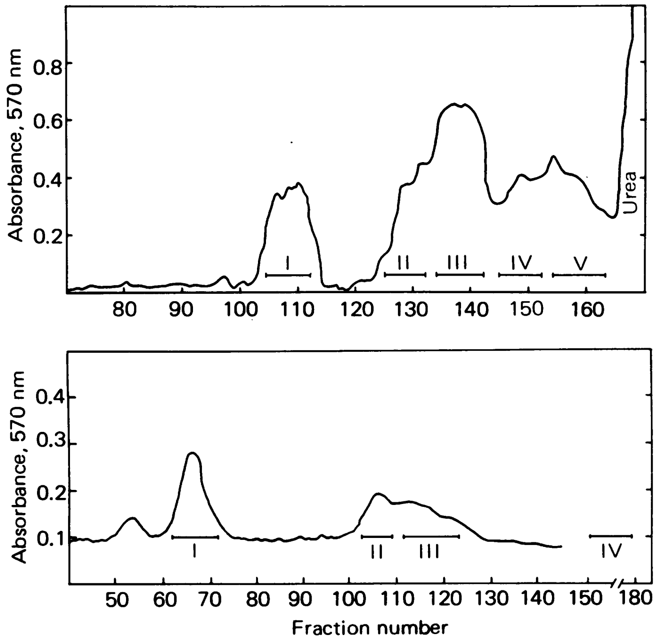

FIG. 1. (*Upper*) Gel filtration of tryptic digest of apocytochrome b_5 (0.8 μmol) on a column of Sephadex G-75 (2.9 × 105 cm) equilibrated with 9 M acetic acid. Fractions of 3 ml were collected at a flow rate of 13 ml/hr, and 200-μl aliquots were analyzed by the ninhydrin method. The membranous peptide (residues 91–133) was present in fraction I. (*Lower*) Gel filtration of 0.5 μmol of CNBr/F_7But digest of photooxidized apocytochrome. Conditions were as described in the upper panel except that the column dimensions were 2.9 × 90 cm. The roman numeral below the indicated fractions corresponds to the respective CB peptide.

Table 1. Amino acid composition of porcine cytochrome b_5

Amino acid	Residues per molecule of protein
Lysine	9.7 (9)
Histidine	7.2 (7)
Arginine	3.6 (3)
Aspartic acid	14.0 (13)
Threonine	10.7 (11)
Serine	10.8 (13)
Glutamic acid	19.3 (18)
Proline	4.1 (4)
Glycine	8.1 (6)
Alanine	9.4 (8)
Cysteine	0 (0)
Valine	7.5 (8)
Methionine	1.4 (1)
Isoleucine	7.8 (8)
Leucine	11.3 (11)
Tyrosine	5.4 (5)
Phenylalanine	4.6 (4)
Tryptophan*	3.4 (4)
Total	133

The samples were hydrolyzed in duplicate for 20 and 92 hr. The values reported for aliphatic hydrophobic amino acids were obtained by analysis of 92-hr hydrolysates; the others were average values of 20-hr hydrolysates. The numbers in parentheses represent the number of residues obtained from the amino acid sequence.

* Tryptophan was determined from a 20-hr hydrolysate using 4 M methanesulfonic acid containing 0.2% 3-(2-aminoethyl)indole.

performed as follows: to 500 nmol of protein 1.0 ml each of 88% formic acid and anhydrous F_7But were added. After addition of 700 mg of solid CNBr, the samples were incubated for 24 hr in the dark. The reagent and solvents were removed with a stream of N_2, and the remaining white material was suspended in 10 ml of H_2O and lyophilized. The dried material was dissolved in 9 M acetic acid and applied to a column of Sephadex G-75 (2.9 × 105 cm) that had been equilibrated with 9 M acetic acid. Chromatography was at 20°, and fractions of 3 ml were collected at a flow rate of 13 ml/hr.

