Changes in lipid phase behaviour in human myelin during maturation and aging

Involvement of lipid peroxidation

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The biophysical properties of human myelin isolated from white matter of patients aged two months to 74 years were investigated using wide-angle X-ray diffraction. The myelin transition temperature increased from 13°C to 65°C as age increased from two months to 17 years, demonstrating an increase in the myelin lipid stability. Following this maturation period, the myelin transition temperature remained constant at 65°C until age 50. Beyond age 50, the transition temperature of myelin decreased by 13°C indicating that myelin stability decreased with aging. During this aging period, the levels of malondialdehyde and conjugated diene increased, indicating an increasing amount of lipid peroxidation. Although evidence is indirect, the results of this investigation strongly suggest that free radicals could be a primary factor in the acceleration of the aging processes in the human brain.

Myelin *Lipid phase* *Aging* *Lipid peroxidation*

1. INTRODUCTION

Aging in the human brain is a complex process characterized by intricate alterations in morphology and neurochemistry [1–3]. Changes in various biochemical substrates have been correlated with changes in neurones and membranes during aging. In the study of these changes, the most important challenge is to determine the functional significance of these alterations in terms of mechanisms responsible for aging. A principal cause of aging proposed initially in [4] involves reactions of free radicals and the formation of peroxidized lipid which lead to age-associated degradation of living tissues. Although several age-related biochemical changes in the nervous system appear to be associated with lipid peroxidation [5,6], little is known about the biophysical consequences of these processes on human myelin [7] during development and aging. Myelin at different stages of development may be selectively vulnerable to a wide range of noxious influences, in particular, to free radicals generated by internal or external factors, some of which may come from the environment. Here, 18 brains from normal individuals aged from 2 months to 74 years were subjected to biophysical investigations to determine changes in lipid phase behaviour of myelin during development and aging. These phase changes were monitored by wide-angle X-ray diffraction, a technique which allows unambiguous detection of gel phase lipid in membranes and thus permits the determination of liquid–crystalline to gel–phase transitions. This study also provides evidence that peroxidation of the nervous system may be involved in aging of the human brain.

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2. MATERIALS AND METHODS

2.1. *Myelin isolation*

The brains of 18 patients who died from accidental deaths or from diseases not affecting the nervous system were obtained within 4–8 h after death. Each brain was examined by a neuropathologist to rule out central nervous system pathology. The cortex was removed, and the white matter was separated from the grey matter. Myelin was prepared as in [8] except that phenylmethylsulphonyl fluoride (PMSF, 10 μM) was present in the sucrose solution throughout the isolation to inhibit protease activity.

2.2. *X-ray diffraction of myelin*

Myelin samples for X-ray diffraction were prepared as in [9]. Wide angle X-ray diffraction patterns were recorded at various temperatures using Cukα radiation from a point-focussed X-ray tube (type PW 2103/01) on a Philips (type 1030) camera under conditions in which the samples retain 50–75% moisture with respect to final dry wt. The lipid phase transition temperature, defined as the highest temperature at which gel phase lipid can be detected, was determined to within 1°C.

2.3. *Analytical procedures*

2.3.1. Malondialdehyde

Levels of malondialdehyde (MDA), a breakdown product of unsaturated fatty acid hydroperoxides, were estimated in the brain homogenates using a modified thiobarbituric acid (TBA) test as in [10]. Results were expressed as nmol MDA/g dry wt of lyophilized homogenate. MDA levels were calculated relative to a standard preparation from the hydrolysis of 1,1,3,3-tetramethoxypropane as in [11].

2.3.2. Conjugated diene

Conjugated diene, which is formed during the peroxidation of unsaturated fatty acids, exhibits spectra characterized by an intense absorption at 233 nm and were estimated by the method in [12].

3. RESULTS AND DISCUSSION

Wide-angle X-ray diffraction patterns recorded at 37°C from specimens of myelin isolated from 2- and 3-month old infants, featured two broad bands centered at Bragg spacings of 4.6 Å and about 10 Å (fig.1A,B). The broad ring at 4.6 Å is derived from liquid–crystalline phase lipid [13] but as yet the 10 Å reflection is not well characterized [14,15]. The broad 4.6 Å reflection indicates that the lipid of the myelin was in a comparatively disordered state at this early stage in the myelin development. This does not, however, preclude heterogeneity of fluidity within the lipid matrix, because X-ray diffraction data is space-averaged and will not depict the physical state of lipids in individual microenvironments. The lipid phase transition temperatures (the highest temperature at which gel phase lipid can be detected) for myelin from 2 and 3 month old infants were 13°C and 25°C, respectively (fig.2A).

The diffraction pattern of myelin from a 5-year-old child (fig.1C) was essentially identical with that for 2 and 3 month old infants, except that it was possible to discern a sharp reflection superimposed on the broad 4.6 Å reflection and centered at a

