

Structure of Cerebrosides.

II. Small Angle X-Ray Diffraction Study of Cerasine

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Cerasine having a molecular weight of 800 differs chemically from phrenosine only in the hydroxyl group attached to the fatty acid tail which is replaced by a hydrogen atom. Nevertheless, remarkable differences between both cerebrosides are detected in the lamellae periodicities. In the range of 23 – 66 °C just one single (instead of two) structure with a similar subcell to the triclinic one component of phrenosine is detected. Between 66 and 87 °C three new components (instead of one in phrenosine) appear. Two of the structures are similar to the two phrenosine-components at low temperature and the tilt angles of their chains with respect to the basal planes can explain the stabilizing capacity of the 201 and 301 netplanes of the paraffin-like subcells respectively. These lattice planes are parallelly aligned to the surfaces of the lamellae. The long period of 58 Å of component II cannot be explained in such a way. This period persists upto 105 °C and coexists from 87 °C with a new component showing a 40 Å-periodicity, which cannot either be explained in the above manner. Paracrystalline distortions of the arrangement of the bilayers can be justified by orientational disorder of the galactose heads.

Introduction

The structural behaviour of cerebrosides – a special class of glycosphingolipids – is of great interest because they occur as one of the main constituents of brain membranes [1, 2] and are often connected to antigenic properties [3]. X-ray diffraction studies of cerebrosides are, however, scarce [4, 5]. A fresh attack to establish the spatial distribution of molecular arrangements by X-ray diffraction analysis has been recently reported in this laboratory [6] for dry phrenosine from beef brain. Special emphasis was given in this study to crystal size, lattice distortions and to the electron density profile analysis across the lipid bilayer. The coexistence of two structures for phrenosine at 23 °C was demonstrated. While structure I showed a 65 Å periodicity (orthorhombic subcell) the bimodal analysis of structure II (51 Å) led to a statistical mixture of 65 Å thick bilayers (orthorhombic) with 49 Å triclinic ones. The presence of a third polymorphic structure (triclinic) with a 51 Å period was, additionally, identified at 66 °C.

The present paper represents a further contribution to the study of the structure of cerebrosides and complements our previous results on phrenosine reported in part I [6]. In this work we offer evidence on the structures arising in dry cerasine at various temperatures between 23 ° and 105 °C by means of SAXS.

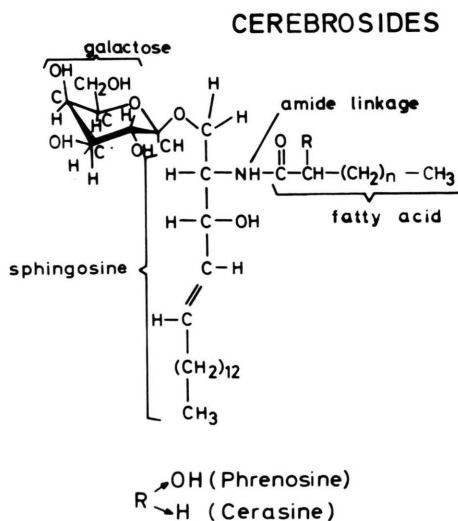


Fig. 1. Chemical constitution of cerebrosides.

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Experimental

The chemical structure of the molecule of cerasine is depicted in Fig. 1. Cerasine has an identical sphingosine tail and a galactose head as phrenosine. The only difference rests on the R substituent attached to the fatty acid chain; R = H for cerasine while R = OH for phrenosine. The investigated cerasine samples were obtained by M. Lomas and Capman [7] by the method of Carter *et al.* [8] and their purity is claimed to be of 96%. The samples in powder form were inserted for X-ray diffraction in Mark-capillaries of 1 mm diameter. Diffraction patterns were recorded with a CuK_α radiation from a Rigaku Denki rotating anode generator (5 KW) using a pinhole Kiessig system. The incident beam measured ~ 0.2 mm at the specimen. The sample-to-film distance used was 182 mm. Patterns were recorded on Gevaert Industrial G-X-ray film with approximately 2 h for the first order SAXS reflections and 120 h exposure time for the higher orders. Intensities and spacings were measured from densitometer traces recorded on a Joyce-Loebel MK III C microdensitometer. Collimation errors were taken into account by deconvolution of the experimental profile with that of the primary beam. The diffraction experiments were conducted at room temperature (23 °C), 66 °, 87 ° and 105 °C respectively, where phase transitions occur.