The amino acid compositions of the peptides and proteins were determined on acid hydrolysates of the samples using a Beckman 121 automatic amino acid analyzer. Automated sequence analyses were performed on a Beckman model 890C Sequencer. The peptide program 102974, supplied by the manufacturer, was used to degrade the isolated peptides. The phenylthiohydantoin derivatives were identified by thin-layer chromatography and quantitated by amino acid analysis after back conversion of derivatives to the corresponding amino acids by hydrolysis in hydriodic acid vapor as described (1). Other experimental procedures have been described earlier (1).

RESULTS AND DISCUSSION

The membranous segment was isolated by gel filtration of a tryptic digest of denatured apocytochrome b_5 (Fig. 1 *upper*). The composition of the native preparation and the isolated membranous segment are shown in Tables 1 and 2. The complete primary structure of this peptide was determined unequivocally by automated sequence analysis (Table 3).

CNBr/F_7But cleavage at tryptophanyl residues of the photooxidized apocytochrome yielded four polypeptides. They were isolated by gel filtration as shown in Fig. 1 *lower*. The amino acid composition and yields of these peptides are given in Table 4. Twenty-one cycles of automated sequence analysis of peptide CBIII indicated that it was derived from the cleavage at tryptophanyl residue 112 and corresponded to the 21

Table 2. Amino acid composition of the tryptic peptide from the membranous segment

Amino acid	Amino acid residues/molecule 20-hr hydrolysis	Amino acid residues/molecule 92-hr hydrolysis
Lysine	1.20 (1)	0.83
Histidine	0.73 (1)	0.65
Arginine	0.20 (0)	0.18
Aspartic acid	3.33 (3)	2.44
Threonine	3.67 (5)	2.72
Serine	6.82 (7)	5.31
Glutamic acid	3.58 (3)	2.95
Proline	1.87 (2)	1.63
Glycine	1.23 (0)	0.81
Alanine	2.80 (3)	2.10
Valine	2.76 (4)	3.80
Methionine	0.85 (1)	0.65
Isoleucine	2.59 (4)	3.94
Leucine	2.82 (3)	2.99
Tyrosine	1.81 (2)	1.53
Phenylalanine	1.08 (1)	0.93
Tryptophan	2.5 (3)	
Yield (%)	80	
Total	43	

The peptide was isolated by gel filtration on Sephadex G-75 as described in the legend of Fig. 1 *upper*. Values reported are the average for samples hydrolyzed in duplicate for 20 and 92 hr. No corrections for the destruction of serine and threonine are incorporated. The values in parentheses refer to the number of residues per molecule of peptide determined from the sequence studies. Tryptophan was determined from a 20-hr hydrolysate using 4 M methanesulfonic acid containing 0.2% 3-(2-aminoethyl)indole.

Table 3. Sequencer analysis of 400 nmol of tryptic membranous peptide, residues 91–133

Cycle	Amino acids identified after HI hydrolysis of PTH	Yield, nmol	Thin-layer chromatography PTH derivative of:
1	Ile	260	Ile/Leu
2	Ala	181	Ala
3	Lys	194	Lys
4	Pro	187	Pro
5	Ala	43	Ser
6	Glu	157	Glu
7	Thr	148	Thr
8	Leu	168	Leu/Ile
9	Ile	152	Ile/Leu
10	Thr	101	Thr
11	Thr	125	Thr
12	Val	91	Val
13	Glu	118	Glu
14	Ala	51	Ser
15	Asp + NH_3	117	Asn
16	Ala	48	Ser
17	Ala	42	Ser
18	Gly + Ala	32 + 41	Trp
19	Gly + Ala	29 + 32	Trp
20	Thr	31	Thr
21	Asp	46	Asn
22	Gly + Ala	21 + 27	Trp
23	Val	34	Val
24	Ile	28	Ile/Leu
25	Pro	27	Pro
26	Ala	44	Ala
27	Ile	40	Ile/Leu
28	Ala	26	Ser
29	Ala	41	Ala
30	Leu	26	Leu/Ile
31	Val	18	Val
32	Val	21	Val
33	Ala	14	Ser
34	Leu	22	Leu/Ile
35	—		Met
36	Tyr	16	Tyr
37	His	12	His
38	Phe	10	Phe
39	Tyr	10	Tyr
40	Thr	8	Thr
41	Ala	7	Ser
42	Glu	9	Glu
43	Asp	8	Asn

PTH, phenylthiohydantoin.

