Scanning Microscopy

Volume 3 | Number 4 Article 23

12-21-1989

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Scanning Microscopy, Vol. 3, No. 4, 1989 (Pages 1231-1239)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0891-7035/89$3.00+.00

BINDING OF IONS TO NUCLEAR CHROMATIN

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(Received for publication May 01, 1989, and in revised form December 21, 1989)

Abstract

Ion concentrations in isolated lymphocyte nuclei subjected to KCl or $MgCl_2$ media of varying ionic strength were measured by X-ray microanalysis. Values were corrected for the contribution of free ions by estimating the volume fraction of the water space morphometrically. The amount of bound cations and Cl was constant and independent of the widely varying free ion concentration.

It is concluded that the mechanism of binding is counterion condensation but with limited cooperativity. In contrast to classical counterion condensation theory, the binding of ions occurs at oppositely charged clusters at the surface of the chromatin. Therefore, both cations and anions are bound and binding cannot be completely delocalized.

The bound ions stabilize the basic chromatin fibre but are not involved in the regulation of the transition between the condensed and decondensed state.

Using earlier data, we estimated the concentration of free cations in rat liver nuclei under in-vivo conditions to be in the order of about 80 mM.

Key words: Chromatin, nuclei, ion binding, counterion condensation, polyelectrolytes.

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Introduction

There is increasing experimental evidence for the binding of a large fraction of monovalent ions to cellular polyelectrolytes. The efflux of potassium from permeabilized cells is grossly restricted (Ling 1984, Kellermayer et al. 1986, Hazlewood and Kellermayer 1988, Cameron et al. 1988). The distribution of K parallels that of cellular proteins in myofibrils (Edelmann 1984, von Zglinicki 1988) and in rat liver nuclei (von Zglinicki and Bimmler 1987). Similar results have been obtained for the chloride anion (Negendank 1984, von Zglinicki and Bimmler 1987, Cameron et al. 1988, von Zglinicki 1988). Moreover, there is evidence that the chemical activity of the ions in nuclei is considerably less than 120 mM (Cameron 1985).

The conclusion is that, at least at physiological ionic strength, both cations and anions are electrostatically bound to the cellular polyelectrolytes. The total amount of ions in a given compartment - the A band in the myocyte, for instance - differs considerably from that in the adjacent I band. Because of the electrostatic shielding of fixed charges the distribution of ions is not according to that predicted by net charge Donnan equilibria. On the contrary, compartments with a higher density of polyelectrolytes contain, in general, both more cations and anions (von Zglinicki and Bimmler 1987, von Zglinicki 1988).

We were interested to learn more about the mode of binding and its consequences for cellular regulation in a physiologically relevant system. Polyelectrolyte theories offer two possible modes of electrostatic binding of small ions to fixed charges: 1. Binding might be non-cooperative and governed by the law of mass action (Katchalski 1971) as in association reactions of oppositely charged small ions. In that case the bound fraction is proportional to the bulk ion concentration. 2. On the other hand,

binding might be cooperative (counterion condensation theory; Manning 1978) which means that a constant fraction of the polyelectrolyte charges is neutralized by bound ions widely independent on the bulk ion concentration.

To discriminate between these possibilities we decided to measure ion concentrations by X-ray microanalysis in isolated lymphocyte nuclei in media with widely varying ionic strength. The free permeability of the nuclear membrane for small ions is well documented (Palmer and Civan 1977), so this model allows free control of the ionic composition of the solution bathing the chromatin fibers. Moreover, nuclei respond to increasing ionic strength with a transition from a homogeneous mixture of condensed and decondensed chromatin regions together with a volume decrease. These changes are known to occur at less than about 1 mM divalent or 75 mM monovalent cations.

Therefore, by comparing ion concentrations measured in homogeneous, condensed, and decondensed chromatin, one can learn more about the regulative importance of ion binding.

Materials and Methods

Human lymphocyte nuclei were isolated as described elsewhere (Ziervogel 1989). Briefly, nuclei were isolated after homogenization of the cells in 0.25 M saccharose, 3 mM $MgCl_2$, 0.2% Triton X-100 (SERVA) by centrifugation through 2.2 M saccharose. They were resuspended in a solution containing 0.25 M saccharose, pH 7.4, and different amounts of either KCl or $MgCl_2$. Nuclei were washed twice in this final solution.

