ELECTRON MICROPROBE MEASUREMENTS OF INTRACELLULAR HYDRATION IN FREEZE-DRIED, PLASTIC-EMBEDDED BIOLOGICAL SOFT TISSUE
F. Ingram, M. Ingram

To cite this version:
F. Ingram, M. Ingram. ELECTRON MICROPROBE MEASUREMENTS OF INTRACELLULAR HYDRATION IN FREEZE-DRIED, PLASTIC-EMBEDDED BIOLOGICAL SOFT TISSUE. Journal de Physique Colloques, 1984, 45 (C2), pp.C2-457-C2-460. <10.1051/jphyscol:19842103>. <jpa-00223770>

HAL Id: jpa-00223770
https://hal.archives-ouvertes.fr/jpa-00223770
Submitted on 1 Jan 1984

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Résumé: Après incorporation d'une dibromoacetophenone dans la résine utilisée pour enrober un tissu congelé et séché, le brome contenu dans la résine sert à mesurer la teneur en eau du tissu. La validité de cette méthode est évaluée par comparaison de mesures faites sur le muscle gastrocnemius de rats dans 4 états d'hydratation, à l'aide de la sonde électronique et par chimie conventionnelle. Les deux séries de mesures ne diffèrent que de 3%.

Abstract: Dibromoacetophenone was complexed into the resin used for embedding freeze-dried tissue. Dilution of embedding plastic by tissue solids was measured with the Br Lα signal in the electron microprobe to provide a measure of local water content. This method of measuring water was evaluated by comparing electron microprobe measurements on samples of gastrocnemius from rats established in four different states of hydration with conventional wet chemistry measurements on companion samples. The two sets of measurements agreed to within 3%.

Introduction

Embedded samples provide several advantages for electron probe microanalysis (EPA) of biological soft tissue. Provided proper technique and materials have been used to insure faithful preservation of morphology and intracellular electrolyte distributions, a relatively rugged, versatile sample is produced with capability for accurate quantification of intracellular electrolytes. We have expanded this capability to permit estimation of tissue water simultaneously with electrolyte measurements.

When an element commonly not associated with biological material is complexed into the plastic used for embedding freeze-dried tissue, the characteristic x-ray intensity for that element is reduced in regions of the plastic block that contain tissue. Provided tissue water has been replaced faithfully by embedding medium, a direct relationship will exist between signal intensity and dilution of the embedding plastic by tissue solids.

Br is an excellent choice for the estimation of tissue water in this manner. The embedding medium is tagged with Br by incorporating 40 mmol kg⁻¹ dibromoacetophenone into EPON 826 monomer. Tissue collection, freeze-drying and osmium vapor fixation are performed in standard fashion, with the initial drying temperature near -80°C and completion of drying at +50°C.

The relationship between the Br characteristic x-ray signal and tissue solids must be established with appropriate standards, because all common methods of tissue dehydration result in a shrinkage of...
all common methods of tissue dehydration result in a shrinkage of tissue volume (1-2). With freeze-dried tissue, the shrinkage appears to be approximately 20% by volume. Calibration of the electron microprobe for tissue water was accomplished by processing and analyzing a set of six albumin solutions with tissue solids fractions (range, 10.7% to 37.7% by weight) in similar manner to tissue (3). A straight line fitted to the data had a slope of 1.027 ± 0.069 with intercept 0.057 ± 0.047 and regression coefficient of 0.991.

The accuracy of the measurement of tissue water with the Br dilution technique was assessed by examining tissue that also could be measured by conventional techniques. For this purpose, skeletal muscle from four different groups of rats was analyzed with the electron microprobe and companion tissue from each animal was analyzed using conventional wet chemistry techniques.

Materials and Methods

Four different states of hydration were established in rats by altering the tissue water levels in subsets of animals from each of two different age groups. Six male Sprague-Dawley rats, approximately 35 days old, and 4 male rats approximately 50 days old, were dehydrated with diarrhea. Samples of gastrocnemius for electron probe microanalysis were collected from each of the four different sets of anesthetized rats with a cold clamp. The contralateral gastrocnemius also was collected into weighed vials for water determination by gravimetric means. The dried tissues then were ground to a fine powder and electrolytes were extracted from the dried tissue with 0.75 N HNO₃ for conventional analysis with flame photometer and Buchler-Cotlove chloridometer.

Tissue frozen with the cold clamp was cut free from the animal and immersed into liquid nitrogen while firmly held in the clamp’s copper jaws. Under liquid nitrogen, fragments were chipped from the frozen surfaces and transferred into one of the 12 sample holders in the freeze-dry apparatus. Frozen tissue was freeze-dried at a temperature near -80°C for two days, slowly warmed to -40°C, and held for a final day at +50°C. The freeze-dried samples were fixed with osmic acid vapor and vacuum embedded in brominated EPON 826 without exposure to the atmosphere.