COOH-terminal residues of the membranous segment isolated from the tryptic digest of the parent protein (Table 5).

These data provided the complete covalent structure of the membranous segment of cytochrome b_5, as well as excluded the possibilities of missing segments in the COOH-terminal portion of the native molecule. Peptide C-4 provided independent overlap data for aligning the membranous segment at the COOH terminus of the heme-binding polar moiety. The structure of this peptide was identical with that previously reported for the horse cytochrome, except that there is a serine in position 95 rather than a valine. The complete amino acid sequence of porcine cytochrome b_5 is given in Fig. 2. The amide assignments in the membranous segment, made by the direct identification of the amino acid phenylthiohydantoin derivatives, were unambiguous.

Table 4. Amino acid composition of peptides produced by CNBr/F_7But cleavage of photooxidized porcine cytochrome b_5

Amino acid	CBI	CBII	CBIII	CBIV
Lysine	4.4 (5)	3.8 (4)		
Histidine	4.3 (5)	0.7 (1)	0.8 (1)	
Arginine	3.0 (3)			
Aspartic acid	7.9 (8)	2.9 (3)	0.9 (1)	1.1 (1)
Threonine	6.2 (7)	1.7 (2)	1.1 (1)	1.1 (1)
Serine	5.3 (7)	2.5 (3)	2.8 (3)	
Glutamic acid	10.6 (12)	5.2 (5)	1.2 (1)	
Proline	2.9 (3)		0.9 (1)	
Glycine	5.7 (6)			0.3
Alanine	4.3 (4)	2.2 (2)	2.2 (2)	
Valine	4.4 (4)	1.3 (1)	2.8 (3)	
Methionine			0.2 (1)	
Isoleucine*	4.1 (5)	0.9 (1)	1.8 (2)	
Leucine	7.5 (8)	0.8 (1)	1.9 (2)	
Tyrosine†	0.9 (1)	1.8 (2)	2.0 (2)	
Phenylalanine	3.0 (3)		0.9 (1)	
Tryptophan	(1)	(1)		(2)
Fractions pooled	62–74	105–108	115–125	150–175
Yield, %	75	60	60	35
Position in sequence	27–108	1–26	113–133	110–112

Peptides were isolated by gel filtration on Sephadex G-75 as described in the legend of Fig. 1 *lower*. Samples were hydrolyzed for 24 hr and duplicate analyses were performed on each hydrolysate. The numbers in parentheses refer to the number of residues per mole of peptide determined from the sequence studies.

* Low isoleucine value was due to incomplete hydrolysis of an Ile-Ile bond in the peptide.

† Tyrosine was calculated from the peak height of its derivative eluting at a position preceding the lysine peak. The ninhydrin color value of the derivative is essentially identical to that of native tyrosine. Hydriodic acid hydrolysis of the phenylthiohydantoin derivative of modified tyrosine regenerates the parent molecule. ^1H nuclear magnetic resonance experiments have indicated proton replacement at the 3 and 5 carbon positions of the molecule.

The importance of detailed characterization of the peptides isolated from the chemical cleavage digest is as follows: First, it confirmed independently the sequence analysis of the peptide obtained by tryptic hydrolysis. Second, while it could be argued that the asparaginyl residue at the COOH terminus of the tryptic peptide was a product of an abnormal tryptic cleavage, the isolation and sequence analysis of peptide CBIII, obtained independently by chemical cleavage of the parent protein, excludes the possibility of a peptide fragment extending beyond asparagine 133. Moreover, the trypsin hydrolysis and CNBr/F_7But cleavage were performed on cytochrome b_5 preparations isolated from two separate livers. The peptides generated from these cytochromes were in complete sequence agreement. These results confirm further the unlikelihood that the parent molecule is longer than is shown in Fig. 2. Third, structure studies on the peptides obtained by the chemical cleavage were in agreement with our previously reported amino acid sequence of the polar moiety of porcine cytochrome b_5 (9, 11).

