SHORT COMMUNICATIONS

BBA 73118

Small angle X-ray diffraction of myelin membrane: Lack of effect of deuterium oxide on myelin

Neutron diffraction of hydrated biological material[1] requires the substitution of most of the water within the tissue by ^2H_2O. If not substituted, the large cross-section of hydrogen for diffuse scattering[2] gives rise to a strong incoherent scatter from the water within the biological material which masks Bragg reflections.

The substitution of ^2H_2O for water in feeding animals frequently leads to toxicity and death[3–8], primarily as a result of neuromuscular and kidney damage. At the molecular level, enzymatic processes are affected[9,10] with alterations in the kinetics of electron transport and oxidative phosphorylation[11,12]. The structural changes in cells have been summarized by MATTY AND DEUTSCH[8] with respect to membranes. DEUTSCH *et al.*[7] also noted disruption of the myelin sheath of the sciatic nerves of moribund mice following ^2H_2O feeding.

We wish to establish the extent of structural changes accompanying substitution of free water of the sciatic nerve with ^2H_2O. Frog (*Rana pipiens*) sciatic nerves were soaked at 4° in a single volume of 25 ml of 0.18 M NaCl in ^2H_2O (pH 7.2) for periods of up to 48 h, or given multiple soakings by immersion in four 25-ml volumes for 12 h each. Control nerves were soaked in 0.18 M NaCl in ^1H_2O. Multiple soakings gave the same results as immersion in a single volume for 48 h.

The X-ray diffraction pattern was recorded on the ^2H_2O substituted sciatic nerves and compared to the X-ray diffraction patterns of normal nerves (Figs. 1a and 1b). The experimental conditions have been described previously[13]. The normal

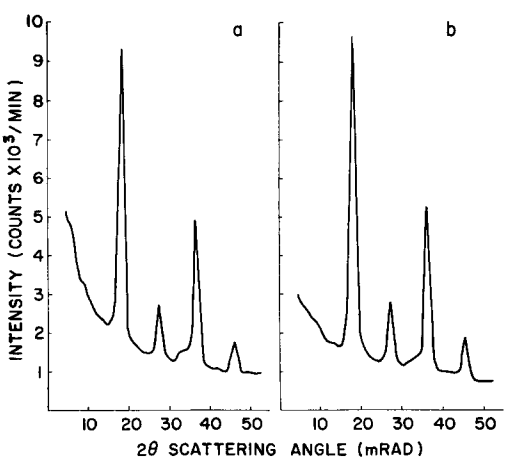

Fig. 1. X-ray diffraction pattern of frog sciatic nerve. a. Normal nerve (XM 536). b. 48 h ^2H_2O substituted nerve (XM 584).

TABLE I

INTENSITIES OF ^2H_2O SUBSTITUTED FROG SCIATIC NERVE

Substitution time (h)	*Number of experiments*	*(h00)*			
		(200) *	*(300)*	*(400)*	*(500)*
0	15	100 ± 17	22 ± 4	48 ± 6	14 ± 2
2	1	115	20	48	17
12	1	108	22	45	14
48	3	113 ± 2	21 ± 3	52 ± 2	13 ± 1

* Average intensities of (200) reflection of normal sciatic nerve normalized to 100. All intensities are compared to this reflection.

TABLE II

INTENSITIES OF HIGHER ORDER REFLECTIONS OF ^2H_2O SUBSTITUTED FROG SCIATIC NERVE

Substitution time (h)	*(h00)* *		
	(600)	*(800)*	*(11.0.0)*
48	0.48	0.31	0.56
	0.12	0.36	0.90
	0.28	0.40	0.84
Average	0.29 ± 0.12	0.36 ± 0.03	0.76 ± 0.14
Control	0.36 ± 0.15	0.36 ± 0.10	0.75 ± 0.25

* Intensities are compared to the (200) reflection which has been normalized to a value of 100.

Bragg spacings (fundamental of 171Å) and intensities (within the experimental error) were observed in all nerves which were ^2H_2O substituted for periods up to 48 h (Tables I and II). The intensity of the 4th and 5th order reflections decreased and the peaks disappeared as the substitution time continued beyond 48 h. The intensity of the 3rd order reflection also decreased but did not disappear.

The absence of detectable structural changes in the myelin membrane in our X-ray diffraction experiments appear to differ markedly with the electron observations of DEUTSCH[7] in the myelin membrane of ^2H_2O fed mice. In the mice experiments, 7–8 days of exposure of ^2H_2O were used, but the substitution of ^2H_2O in the tissue could not be nearly as complete as in our experiments.

In our experiments, nerves only showed detectable changes after 3 days immersion in isotonic NaCl–^2H_2O (pH 7.2). The control (0.18 M NaCl–^1H_2O, pH 7.2) showed similar changes after 4 days, suggesting that the deterioration was related more to the unbuffered and unbalanced salt composition rather than the substitution of ^1H_2O by ^2H_2O. Previous workers[7,8] have suggested that the observed changes in membrane structure are either due to "differences in shape of the molecular units, resulting from the replacement of the hydrogen atom by deuterium . . . or changes occur in enzyme systems which, probably, regulate the aggregation of these units"[7]. Our results do not differentiate between these possibilities because, under substitution conditions, metabolic activities quickly cease and there should be little incorporation

of deuterium (except by exchange) into the membrane structural components. In addition, a small incorporation is to be expected because it is known that myelin has a long turnover time for its membrane components[14]. However, within the available resolution of the small angle X-ray diffraction method (the electron density distribution has a resolution of 8Å using the 11th order reflection) substitution by ^2H_2O produces no detectable structural changes. It should be emphasized that the X-ray analysis of myelin membrane is far more revealing of the inner structure than is electron microscopy of myelin. The lack of change associated with ^2H_2O substitution may be related to our recent findings from X-ray diffraction of myelin[13,15], that there is no significant unbound water space between pairs of membranes and that at least part of the protein portion of each membrane layer must be hydrated. The result of this study suggests that replacement of this bound water produces no large scale structural changes in the membrane protein–lipid complexes.

Further work is in progress to examine possible changes in the wide angle pattern of myelin following ^2H_2O substitution. The apparent inertness of the myelin structure to ^2H_2O substitution encourages further exploration of the neutron diffraction data of myelin where ^2H_2O substitution is essential.

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