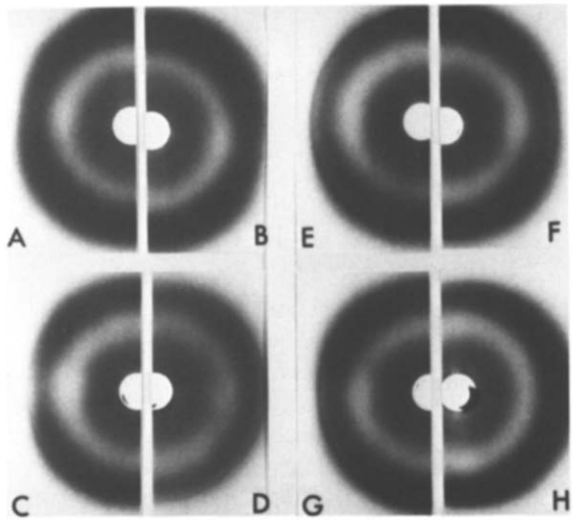

Fig.1. Wide-angle X-ray diffraction patterns for isolated myelin fractions from human brain. Myelin patterns were recorded at 37°C. (A) and (B) Myelin patterns from 2 and 5 month old infants featuring (from outside to inside) two broad bands centered at Bragg spacings of 4.6 Å and about 10 Å. (C), (D), (E), (F), (G) and (H) Patterns for myelin isolated from 5, 8, 17, 30, 50 and 74 year old human brain featuring (from outside to inside) a sharp band centered at a Bragg spacing of 4.15 Å and two broad diffuse bands centered at Bragg spacings of 4.6 and about 10 Å.

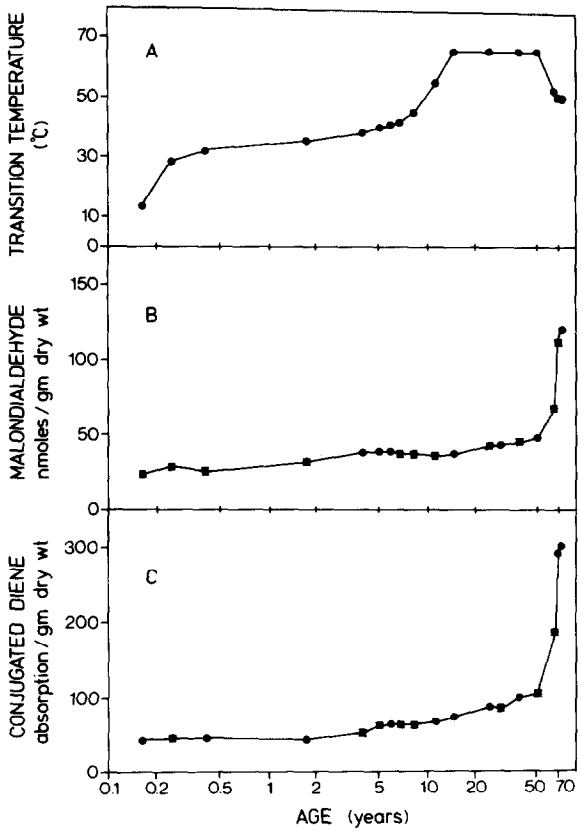

Fig.2. (A) Changes in transition temperature of myelin from human brain of different ages. Transition temperatures were determined by wide-angle X-ray diffraction. Means and standard errors of the means for separate experiments are shown; $n = 3$. (B) Changes in malondialdehyde levels in tissues from human brain of different ages. Means and standard errors of the means for separate experiments are shown; $n = 3$. (C) Changes in conjugated diene absorption levels in tissues from human brain of different ages. Means and standard errors of the means for separate experiments are shown; $n = 3$.

Bragg spacing 4.15 Å. This sharp ring was appreciably intensified in patterns for 8 year olds (fig.1D) and even more pronounced for 17 year olds (fig.1E) and 30–50 year olds (fig.1F,G). The presence of the sharp 4.15 Å reflection in patterns for myelin from white matter of mature brains indicated that portions of the lipid had undergone a phase change from a disordered to an ordered phase as development of myelin advanced. In this case, the transition to a crystalline state may be due to chemical or structural changes (or both) in the membranes during development of the myelin, since all the diffraction patterns were recorded isothermally as a function of maturation. The lipid phase transition temperature increased steadily as maturation progressed reaching a maximum at the age of 17 years. The transition temperature then remained constant at 65°C until age-related changes occurred. Thus, throughout maturation, there is an increase in myelin stability at physiological temperatures. A role for metals, in particular Ca^{2+}, in the compaction of myelin has been demonstrated [16]. During this period, lipid peroxidation did not appear to be particularly significant (fig.2B,C).

As aging progressed (beyond 50 years), the sharp ring at 4.15 Å became less intense (fig.1H) and the lipid phase transition temperature decreased by 13°C at age 64 (fig.2A). This would suggest that during the aging process, the myelin lipid becomes less ordered. This alteration was most marked in the myelin from a 74 year old brain in which the lipid phase transition temperature was 15°C lower than that of normal adult myelin from younger ages (fig.2A).

The instability of the myelin lipid bilayer in the aged brain possibly reflects an increased degree of unsaturation in the long acyl chains of major glycosphingolipids found in aged myelin [7,17]. Our results indicate that lipid peroxidation in the aged brain is strongly associated with this age-related increase in disorder of the myelin lipid bilayer. The levels of MDA, a product of lipid peroxidation, showed a sharp increase in brain homogenates (fig.2B). This increase in MDA accompanied the increased instability of the myelin lipid bilayer, as determined by a decrease in transition temperature. Conjugated diene, another indicator of lipid peroxidation, was increased during aging also in a similar manner (fig.2C), thus substantiating the contention that lipid peroxidation is correlated with the alteration in biophysical properties of human myelin membrane during aging.

Although the precise mechanism of the cause of aging is not well understood, our finding of an increase in MDA and conjugated diene in the human brain are in agreement with the suggestion that reactions with free radicals are involved during the aging process [4,5,18–21]. The central nervous system may be particularly susceptible to degrada-

tion by such free radicals. Such biological perturbations, if they occur in highly specialized tissues such as myelin, might enhance the aging process.

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