Short Communications

Potentiometric iodometric determination of plasmalogen

Difficulties in detecting the visual end point in the iodination procedure of RAPPORT AND LERNER[1] for the determination of plasmalogen were noted in titrating highly colored lipid extracts. The end point also could not be determined reproducibly with colorless samples containing less than 2 μmoles plasmalogen. In addition it was desired to follow the concentration of plasmalogen and other enol ethers in the course of reactions with colored thiols and thiol acids. For these reasons the iodination procedure has been adapted for potentiometric end-point detection.

The advantages of the potentiometric method are: (a) Higher accuracy and precision especially in colored samples. (b) Smaller samples may be used. (c) Elimination of the two-phase system during titration and therefore elimination of the need for vigorous agitation by nitrogen bubbling which we have found causes iodine losses.

Fresh rat tissues and beef adrenals obtained directly from the slaughter house (trimmed and dissected into cortex and medulla at 0–4°) were extracted immediately by the method of FOLCH, LEES AND SLOANE-STANLEY[2]. These washed total-lipid extracts were taken to dryness *in vacuo* at 40°, redissolved in $CHCl_3$–MeOH (2 : 1, v/v), filtered and made up to volume to contain 1–3 μmoles plasmalogen/ml. To 1.00 ml of this solution in a 10-ml beaker is added 1.00 ml 0.01 *N* aq. I_2. This is mixed by swirling for 30 sec at room temperature and then diluted with 3.0 ml 95 % ethanol. The micro platinum and calomel electrodes of a standard line-operated pH meter (Beckman model H-2 was used in this study) are immersed in the solution and titration of the excess I_2 is carried out rapidly with 0.1 *N* $Na_2S_2O_3$ supplied from a 0.1-ml ultramicroburet. Good agitation by a magnetic stirrer is essential.

Beginning at about 0.01 ml before the end point the titrant is added in 0.001 ml increments and the e.m.f. is recorded after each increment. The end point is determined graphically in the usual way from the S-shaped titration curve, the end point occurring at about 150 mV. In these very dilute I_2 solutions the visual end point occurs approximately 10 % before the true end point determined potentiometrically. The potentiometric end point is determined to 0.0001 ml and the absolute precision of the method is $\pm$ 0.0002 ml; therefore, 0.5 μmole can be determined with a precision of $\pm$ 2 % and 3 μmoles with a precision of $\pm$ 0.3 %. These values vary from one tissue to another. For all tissues studied (see Table I) with varying plasmalogen concentrations both in the tissue and the final lipid solution, the average precision was $\pm$ 1.3 %; however, plasmalogen in a sheep-brain extract was determined at the 1 μmole level with a precision of $\pm$ 0.3 %.

All tissues studied were also analyzed for long-chain fatty aldehydes by the *p*-nitrophenylhydrazone colorimetric procedure[3]. The average value for all tissues, exclusive of rat liver, of the molar ratio of I_2 to *p*-nitrophenylhydrazone is 0.96. This is compared to the findings of RAPPORT AND LERNER[1] who obtained 1.08 for this ratio.

TABLE I

PLASMALOGEN CONTENT OF RAT TISSUES AND BEEF ADRENALS

The water content was obtained by drying 500 mg tissue samples for 20 h at 70° *in vacuo*. Lipid weights were obtained by drying aliquots of the reconstituted $CHCl_3$–MeOH extract under the same conditions. Lipid P was estimated by the method of FISKE AND SUBBAROW[4]. The plasmalogen values, reported as μmole/g dry wt., are averages of at least 4 determinations except in the case of the *p*-nitrophenylhydrazone values for beef adrenals where only two values were averaged. The plasmalogen as per cent of total lipid was calculated assuming 750 for the molecular weight of plasmalogen.

