The Molecular Weight and Amino Acid Content of Microsomal Cytochrome b_5

ROSINE BOIS-POLTORATSKY* and ANDERS EHRENBERG

Medicinska Nobelinstitutet, Biokemiska Avdelningen, Karolinska Institutet, Stockholm, Sweden

Cytochrome b_5 was first isolated by Strittmatter and Velick [1,2] from calf liver microsomes. The molecular weight was 12 700 based on heme content and 14 400 as determined in the ultracentrifuge.[2] Recently Poltoratsky and Chaix [3-5] purified cytochrome b_5 from pig liver according to a slightly different method. It was of interest to determine the molecular weight and hydrodynamic homogeneity of the latter preparation.

The saltfree enzyme was stored in the lyophilized state. Before measurements it was dissolved in and dialyzed overnight against 0.05 M Na-phosphate pH 7.0, 1 % NaCl. All dialyses were carried out in acetylated Visking tubings,[6] which were impermeable to cytochrome b_5. The enzyme was characterized by A(556 mμ, red)/A(280 mμ, ox) = 1.00.

Experiments were made in a Spinco ultracentrifuge model E with phase plate schlieren optics and rotor temperature control (18–21°C was used). For the determination of S and D, experiments were made at 59 780 and 29 500/3 rpm, respectively. A synthetic boundary cell with rubber valve was used. All results were corrected to 20°C and water as solvent.

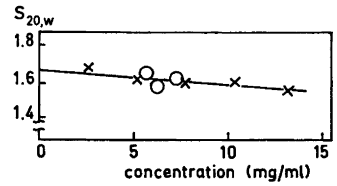


Fig. 1. Concentration dependence of the sedimentation coefficient of cytochrome b_5. ×: pH 7.0; ○: pH 5.4 (5.6 mg/ml), pH 7.35 (7.3 mg/ml) and pH 9.45 (6.3 mg/ml). The line is drawn by the least square method.

The preparation appeared to be homogeneous as judged from sedimentation patterns after 120 min. A single and symmetrical peak was observed at all protein concentrations and pH values studied (*cf.* Fig. 1). The sedimentation coefficient was found to depend slightly on the concentration a(mg/ml): $S_{20,w} = 1.67(1-0.0031\ a)$ S. $S_{20,w}^{\circ} = 1.67$ S is the value extrapolated to infinite dilution (Fig. 1).

The diffusion coefficient was determined as described earlier.[7] The "maximum ordinate-area" method gave constantly about 5 % lower value than the "maximum ordinate" method. No dependence on concentration or pH was detected. The mean of all the determinations is $D = 11.6 \times 10^{-7}$ cm² sec⁻¹.

Strittmatter [2] determined the partial specific volume (V) of cytochrome b_5 to be 0.723 cm³g⁻¹ using the gradient method. From the amino acid composition (Table 1) we calculate for the apoenzyme $V = 0.731$ cm³g⁻¹. Since the effect on V on the heme group in the holoenzyme is uncertain, we have used the figure 0.723 cm³g⁻¹ for calculation of M. From S, D, and V we obtain $M = 12\ 700$.

M was also derived from S/D determined from the Archibald boundary condition at the meniscus using the method described earlier.[7] The result is shown in Fig. 2. The slight increase during the earlier period of the experiment is probably an experimental artefact.[7] The plateau of the curve gives $M = 12\ 900$ (± 200) in good agreement with the value from S, D, and V. Since there is no significant decrease with time of the observed M, we conclude that the sample is essentially homogeneous with respect to molecular weight.

M of cytochrome b^5 evaluated from the present study in the ultracentrifuge, 12 800

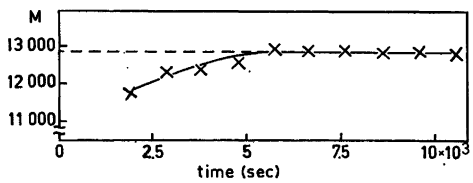


Fig. 2. Time dependence of cytochrome b_5 molecular weight calculated at the meniscus by Archibald's method.[7] Protein concentration 10.4 mg/ml. Time is measured from the instance when the speed 23 150 rpm was obtained.