Results

An example of the SAXS diffraction pattern that was recorded at 87 °C is shown in Fig. 2. In Table I

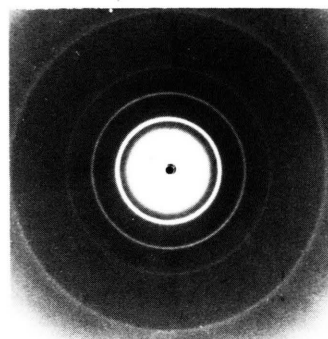


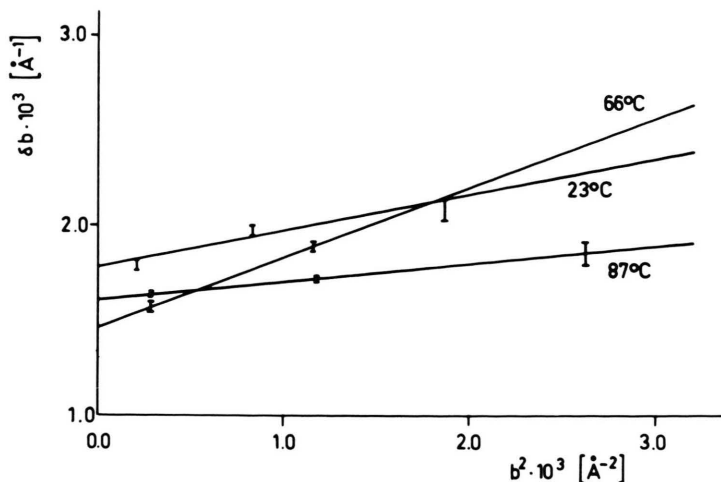
Fig. 2. An example of an SAXS pattern recorded from dry cerasine at 87 °C. The dominant periodicity is 58.4 Å.

are collected the observed positions of the reflections. Five different structure components, I, I', II, III, IV, were observed depending on temperature. At 23 °C the first three orders of a 69.6 periodicity (structure I) can be identified. The 5th and the 7th orders are too diffuse and weak and could not be photometrically recorded. Between 66 and 87 °C structure I disappears and three new components, I', II and III, appear instead. Structure II offers the first four orders (plus the sixth one) of a 58.4 Å periodicity (only the two first orders could be photometrically recorded). Structure I' exhibits only one order of a 66.3 Å periodicity and has obviously to do with structure I of phrenosine (65 Å). Structure III exhibits also only one order of a 50.1 Å periodicity. At 87 °C structure II still remains and a new structure IV with only one reflection at ~ 40 Å emerges. The appearance of only a few orders of the above

Structure	$b_{\text{exp}} [\text{\AA}^{-1}]$	$d_{\text{exp}} [\text{\AA}]$	$b_{\text{cal}} [\text{\AA}^{-1}]$	$d_{\text{cal}} [\text{\AA}]$	$(b_{\text{cal}} - b_{\text{exp}}) \times 10^4 [\text{\AA}^{-1}]$
I 23–66 °C	0.01436	69.6	0.01441	69.4	0.5
	0.02884	34.7	0.02883	34.7	–0.1
	0.04324	23.1	0.04324	23.1	0.0
	0.0715	14.0	0.07207	13.3	5.7
	0.1004	10.0	0.10089	10.0	4.9
I' 66–87 °C	0.01508	66.3	–	–	–
II 66–105 °C	0.01713	58.4	0.01761	56.8	4.8
	0.03415	29.3	0.03522	28.4	10.7
	0.05130	19.5	0.05283	18.9	15.3
	0.0696	14.3	0.07044	14.2	8.4
	0.10566	9.5	0.10566	9.5	0.0
III 66–87 °C	0.01997	50.1	–	–	–
IV 87–105 °C	0.01976	40.1	–	–	–

Table I. Location of the observed SAXS reflections of the five structures of cerasine.

Fig. 3. Corrected integral widths of the SAXS reflections against the square of reciprocal vector b for structure I at 23 °C and structure II at 66 and 87 °C.



periodicities suggests the presence of distortions within the lamellar stacks causing deviations from the fully crystalline state. According to the theory of paracrystals [9] the paracrystalline distortions, g , and the coherent length of lamellar stacks, D , of these macroparacrystallites (MPC's) [6] normal to the bilayer can be straight-forwardly derived from the expression:

$$\delta\beta = 1/D + \frac{\pi^2 g^2 n^2}{P} \quad (1)$$

where $\delta\beta$ is the integral width, and n the order of each reflection. The paracrystalline distortion, g , of such a superlattice is defined by the relative fluctuation of lamellar distances $g = \sqrt{\bar{P}^2/\bar{P}^2 - 1}$. Fig. 3 shows the plot of $\delta\beta$ against b^2 for the three orders of the 69.6 Å periodicity at 23 °C (structure I) and for the order of the new ~ 58 Å periodicity appearing at 66 and 87 °C (structure II). The average size of the stacks of lamellae, D , and the, g , lattice distortions were calculated from the intercept and the slopes of the straight lines respectively. Table II summarizes the D , g and $N = D/P$ values for the three temperatures derived from Eqn (1) and the corresponding periodicity P from SAXS. The observed g -values are

Table II. Long period, P , coherent mean size of lamellar stacks, D , number of bimolecular lamellae, $N = D/P$, and percent paracrystalline distortion, g .