For the estimation of nuclear volumes, the diameter of 100 nuclei each was measured in phase contrast at a magnification of 2400 using an optical microscope.

For X-ray microanalysis, a small pellet was mounted on a silver pin and injected into liquid propane chilled by liquid nitrogen with a velocity of 4.9 m/sec. Cryosections about 200 nm thick were cut at a temperature around 150 K in an LKB Cryonova modified to fit onto an LKB III ultramicrotome. Sections were freeze-dried overnight at a pressure of less than 10^{-4} Torr, carbon coated and examined in a Siemens ELMISKOP 102 equipped with a Kevex 7000 at 80 kV and ambient temperature. Concentrations per dry weight were computed according to the Hall method using aluminum-carbon foils as standards (von Zglinicki 1983). Element concentrations were finally expressed as M(element)/M(phosphorus). Measurements in the low concentration range (C_o = 0.25 mM $MgCl_2$ or $\leq$75 mM KCl) were done with a spot diameter of 2 μm. In the high concentration range regions of condensed and decondensed chromatin could be discriminated and measurements were done with 0.5 μm spot diameter. At least 10 nuclei per group were measured.

For morphometrical estimation of the volume densities of chromatin fibers $V_{v,c}$ and water $V_{v,w}$ the nuclear suspension was diluted 1:1 with 5% glutaraldehyde in 60 mM CCP (dimethylarsinic acid sodium salt) buffer. After 15 min fixation, nuclei were pelleted and further processed for conventional electron microscopy. Ten micrographs per sample were randomly taken at a final magnification of 80000. A square grid with 3 cm spacing was used for estimation of the volume densities by point counting (Weibel 1979).

Our basic assumption was that the nuclear water, at least if more apart from the chromatin fibers than the Debye-Hueckel length, is not structured and that the ion concentrations there are equal to those in the bathing solution. Close to the chromatin the concentration of ions is higher due to electrostatic interactions and the amount of this electrostatically bound fraction of ions will be estimated. It is presupposed that due to eutectic growth of ice crystals all ions dissolved in the nuclear water will be precipitated onto the nuclear polyelectrolytes during freezing. No ions were lost during freeze-drying, therefore.

The concentration of bound ions C_b is then simply the difference between the measured total ion concentration C_m and the concentration C_p of ions precipitated from the bulk water. All concentrations are expressed as concentrations per dry mass of chromatin, that means, C_p is the bulk ion concentration (C_o) times the volume of nuclear water (V_w) divided by the mass of chromatin (M_c). With

$$\rho = \frac{M_c}{V_c} \qquad (1)$$

ρ ... solid density of chromatin
V_c ... volume of chromatin

and

$$V_{v,c} = \frac{V_c}{V_n} \qquad (2a)$$

$$V_{v,w} = \frac{V_w}{V_n} \qquad (2b)$$

V_n ... volume of nuclei

one arrives at:

$$C_b = \frac{C_m * \rho - (V_{v,w} / V_{v,c}) * C_o}{\rho} \qquad (3)$$

with C_b in mmol/kg dry weight

C_m ... total ion concentration in chromatin (mmol/kg dry weight)

C_o ... bulk ion concentration in mM

ρ was taken as 1.33, the mean solid density of biological matter.

Standard errors of the estimates were calculated according to the Gaussian law of error propagation. Results were compared by an analysis of variance and the critical difference between means (CDM) was computed for the 5% significance level.

Results

First we tested the normal behavior of our nuclear preparation by measuring the volume response to mono- and divalent cations (Fig 1). Removal of the nuclear membrane by Triton X-100 does not change this volume response (Fig 1c).

The morphology of the isolated nuclei is shown in Fig 2. The structural transition induced by high salt concentrations is obvious even in the frozen-dried cryosections. From the conventional micrographs (Figs 2c,d) it is evident that only the highest packing level changes, while the basic 20...30 nm chromatin fiber remains unchanged over the whole range of ion concentrations studied.

