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*Session II. LAMMA Applications in Biomedical Microprobe Analysis*

# Selective Accumulation of $Li^+$, $Na^+$, $K^+$, $Rb^+$, and $Cs^+$ at Protein Sites of Freeze-Dried Embedded Muscle Detected by LAMMA

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**Nachweis der selektiven Anreicherung von $Li^+$, $Na^+$, $K^+$, $Rb^+$ und $Cs^+$ an Protein gefriergetrockneter, eingebetteter Muskeln mit Hilfe von LAMMA**

**Key words:** Nachw. von Alkaliionen an Protein, Biolog. Material; Massenspektrometrie, Lasermikrosonde

## Introduction

The question whether $K^+$, the main cellular cation, is homogeneously distributed in the striated muscle cell or if it is preferentially localized at certain proteins – as predicted by the association-induction hypothesis (Ling [9]) – has been investigated during the last 5 years by several independent techniques. Results consistently showed the following: The alkali-metal ions $K^+$, $Rb^+$, $Cs^+$ but also $Tl^+$, which all are accumulated in living muscle cells by most probably the same mechanism (Ling [10]), are preferentially localized within the myosin-rich *A* band as well as in the *Z* lines. The ions appear to be accumulated at the same sites where uranyl and lead ions as employed in conventional EM staining techniques are bound. The results support the idea that such ions are bound to free $\beta$- and $\gamma$-carboxyl groups of aspartic and glutamic acid residues of the cellular proteins (Edelmann [1], Ling [11], Edelmann [4]).

At first sight this conclusion seems to contradict earlier experiments which showed that isolated actomyosin and muscle homogenates do not bind a significant amount of alkali-metal ions (Szent-Györgyi [12], Erdös [7], Lewis and Saroff [8], Szentkuti and Giese [13]). It may be doubted however, if the chemical and physical properties of proteins are identical in either living cell or after isolation. It appears well conceivable that ion binding capability is lost during the isolation procedures. In order to investigate this possibility a new kind of *in vitro* experiments was designed: Sections of freeze-dried and plastic embedded frog muscle fibres were exposed to aqueous solutions of alkali-metal salts. Using energy dispersive X-ray microanalysis and LAMMA it was found that these sections showed preferential accumulation of $K^+$ and $Cs^+$ over $Na^+$ on specific protein sites in the A bands (Edelmann [5]).

In this presentation I shall demonstrate the highly reproducible selectivity of ion accumulation in muscle sections after exposure to solutions containing the alkali-metal ions $Li^+$, $Na^+$, $K^+$, $Rb^+$ and $Cs^+$.

## Methods

Freeze-drying and plastic embedding of frog sartorius muscle was carried out as described in earlier papers (Edelmann [2, 3]). Sections 0.2 µm thick were wet cut with a diamond knife and collected on Formvar-coated grids. The sections were exposed to aqueous solutions containing LiCl, NaCl, KCl, RbCl, and CsCl (pH adjusted to 7.0 with TRIS buffer) in the following manner: Grids bearing the sections were floated face down onto droplets of the solution for 5 min. The grids were picked up with a pair of forceps and vigorously moved through the air to remove the adhering fluid by centrifugal force. This method yielded sections with larger areas virtually free of remaining salt crystals when observed under the TEM. Elemental analysis was carried out by means of a LAMMA 500 instrument. Gelatin standards were prepared as described by Roomans and Sevéus (1977).