Structure	T [°C]	P [Å]	D [Å]	N	g [%]
I	23	69.6	562	8	1.7
II	66	58.4	685	12	2.5
II	87	58.1	625	11	1.3

of the same order as those found in the phrenosine case. They are, however, larger than in stacks of paraffin lamellae [10]. In our previous investigation [6] it was suggested that conformational disorder of the galactose heads could be responsible for such larger values.

Discussion

The present study substantiates our earlier results with phrenosine and offers a further variety of crystal forms for dry cerasine. The long spacings of the five structures of cerasine are depicted as full lines in Fig. 4 and compared with the three structures of phrenosine (broken lines). Two groups of long spacings can be distinguished: On the one hand structures I and I' of cerasine and I of phrenosine having long periods $P \sim 68$ Å, and on the other structure III of cerasine and structures II and III of phrenosine showing periods in the vicinity of 50 Å. In our previous publication [6] (part I of this series) these predominant periods were explained by the stabilizing

Table III. The triclinic subcell of structure I of cerasine compared with the triclinic subcell of one component of structure II of phrenosine.

Cerasine	Phrenosine	
23 °C	23 °C	66 °C
$a = 4.99 \pm 0.02$	4.73 ± 0.02	4.74 ± 0.02
$b = 4.50 \pm 0.02$	4.60 ± 0.02	4.71 ± 0.02
$c = (2.55)$	(2.55)	
$\alpha = 106^\circ\text{C}$	106°C	
$\beta = 83^\circ\text{C}$	85°C	
$\gamma = 74^\circ\text{C}$	72°C	

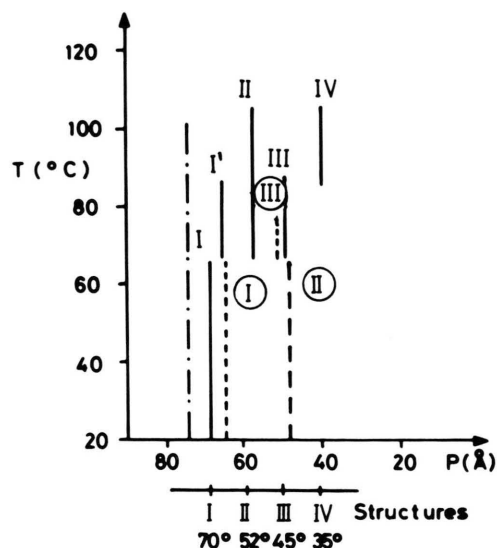


Fig. 4. Observed periods, P , of cerasine as a function of temperature. The dotted line on the left hand side represents the spacing expected from extended chains with a normal position of the galactose heads in a vertical structure (bimolecules perpendicular to basal plane). The angles thus calculated indicate the various obliquities arising for the various periodicities. The periodicities obtained for phrenosine are also shown for comparison (— —).

capacity of the hydrocarbon tails which pack within a substructure which may be orthorhombic and/or triclinic similarly to the paraffin case. Table III

shows the cell dimensions of the triclinic modification I of cerasine which are indeed similar to the triclinic modification of phrenosine. The tilt angles, φ , (angle between the chain direction and the basal phase) are also given in Fig. 4 by $\cos(90 - \varphi) = P/L$ under the assumption that L = twice the length of the bilipid molecule plus end-to-end gap between a bilayer, has a value of 74 Å. The length of 74 Å depends of course on the orientation of the galactose heads relative to the aliphatic chains and therefore small variances of φ may occur. The tilt angle of $\sim 68^\circ$ leads, hence, to the 201 paraffin netplanes parallel to the lamellae surface, while the angle $\sim 45^\circ$ to the (301)-netplanes. Variations of the observed long period can be explained by special orientations of the galactose heads, as discussed in the foregoing paper [6]. Solely the new structures III and IV of cerasine deviate from these two main groups of spacings. The reason for these deviations cannot presently be given, because just the first order reflections were available.

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