Table 1 gives the measured ion concentrations in the nuclear chromatin. Displaying concentrations per phosphorus atom eliminates the errors in the background estimation and is the most natural way in this system. Concentrations of ions bound to the chromatin are obtained by subtracting the amount of freely dissolved ions (see Materials and Methods). This correction can be neglected in the $MgCl_2$ case because of the small bulk ion concentration.

In the KCl solutions, the volume fraction of chromatin was estimated by morphometry of conventional micrographs (Fig 3). The volume density of water $V_{v,w}$ is then $V_{v,w} = 1 - V_{v,c}$. It was found that even extremely swollen nuclei at the lowest ionic strength do not change their volume during fixation if a fixative with high glutaraldehyde and low buffer concentration was used (Fig 4).

Concentrations of ions bound to the chromatin in KCl and $MgCl_2$ solution are given in Fig 5. There are no significant differences between ion concentrations in condensed and decondensed regions. Moreover, the slopes of all regression lines are not significantly different from zero. Therefore, it has to be estimated that we have 1.01 ± 0.06 potassium ions and 0.39 ± 0.04 chloride ions bound per phosphorus atom in the KCl solutions and 0.19 ± 0.02 magnesium ions plus 0.05 ± 0.01 chloride ions bound per phosphorus atom in the $MgCl_2$ solutions independent of the salt concentration.

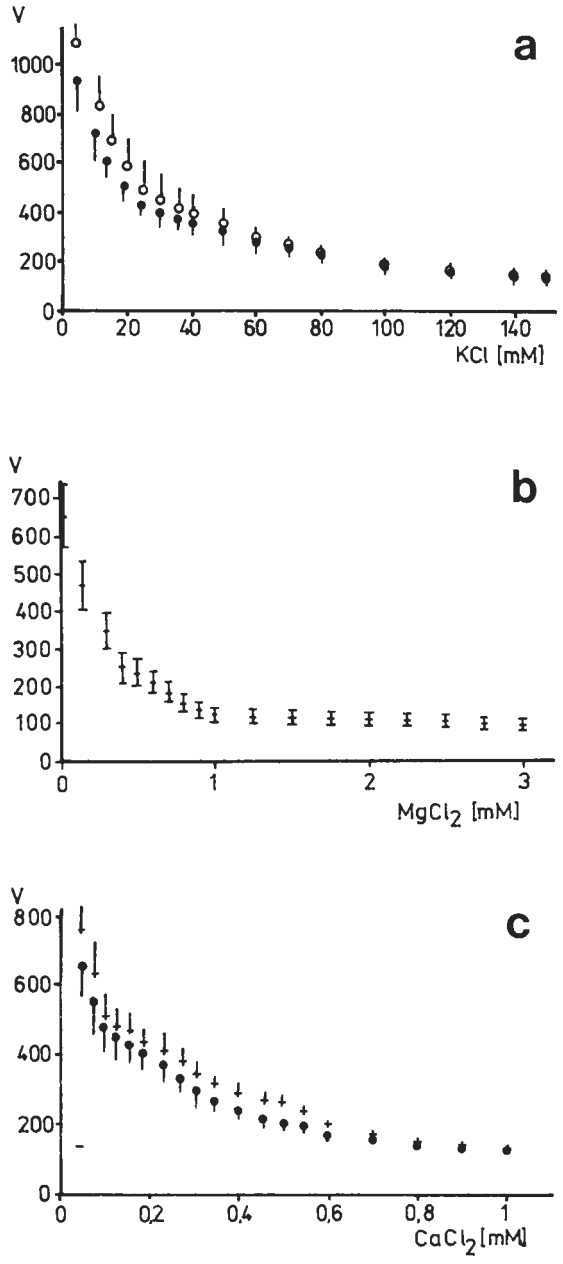

Fig 1: Volume V (in μm^3) of isolated lymphocyte nuclei (mean ± SEM) in response to the ion concentration in the bathing medium. a) nuclear volume vs KCl concentration. The solutions contain 5 mM Tris, pH 7.4. For the lower curve, the osmolarity of the bathing solution was adjusted to 290 mOsm using saccharose. b) Nuclear volume vs $MgCl_2$ concentration. Solutions contain 250 mM saccharose, 5 mM Tris, pH 7.4. c) Nuclear volume vs $CaCl_2$ concentration. Composition of solutions as in b). For the lower curve, the nuclear membranes were permeabilized by adding 1% Triton X-100.