Numerous difficulties were encountered in obtaining protein preparations suitable for sequence analysis. In particular, while cytochrome preparations appeared homogeneous when examined by sodium dodecyl sulfate gel electrophoresis, quantitative end group determination by automated sequence analysis revealed the presence of non-cytochrome b_5 proteins. These contaminants were responsible for the slightly higher molecular weights reported for earlier isolations (11). Preparations that contained no detectable NH_2-terminal residue

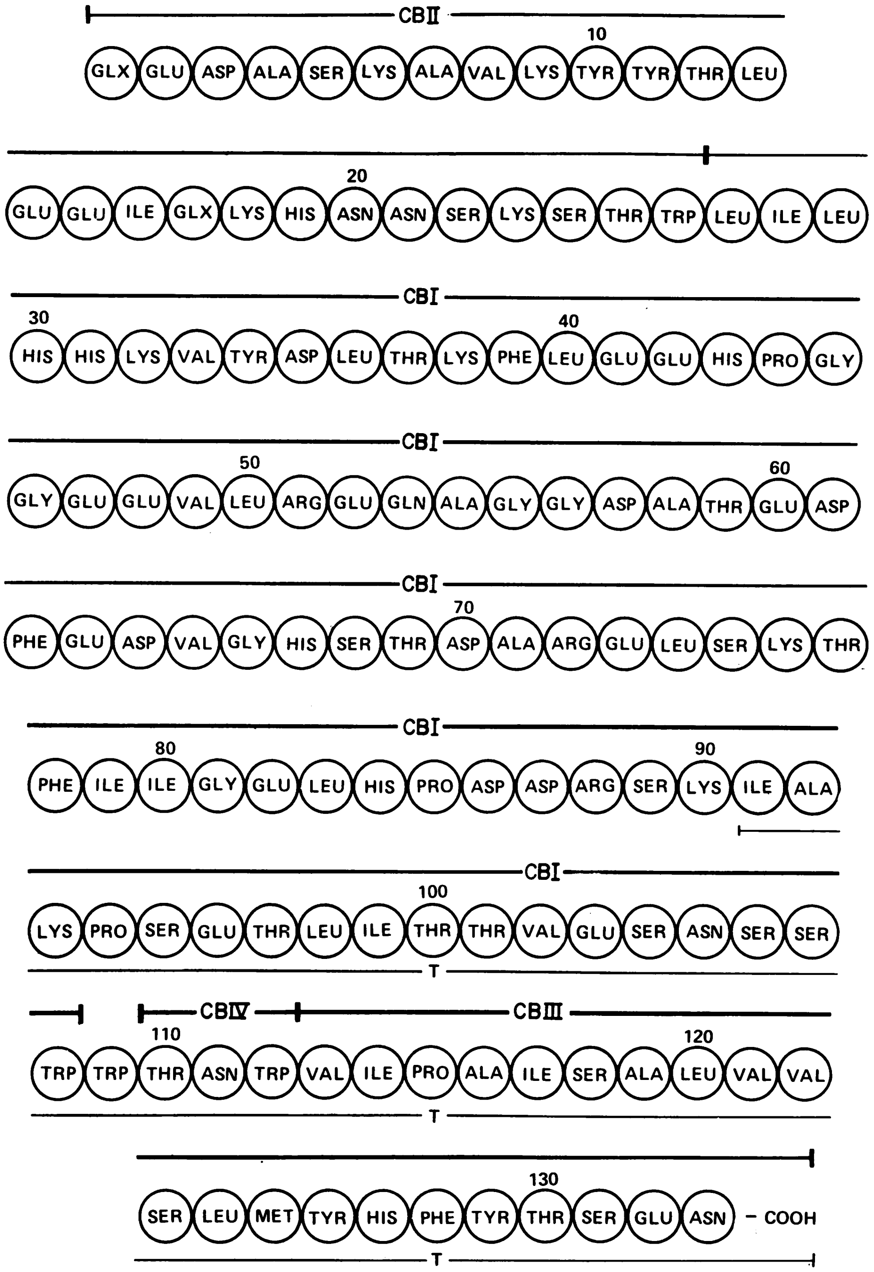

FIG. 2. The complete amino acid sequence of porcine cytochrome b_5. The alignment of residues 1–90 is from the work of Nobrega and Ozols (9) and Ozols (11). T represents the tryptic peptide, and CB designates peptides obtained by CNBr/F_7But cleavage of photooxidized apocytochrome. The amino terminal residue of the protein is masked, and the assignment for residues 1–4 is tentative (8).