## Results

Electron micrographs of sections exposed to solutions containing (mM) $Li^+50$, $Na^+50$, $K^+10$, $Rb^+10$, and $Cs^+10$ are shown in Fig. 1. The holes in these sections are generated by the focused laser beam of a LAMMA 500 instrument. Mass spectra of the respective evaporated material are given in Fig. 2 together with the mass spectrum obtained from a 0.3 µm thick section of a gelatin standard. The alkali-metal ion concentration in the standard were the same as in the solution to which

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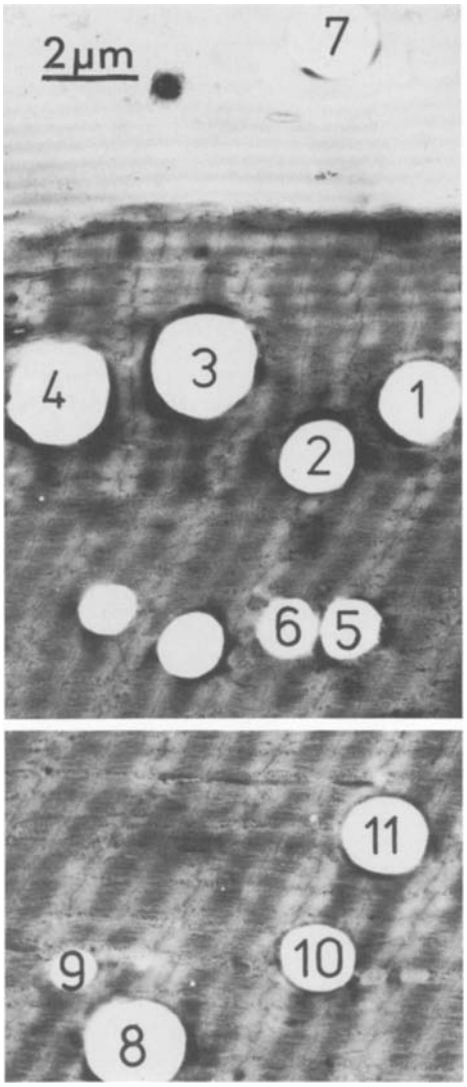


**Fig. 1.** Perforated sections of frog sartorius muscle

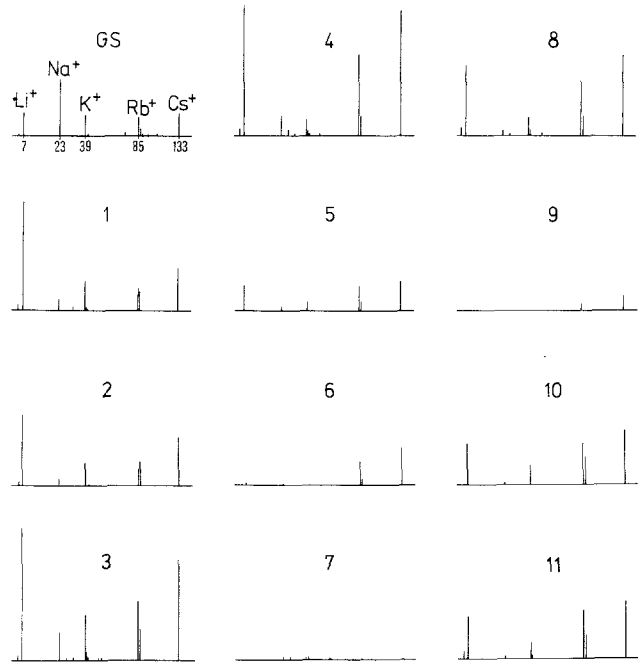


**Fig. 2.** LAMMA-spectra recorded from a muscle specimen. The numbers correspond to the respective probing areas shown in Fig. 1. The spectrum labelled GS stems from a gelatin standard containing (mM) $Li^+50$, $Na^+50$, $K^+10$, $Rb^+10$ and $Cs^+10$

the muscle sections had been exposed. Ion selectivities can be deduced by comparing the mass spectra of the specimen with that of the relevant standard: The following order of selective ion uptake was found

$Li^+ > Cs^+ > Rb^+ > K^+ > Na^+$ (spectra *1*, *2*, *3*, *4*) or
$Cs^+ > Li^+ > Rb^+ > K^+ > Na^+$ (spectra *5*, *8*, *10*, *11*).