* Permanent address: Laboratoire de Chimie Biologique de la Faculté des Sciences, 96 Boulevard Raspail, Paris 6e, France.

Table 1. Comparison between cytochrome *c* and cytochrome b_5 amino acid composition.

Amino acid	Amino acid residues per mole of cyt. *c* **	Amino acid residues per mole of cyt. b_5 (M = 13 100)
Lys	19	7.9
His	3	5.2
Arg	2	2.3
Asp	8	10.3
Thr	10	6.8
Ser	0	4.6
Glu	9	13.7
Pro	4	2.5
Gly	15	7.4
Ala	6	4.3
Val	3	5.8
Met	2	0.8
Ileu	6	4.9
Leu	6	8.3
Tyr	5	2.7
Phe	4	3.7
Cys	2	0.1
Try	1	1 *

* According to Strittmatter.[2]

** According to Margoliash *et al.*[9] for beef heart cytochrome *c*.

(mean value), is smaller than the value 15 000 based on iron content and dry weight determinations. It is possible that our sample was contaminated with some apoprotein of cytochrome b_5. Our determination of M of pig liver cytochrome b_5 in the ultracentrifuge gives the most reliable result, 12 800. This compares well with the value 12 700 obtained by Strittmatter [2] for calf liver cytochrome b_5 from the measured heme content, which he considered to be the best of his experiments.

In Tables 1 and 2 the amino acid composition and molecular properties of cytochromes *c* and b_5 are compared. The similarities suggest that the two molecules are very similar in size and shape. In the amino acid contents there are both similarities and differences. There are more histidine residues in b_5 than in *c*. b_5 contains at least one methionine residue but there are no cysteine residues. The molar ratio of basic to acidic residues is different in cytochrome b_5 and in cytochrome *c* (0.6 and 1.4, respectively), which is in accord with the difference in their isoelectric points (below pH 6 for cytochrome b_5 and pH 10.6 for cytochrome *c*). One cause of this low ratio, in cytochrome b_5, could be losses of lysine or arginine during the digestion with pancreatic enzymes (trypsin), which is necessary to release the firmly bound cytochrome b_5 from the membranes of the endoplasmic reticulum. However, it should be observed that a value of 0.5—0.55 has recently been found for the ratio of basic to acidic residues present in the whole membranes.[8]

Table 2. Comparison of hydrodynamic properties of cytochromes *c* and b_5.

	unit	cyto-chrome *c*[7]	cyto-chrome b_5
$S_{20,w}^{\circ}$	S	1.71 [a]	1.67
$D_{20,w}$	F	11.4	11.6
V	cm^3g^{-1}	0.728 [b]	0.723 [2,b]
		0.741 [c]	0.731 [c]
$M_{analysis}$		13 000	15 000 / 12 700 [2]
$M_{S \text{ and } D}$		13 300	12 700 / 14 400 [2]
$M_{S/D}$		13 300	12 900
f/f_0		1.19	1.19

[a] At 10 mg/ml, concentration dependence was not determined.

[b] Measured values.

[c] Values calculated from amino acids.

Acknowledgement. This investigation was supported by grants from *United States Public Health Service* (AM-05895) and *Statens Medicinska Forskningsråd.*

R. B-P, fellow of the "Centre National de la Recherche Scientifique", is grateful to the "Comission d'attribution des Bourses de Recherche Scientifique de l'O.T.A.N" for financial support during her stay in Stockholm.

1. Strittmatter, P. and Velick, S. F. *J. Biol. Chem.* **221** (1956) 253.
2. Strittmatter, P. *J. Biol. Chem.* **235** (1960) 2492.
3. Poltoratsky, R. and Chaix, P. *Bull. Soc. Chim. Biol.* **46** (1964) 867.
4. Poltoratsky, R. and Chaix, P. *Compt. Rend.* **258** (1964) 3787.
5. Poltoratsky, R. and Chaix, P. *Bull. Soc. Chim. Biol.* **48** (1966) 449.
6. Craig, L. C. In Alexander, P. and Block, R. J. *Analytical methods of Protein Chem-*

istry, Vol. 1, Pergamon, London 1960, p. 117.
7. Ehrenberg, A. *Acta Chem. Scand.* **11** (1957) 1257.
8. Manganiello, V. C. and Phillips, A. H. *J. Biol. Chem.* **240** (1965) 3951.
9. Margoliash, E., Smith, E. L., Kreil, G. and Tuppy, H. *Nature* **192** (1960) 1125.