Tissue	*No. of animals*	*Water %*	*Lipid % dry wt.*	*Lipid P μmole/g dry wt.*	*Plasmalogen (I_2 titration) μmole/g dry wt.*	*Plasmalogen (as p-nitrophenylhydrazone)*	*Plasmalogen % of total lipid*	*μmole I_2 / μmole hydrazone*
Rat brain	7	78.7	40.0		55.2 ± 0.6	52.8 ± 0.9	10.3	1.05
Rat brain	10	78.8	34.8		48.9 ± 0.8	48.9 ± 2.0	10.6	1.00
Rat brain	10	78.9	34.8		51.9 ± 0.9	48.3 ± 2.7	11.2	1.07
Rat heart	8	79.1	18.0		11.7 ± 0.2	12.4 ± 0.05	4.9	0.94
Rat heart	10	78.0	16.0		12.2 ± 0.1	13.8 ± 0.3	5.8	0.88
Rat liver	2	68.8	16.7		2.13 ± 0.02	2.96 ± 0.10	0.96	0.72
Rat liver	5	69.4	15.0		2.19 ± 0.07	3.13 ± 0.20	1.1	0.70
Rat lung	8	80.0	20.0		14.4 ± 0.1	17.4 ± 1.4	5.4	0.83
Rat lung	10	80.2	17.9		15.3 ± 0.1	15.0 ± 1.9	6.4	1.02
Rat lung	10	79.5	19.0		17.1 ± 0.5	16.2 ± 0.6	6.8	1.06
Rat kidney	8	78.1	27.1		12.2 ± 0.15	13.0 ± 0.3	3.4	0.94
Rat kidney	10	77.1	23.5		11.0 ± 0.2	10.5 ± 1.8	3.5	1.04
Rat kidney	10	77.6	31.1		12.5 ± 0.5	14.2 ± 0.8	3.0	0.88
Rat skeletal muscle	4	76.0	17.5		5.28 ± 0.05	5.9 ± 0.3	2.3	0.90
Rat skeletal muscle	4	75.5	17.3		5.36 ± 0.13	6.17 ± 0.16	2.3	0.87
Beef adrenal cortex	3	78.2	20.4	178	22.3 ± 0.5	23.3	8.2	0.96
Beef adrenal medulla	6	79.1	14.9	141	25.4 ± 1.2	24.5	12.8	1.04
Beef whole adrenal	1	78.2	21.4	195	25.2 ± 0.9	27.5	8.8	0.92

The discrepancy between these two ratios may be partially accounted for by the general differences in technique employed in the two iodination methods.

The large reproducible difference between the two analytical methods for plasmalogen in rat liver is unexplained. RAPPORT AND LERNER also found that rat liver gave lower molar ratios of iodine to *p*-nitrophenyl-hydrazone than the other rat tissues studied. We believe this observation has significance in regard to plasmalogen metabolism and is under further investigation.

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Thermal denaturation of deoxyribosenucleic acid isolated from a thermophile

In the course of an investigation of the thermal stability of highly polymerized deoxyribosenucleic acids, isolated from various microorganisms, it was found that the denaturation of the DNA was related to the overall mole percent composition of guanine plus cytosine in the sample[1]. It was thought of interest to study the heat denaturation of polymerized DNA isolated from a thermophile grown at an elevated temperature. *Bacillus stearothermophilus* was grown in the basal medium of BAKER *et al.*[2] supplemented with 0.5 % acid-hydrolyzed casein in shallow players at 55° for 16 h (cells mostly in the vegetative state). The cells were harvested, washed, lyophilized and send to us by Dr. BAKER[2]. The guanine plus cytosine content of the DNA of *B. stearothermophilus* has been found to average 46.7 %[2,3]. The denaturation of *Escherichia coli* (K-12) DNA, which has a base composition (50 % guanine plus cytosine) similar to that of *B. stearothermophilus*, was studied simultaneously for comparison. DNA was isolated from both organisms by first disrupting the vegetative cells with sodium lauryl sulfate followed by deproteinization with chloroform and isoamyl alcohol*. The sedimentation coefficient, $S_{20,w}$, measured at infinite dilution was found to be 18.3 for *B. stearothermophilus* and 33.4 for *E. coli*. Thermal denaturation of the DNA was studied by determining the absorbance–temperature profile n a thermostated Beckman Spectrophotometer Model DU at 260 mμ. DNA dissolved in 0.15 *M* NaCl plus 0.015 *M* sodium citrate, pH 7, was placed in glass-stoppered

Abbreviation: DNA, deoxyribosenucleic acid.
* A detailed description of this method will be published shortly.