Spectra *6* and *9* do not show prominent $Li^+$ peaks. Spectrum *7* is obtained from the embedding medium indicating that only minute amounts of alkali-metal ions were present in or at the resin material.

## Discussion

### *Staining*

The present investigations show that alkali-metal ions can be used to stain sections of freeze-dried and embedded biological material. Staining of muscle sections is reproducible and the staining pattern is similar to conventional staining patterns. The staining of the muscle sections is due to the fact that under the conditions described large amounts of the electron dense $Rb^+$ and $Cs^+$ ions are accumulated at those sites which bind uranium and lead in conventional staining techniques (Edelmann [5, 6]).

By comparing the staining intensity of sections and/or the accumulated amount of alkali-metal ions in sections of varying thickness (so far up to 0.3 µm) it is evident that the ions can reach the protein binding sites inside the section: ion accumulation is proportional to the section thickness.

### *Selective Accumulation*

The accumulation of the alkali-metal ions at the muscle protein sites of the sections occurs with a characteristic and reproducible selectivity as shown by the LAMMA technique. Most important is the observation reported earlier that the ion selectivity for 2 or 3 ion species may be changed by addition of a further ion species (Edelmann [5]). This phenomenon is now under further investigation. Under the conditions described the order of selectivity is (with one exception) such that the less hydrated heavy ions are preferred over the more hydrated light ions: $Cs^+ > Rb^+ > K^+ > Na^+$. $Li^+$

however, with a mass number of *7* and the largest hydration is also accumulated to a large extent. According to calculations and considerations presented in the association-induction hypothesis it is conceivable that a relatively strong binding of $Li^+$ at certain binding sites diminishes the electronegativity of other sites by an inductive effect. Then, due to the weak influence of these sites on their environment the cations which are captured with the highest probability are those with the smallest hydration (for details see Ling [9], Part III).

The spectra *6* and *9* of Fig. 2 show no $Li^+$ peaks. These spectra were obtained from areas near previously shot holes (see Fig. 1). It has to be checked if the loss of $Li^+$ is eventually due to ion movements induced in the immediate neighbourhood of a probe area of interest. In this conncetion it is noteworthy that irradiation of a section with an electron beam and subsequent exposure to normal air humidity causes considerable ion movements.

The results reported here demonstrate that the LAMMA technique is especially well suited to investigate the accumulation of alkali-metal ions in sections of freeze-dried and plastic embedded biological material: all alkali-metal ions can be detected simultaneously with a high sensitivity. This opens new access to systematic *in vitro* studies of selective ion binding to cellular proteins.

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## References

1. Edelmann. L.: Physiol. Chem. Phys. **9**, 313–317 (1977)
2. Edelmann, L.: J. Microsc. (Oxford) **112**, 243–248 (1978)
3. Edelmann, L.: Mikroskopie **35**, 31–36 (1979)
4. Edelmann, L.: Histochemistry **67**, 233–242 (1980)
5. Edelmann, L.: Physiol. Chem. Phys. **12**, 509–515 (1980)
6. Edelmann, L.: Electron microscopic demonstration of potassium binding sites in muscle. In: International cell biology 1980–1981, ed. H. G. Schweiger, pp. 941–948. Berlin, Heidelberg, New York: Springer
7. Erdös, R.: Acta Physiol. Hung. **1**, 33–38 (1946)
8. Lewis, M. S., Saroff, H. A.: J. Am. Chem. Soc. **79**, 2112–2117 (1957)
9. Ling, G. N.: A physical theory of the living state: The association-induction hypothesis. Waltham: Blaisdell 1962
10. Ling, G. N.: Physiol. Chem. Phys. **9**, 217–222 (1977)
11. Ling, G. N.: Physiol. Chem. Phys. **9**, 319–327 (1977)
12. Szent-Györgyi, A.: Acta Physiol. Scand. **8**, Suppl. 25 (1945)
13. Szentkuti, L., Giese, W.: Health Physics **26**, 343–347 (1974)

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