when subjected to several cycles of sequence analysis, however, yielded amino acid compositions that indicated that the native cytochrome contained between 135 and 145 residues (Table 1). Nonetheless, attempts to isolate the membranous segment by tryptic or chymotryptic hydrolysis using conventional conditions were often complicated by abnormal or incomplete cleavages. In many instances the peptides were lost during column chromatography or were recovered in low yield.

While chymotryptic hydrolysis of denatured cytochromes yielded many cleavage products refractory to separation, it was found that tryptic hydrolysis, when performed on urea-denatured apocytochrome, generated intact membranous segment. Since our attempts to isolate peptides comprising the membranous segment from chymotryptic digests were generally fruitless, considerable efforts were directed to obtain chemical cleavage at the single methionyl residue in the membranous segment. CNBr cleavages of native porcine, equine, bovine, and rat cytochromes b_5 were unsuccessful, even when 70 or 80% formic acid was used as the solvent. Cleavage of urea- or guanidine•HCl-denatured proteins by CNBr in 88% formic acid generated peptides in too low yields for quantitative primary structure analysis. These results exemplify the unusual properties of the membranous segment. For example, tryptic fragmentation of native equine cytochrome b_5 generated the heme peptide (residues 7–90) and a membranous segment about 37 residues long. Both peptides remained fully soluble in the dilute aqueous buffers used during hydrolysis. Gel filtration or ion-exchange chromatography of such digests in these buffers, however, yielded only the heme peptide (1). Lyophilized digests containing the membranous segment were insoluble in neutral buffers and dissolved very slowly even in 9 M acetic acid. In light of such findings, we performed CNBr cleavage in formic

Table 5. Sequencer analysis of 170 nmol of peptide CBIII, residues 113–133

Cycle	Amino acids identified after HI hydrolysis of PTH	Yield, nmol	Thin-layer chromatography PTH derivative of:
1	Val	23	Val
2	Ile	15	Ile/Leu
3	Pro	16	Pro
4	Ala	22	Ala
5	Ile	17	Ile/Leu
6	Ala	8	Ser
7	Ala	17	Ala
8	Leu	20	Leu/Ile
9	Val	7	Val
10	Val	7	Val
11	Ala	6	Ser
12	Leu	12	Leu/Ile
13	—		Met(O_2)
14	Tyr	9	Tyr
15	His	5	His
16	Phe	8	Phe
17	Tyr	8	Tyr
18	Thr	7	Thr
19	Ala	4	Ser
20	Glu	5	Glu
21	Asp	5	Asn

PTH, phenylthiohydantoin; Met(O_2), methionine sulfone.

acid/F_7But solution. These solvent conditions resulted in not only methionyl, but tryptophanyl cleavage as well, both in high yield. Cleavage of methionyl bonds by CNBr/F_7But treatment could be prevented completely by photooxidation of these residues, sensitized by methylene blue. In photooxidized apocytochromes, the cleavage was directed specifically towards tryptophanyl residues. The specificity of this cleavage was confirmed using the polar moiety of cytochrome b_5 from several species with known primary structures. An extensive description of this method and its application to other proteins as well will be described separately.

The primary structure of the membranous segment could not be established without Sequencer analysis. Manual subtractive-Edman degradations were consistently unsuccessful, and only three steps of sequential dansyl-Edman degradation could be carried out (10). It was not possible to determine the sequence beyond this point without introducing ambiguity. Automated sequence analysis of peptide CIII (residues 113–133) resulted repeatedly in low initial yields, with repetitive yields averaging 92%. Due to the remarkably low background and overlap pattern observed in these degradations, the possibilities of an impure peptide and incomplete coupling, cleavage, or extraction of residues appear unlikely. Thus, at present we have no simple explanation for these findings. Sequence analyses of the membranous segments isolated from tryptic digests also resulted in repetitive yields that were lower than expected (91–95%). Degradation of apomyoglobin using the same instrument gave repetitive yields of 96–98%.