Blocks of embedded tissue were cut to expose the muscle fibers in cross section. Cut blocks were mounted in brass sample holders with the cut surface oriented perpendicular to the electron beam. A fragment of quartz and a crystal each of NaCl, KCl, KBr and PbS were mounted on each sample holder, and the ensemble was overcoated with approximately 20 nm carbon in a vacuum evaporator. Electron probe microanalysis was conducted with a focused, 50 nA, 10 keV electron beam in a fully automated, four spectrometer, Applied Research Laboratories, model SEMQ, electron probe microanalyzer. Data were collected as line-scan profiles and the average signal across a cell was integrated and used to represent the average concentration in a given fiber. Twenty to 22 fibers from each animals were analyzed for K Kα, Cl Kα, and S Kα with pentaerythritol (PET) diffraction crystals, and for Na Kα and Br Lα with thallium acid phthalate (TAP) diffraction crystals. Continuum from the 4.2 to 7.2 keV region of the energy spectrum was monitored simultaneously with data collection using a KEVEX Si(Li) energy detector system (4). Background for each data line was calibrated against the continuum signal to provide a number to represent background that was collected simultaneously with on-peak data at each location where data were collected. The region below 2 keV in the energy spectrum was blocked with an absorber mounted over the Si(Li) energy detector.
Electron microprobe signals were calibrated by reference to counting rates on albumin standards through pure crystal secondary standards (5). The same tissue sample preparation and electron microprobe operating parameters were used for tissue analysis as had been used for calibration.

Portions of each sample block destined for analysis were "stabilized" with a rastering 150 µm diameter, 200 nA, 10 keV electron beam scanning with a 600X magnification. This permitted stable x-ray count rates with little apparent sample damage from electron beam bombardment during subsequent analysis.

Results

Conventional wet chemistry measurements on whole muscles must be corrected for extracellular fluid and extracellular solids to provide estimations of intracellular electrolyte and water concentrations. It is common to assume that intracellular [Cl] is nearly zero and use tissue [Cl] to estimate extracellular volume. The electron microprobe measurements confirmed that [Cl] was indeed nearly zero in the tissue analyzed for this study. Extracellular electrolyte concentrations are derived from plasma values. The relationships recommended by Cheek et al. were used to correct for plasma solids and the Donnan factor for ions distributed across capillary walls (5):

\[ [\text{Na}]_e = \frac{[\text{Na}]_p}{W_p} \times 0.95 \]

and

\[ [\text{Cl}]_e = \frac{[\text{Cl}]_p}{W_p} \times 1 \]

where \([\text{Na}]_p\) and \([\text{Cl}]_p\) are plasma concentrations, \([\text{Na}]_e\) and \([\text{Cl}]_e\) are extracellular concentrations, and \(W_p\) is plasma water fraction.

The following table presents both the electron microprobe results and the estimation of intracellular [K] and water from conventional measurements using CI space to correct for extracellular volume:

<table>
<thead>
<tr>
<th>EPA</th>
<th>35-Day Rats</th>
<th>50-Day Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (n=4)</td>
<td>Dehydrated (n=6)</td>
</tr>
<tr>
<td>[K] (mM)</td>
<td>115 ± 9*</td>
<td>117 ± 16</td>
</tr>
<tr>
<td>[Cl] (mM)</td>
<td>4.1 ± 2.1</td>
<td>4.1 ± 2.0</td>
</tr>
<tr>
<td>[Na] (mM)</td>
<td>7.5 ± 4.7</td>
<td>4.5 ± 2.5</td>
</tr>
<tr>
<td>[S] (mM)</td>
<td>128 ± 7</td>
<td>144 ± 7</td>
</tr>
<tr>
<td>(H_2O) (fraction)</td>
<td>.811 ± .019</td>
<td>.784 ± .020</td>
</tr>
</tbody>
</table>

Conventional Wet Chemistry

<table>
<thead>
<tr>
<th>EPA</th>
<th>35-Day Rats</th>
<th>50-Day Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (n=4)</td>
<td>Dehydrated (n=6)</td>
</tr>
<tr>
<td>[K] (mM)</td>
<td>99 ± 6.4</td>
<td>107 ± 6.4</td>
</tr>
<tr>
<td>(H_2O) (fraction)</td>
<td>.783 ± .008</td>
<td>.767 ± .007</td>
</tr>
</tbody>
</table>

*Mean ± S.D.
Discussion

There is excellent agreement between the electron microprobe measurements of electrolytes and those obtained with conventional wet chemistry on companion tissue. There also is good agreement in the determination of tissue water using the two methods. The electron microprobe values of tissue water average approximately 3% higher than those obtained by conventional gravimetric methods. While this difference is within the limits of uncertainty of the calibration of the electron microprobe and may appear to be a systematic error, the discrepancy may result from basic differences in the two methods for the determination of tissue water content. Maintaining the sample for one day at +50 °C in a vacuum after freeze-drying may not remove the same amount of tissue water as is removed when tissue is dried in an oven to constant weight at 105°C. Alternatively, the discrepancies also may result from assumptions made in the computation of intracellular water from the gravimetric measurements. In the latter case, a correct computation is possible only if the contribution made by extracellular solids to total tissue solids is known. No correction for extracellular solids was included with the gravimetric measurements presented here. Because they are more direct, the electron microprobe determination of tissue water may be more accurate than conventional estimations.

The primary assumptions made with this technique are: 1) all tissue water is removed by freeze-drying; 2) embedding plastic faithfully replaces tissue water; 3) distortions, such as shrinkage, that accompany tissue preparation, are accurately reflected in fabricated albumin standards, and are the same from one type tissue to another; and 4) the reduction of Br x-ray signal results primarily from dilution of plastic by tissue solids and not counting losses from matrix effects. The work presented here and the calibration data presented earlier (3) suggest that these are reasonable assumptions and that the electron microprobe is capable of accurate measurement of tissue water.

Acknowledgments

This work is a publication of the USDA/ARS Children’s Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children’s Hospital, Houston, Texas.

References