SC 2195

Phospholipid and plasmalogen synthesis in rat-brain homogenates

The plasmalogen phospholipids are characterized by the presence of an unsaturated ether moiety. It has been suggested that the precursor of the unsaturated ether linkage could be the long-chain aldehyde[1,2], which may form a hemiacetal that could, by dehydration, give the unsaturated ether[1]. This hypothesis is supported by the report that brain possesses an enzyme that deacylates and reduces palmityl-CoA to give palmitaldehyde[3].

To test the possibility that aldehydes are the source of the unsaturated ether in plasmalogens, the biosynthesis has been studied in brain of male rats 14–21 days old, a tissue rich in plasmalogen[1,2]. Incorporation studies were carried out using [1-^{14}C]palmitic acid, [1-^{14}C]stearic acid and [1-^{14}C]palmitaldehyde; the acids were obtained from the New England Nuclear Corp. The [1-^{14}C]palmitaldehyde was prepared by lithium aluminum hydride reduction of the *N*-methyl anilide of [1-^{14}C]-palmitic acid according to the method of WEYGAND *et al.*[6], with a yield of about 50 %. The radioactive palmitic acid (200 μC) was diluted to 50 mg with carrier palmitic acid and converted to the acid chloride with thionyl chloride, which in turn was reacted with *N*-methylaniline to give the corresponding amide. This was purified by sublimation and verified by infrared spectrum and melting point.

The brains of decapitated animals were rinsed in cold 0.1 M Tris (pH 7.8) and small pieces homogenized in 3 vol. of the buffer (Servall Omni-Mix) at 2°. After centrifugation (0°) at 2200 × *g* for 30 min, the supernatant was retained and the

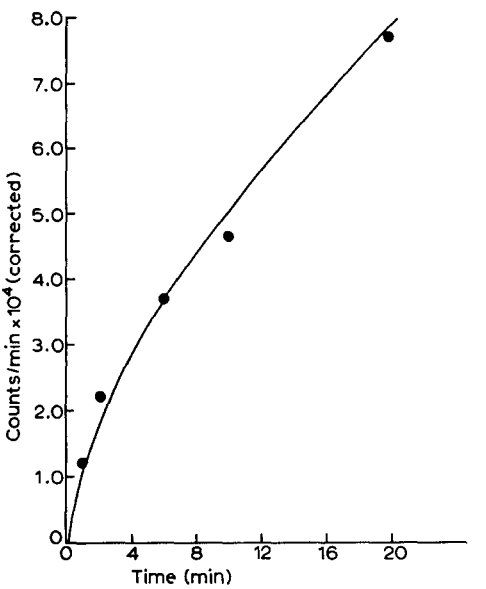

Fig. 1. The rate of incorporation of [1-^{14}C]palmitic acid into total phospholipid by rat-brain homogenate. Each reaction was carried out in a tapered centrifuge tube at 37°, the final vol. being 1.5 ml. Each tube contained, in μmoles: $MgCl_2$, 5.0; $MnCl_2$, 0.5; ATP, 5.0; CoA, 0.5; α-glycerophosphate, 5.0; cytidine triphosphate, 0.5; [1-^{14}C]-palmitic acid, 0.5 (specific radioactivity, 10 μC/μmole); Tris (pH 7.8), 120. The reaction was initiated by the addition of rat-brain homogenate supernatant (6.0 mg protein) and ended by addition of 7 ml of ethanol after the appropriate time interval. The results expressed are in counts/min, after correcting for background and the zero-time blank.