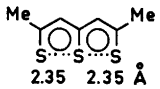
Received August 26, 1966.

The Sulphur-Sulphur Bonds in an Unsymmetrical Thiothiophthene Derivative

ASBJØRN HORDVIK, EINAR SLETTEN and JORUNN SLETTEN

Chemical Institute, University of Bergen, Bergen, Norway

Bezzi *et al.*[1,2] in 1958 discovered the thiothiophthene "no-bond resonance" system through an X-ray crystallographic study of the dimethyl derivative (I).

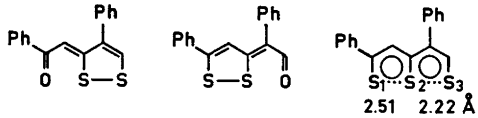


(I)

They found the sulphur atoms to be colinear and equally spaced with sulphur-sulphur distances of 2.36 Å (later,[3] 2.35 Å was reported), as compared with the value 2.10 Å for a sulphur-sulphur single bond in a *cis*-planar disulphide group.[4]

The electronic structure of the thiothiophthene ring system has been calculated by Giacometti and Rigatti,[5] in terms of sulphur-sulphur bonds made up from both fractional σ and π bonds.

Klingsberg [6] has shown that (II) and (III) react with phosphorus pentasulphide to yield the same compound (IV), and preliminary results of a crystal structure analysis of this unsymmetrical thiothiophthene derivative are given here.



(II) (III) (IV)

Table 1. Atomic coordinates, in fractions of corresponding cell edges.

	x	*y*	*z*
S_1	0.3248	0.1634	0.2569
S_2	0.1275	−0.1060	0.0763
S_3	−0.0450	−0.3437	0.0870
C_1	0.3757	0.1877	0.1142
C_2	0.3016	0.0679	−0.0337
C_3	0.1887	−0.0778	−0.0649
C_4	0.1129	−0.2054	−0.2209
C_5	−0.0027	−0.3362	−0.2363
C_6	0.4988	0.3425	0.1444
C_7	0.5706	0.3233	0.0336
C_8	0.6827	0.4677	0.0565
C_9	0.7216	0.6275	0.1997
C_{10}	0.6530	0.6460	0.3098
C_{11}	0.5405	0.5020	0.2865
C_{12}	0.1560	−0.1951	−0.3481
C_{13}	0.0667	−0.1818	−0.4511
C_{14}	0.1088	−0.1762	−0.5736
C_{15}	0.2367	−0.1812	−0.5990
C_{16}	0.3265	−0.1945	−0.4985
C_{17}	0.2869	−0.2016	−0.3751

The crystals are triclinic, and belong to the space group $P\bar{1}$ with $a = 10.18$ Å, $b = 8.52$ Å, $c = 10.29$ Å, $\alpha = 118.8°$, $\beta = 94.3°$, $\gamma = 101.1°$.[7] There are two mole-

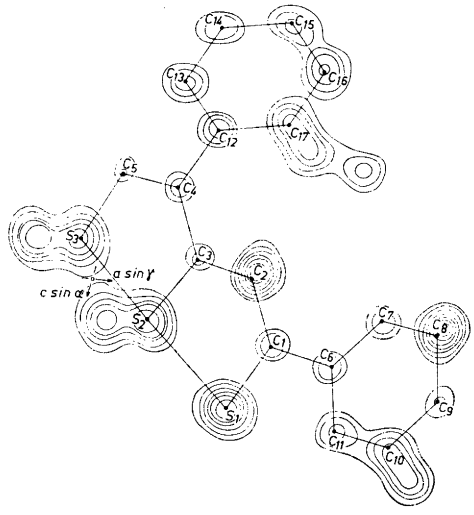


Fig. 1. Electron density projection of (IV) along the *b* axis. Contour intervals, 1 e·Å^{-2} for carbon and 3 e·Å^{-2} for sulphur. Lowest contour 5 e·Å^{-2}.