The results of a predictive analysis for conformational features in the membranous segment are presented in Table 6. An interesting result of this analysis is the prediction of an unusual region comprising residues 104–112. This peptide, with a cluster of tryptophanyl residues, appears to constitute four overlapping β-turn structures, suggesting that the polypeptide possesses a reverse turn geometry. The predictions presented here, however, must be interpreted with caution since the conformational parameters employed were derived from studies on globular hydrophilic proteins and may not be necessarily extendable to membranous peptides.

Table 6. Predictive analysis of conformational features in the membranous segment of cytochrome b_5*

Peptide segment	$[P_\alpha]$	$[P_\beta]$	$[P_t]$	$[p_t]$	Predicted conformation
97–102	0.97	1.36			β-Sheet
104–107	0.74	0.78	1.59	8.5×10^{-5}	β-Turn
105–108	0.82	0.94	1.48	1.86×10^{-4}	β-Turn
106–109	0.92	1.06	1.33	8.8×10^{-5}	β-Turn
109–112	0.92	1.20	1.22	1.85×10^{-4}	β-Turn
116–120	1.20	1.06			α-Helical
121–129	1.0	1.30			β-Sheet

* The predictions are based on the conformational parameters described by Chou and Fasman (13) and Fasman *et al.* (14). $[P_\alpha]$, $[P_\beta]$, and $[p_t]$ are, respectively, the average conformational potential for the computed region to be in the α-helix, β-sheet, and β-turn conformations. $[p_t]$ is the relative probability that a tetrapeptide will form a β-turn. Tetrapeptides with calculated values exceeding 0.5×10^{-5}, as well as satisfying the condition $[p_t] > [P_\beta]$ and $[P_\alpha] < 1.0$, are predicted to exist in β-turn conformation.

The determination of the complete covalent structure of the native cytochrome b_5 permits the construction of models describing the orientation of this protein in the membrane, and allows for designing experiments which will identify the peptide segments in the cytochrome molecule necessary for interactions with the reductase and desaturase, as well as with membrane lipids.

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1. Ozols, J., Gerard, C. & Nobrega, F. G. (1976) *J. Biol. Chem.* **251,** 6767–6774.
2. Shimakata, T., Mihara, K. & Sato, R. (1972) *J. Biochem.* (*Tokyo*) **72,** 1163–1174.
3. Strittmatter, P., Spatz, L., Corcoran, D., Rogers, M. J., Setlow, B. & Redline, R. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4565–4569.
4. Cohen, B. A. & Estabrook, R. W. (1971) *Arch. Biochem. Biophys.* **143,** 37–65.
5. Oshino, N. & Sato, R. (1971) *J. Biochem.* (*Tokyo*) **69,** 169–180.
6. Strittmatter, P., Rogers, M. J. & Spatz, L. (1972) *J. Biol. Chem.* **247,** 7188–7194.
7. Nolan, C. & Margoliash, E. (1968) in *Annual Review of Biochemistry*, eds. Boyer, P. D., Meister, A., Sinsheimer, R. L. & Snell, E. E. (Annual Reviews, Inc., Palo Alto, CA), Vol.. 37, pp. 727–790.
8. Ozols, J. (1970) *J. Biol. Chem.* **245,** 4863–4874.
9. Nobrega, F. G. & Ozols, J. (1971) *J. Biol. Chem.* **246,** 1706–1717.
10. Ozols, J. (1972) *J. Biol. Chem.* **247,** 2242–2245.
11. Ozols, J. (1974) *Biochemistry* **13,** 426–434.
12. Ozols, J. (1975) in *First International Conference on Solid Phase Methods in Protein Sequence Analysis*, ed. Laursen, R. H. (Pierce Chemical Co., Rockford, IL), pp. 221–228.
13. Chou, P. Y. & Fasman, G. D. (1974) *Biochemistry* **13,** 211–222, 222–245.
14. Fasman, G. D., Chou, P. Y. & Adler, A. J. (1976) *Biophys. J.* **16,** 1201–1238.