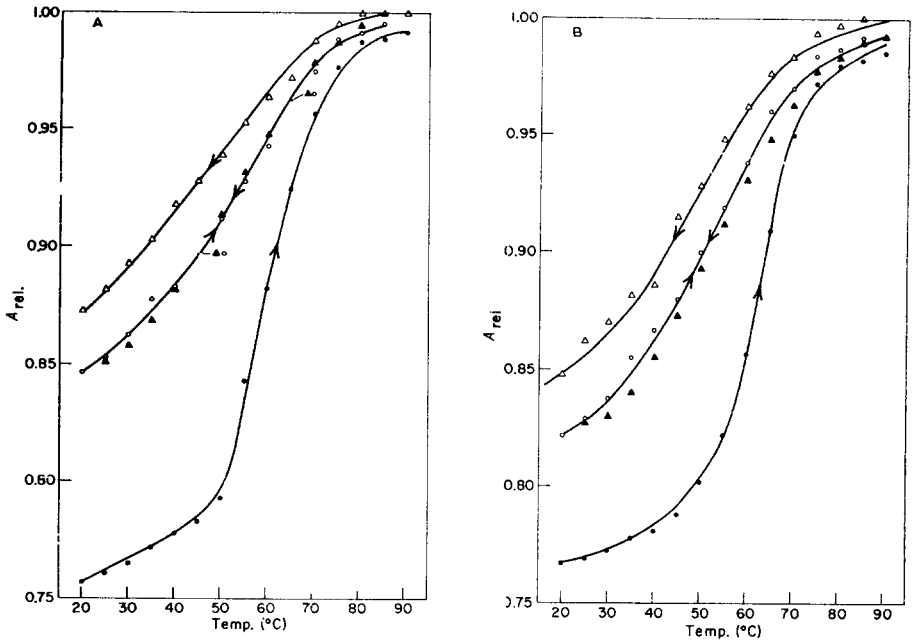

Fig. 2. Effect of repeated heating and cooling (hysteresis) on relative absorbancy–temperature profiles of TMV-RNA. All experiments in standard saline–citrate/100 plus Mg^{2+}(10^{-3} M). A, in H_2O; B, in D_2O; ●, 1st cycle heat; ○, 1st cycle cool; ▲, 2nd cycle heat; △, 2nd cycle cool.

Effect of base composition on T_m of transfer RNA

It had previously been reported that the thermal transition of isolated transfer RNA was relatively broad and that there appeared to be little, if any difference, between different molecular species varying significantly in base composition and very profoundly in partition coefficients[33,34]. This was true, as well, of our experiments in standard saline–citrate although there were indications of a statistically significant difference in T_m (Table IV). The observation that quite sharp profiles and more accurate estimates of T_m could be obtained in standard saline–citrate/100 containing 10^{-3} M Mg^{2+} permitted a critical examination of the problem (Table IV and Fig. 4): there are indeed significant differences in the T_m of the three molecular species, and on the basis of the presently available, quite limited evidence there appears to exist a direct correlation of these differences with base composition (increasing T_m with an increase in the content of guanine plus cytosine). This is the well-known correlation discovered by MARMUR AND DOTY for DNA[18,23,26,35]. An analoguous correlation for RNA of high molecular weight (TMV and ribosomal) had previously been reported by SPIRIN[36], and by WALLACE AND TS'O[37]. The values of T_m for a nucleic acid containing 50 mole % guanine plus cytosine and the slope of the correlation lines are: for DNA in standard saline–citrate[18,23,24] –89.5° and 0.42° per percent guanosine plus cytosine; for DNA in 10^{-3} M phosphate[18] –70° and 0.42° per percent guanosine plus cytosine; for high molecular weight RNA in 0.1 μ phosphate[36] –52° and 0.5° per percent guanosine plus cytosine; for transfer RNA in standard saline–citrate –55° and 1° per percent guanosine plus cytosine; for transfer RNA in standard saline–citrate/100 containing

TABLE IV

THERMAL TRANSITION PARAMETERS FOR TRANSFER RNA

All experimental conditions similar to those of Table II.

Transfer RNA	Composition*		Buffer	Pre-incubation at 50°	H_2O			D_2O		
	$G+C$	$\frac{G+C}{A+U}$**			T_m	$\Delta_{1/2}$	R_Δ (%)	T_m	$\Delta_{1/2}$	R_Δ (%)
Alanine	63.6	1.75	Standard saline–citrate/100, Mg^{2+}	–	72.0	15.5	27	73.5	13.0	28
				–	75.5	14.0	26	73.0	10.0	28
				+	71.0	32.5	29	71.5	13.5	30
				+	74.0	15.5	25	73.0	12.0	30
			Mean ± σ		73.1±2.0	19.4±5.0	26.8±1.7	72.8±0.8	12.1±1.5	29.0±1.1
			Standard saline–citrate	–	68.0	36.0	32	67.5	37.0	30
				+	68.0	36.0	31	68.0	38.5	33
Tyrosine	56.9	1.32	Standard saline–citrate/100, Mg^{2+}	–	52.5	13.5	28	53.0	11.5	25
				+	52.5	14.0	30	52.5	14.0	30
			Standard saline–citrate	–	62.5	42.5	31	62.0	33.0	24
				+	63.5	37.0	31	63.0	35.5	26
Valine	56.0	1.28	Standard saline–citrate/100, Mg^{2+}	–	48.5	19.0	23	49.5	15.0	26
				+	49.0	18.0	23	50.0	15.0	27
			Standard saline–citrate	–	59.0	33.5	27	61.0	30.5	28
				+	60.5	33	28	61.5	32	29

* Refs. 33, 34

** Includes ψU.