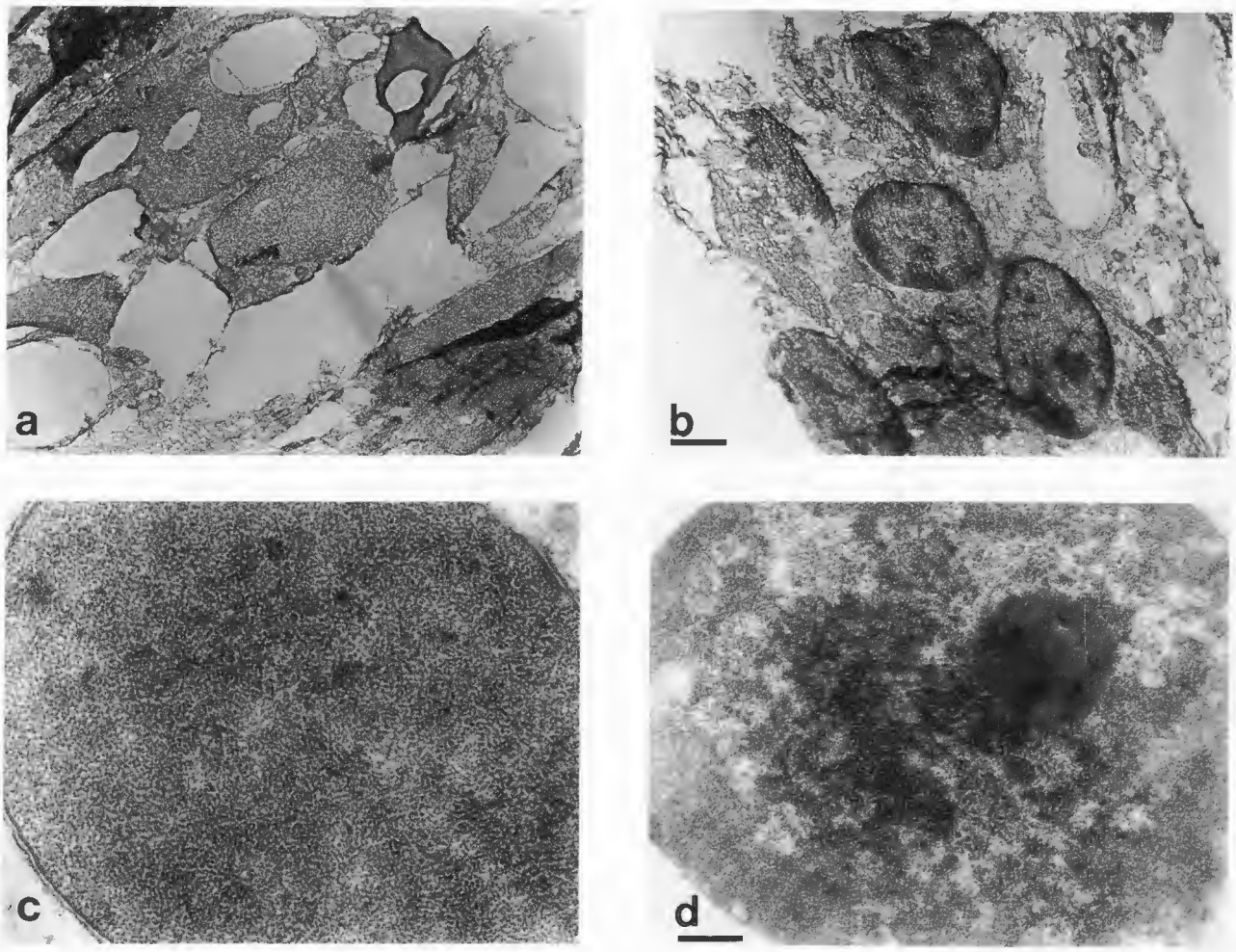

Fig 2: Morphology of isolated lymphocyte nuclei. a) and b): frozen-dried cryosections; Bar = 2 μm. c) and d): conventional electron micrographs; bar = 0.5 μm. a) and c): C_o = 7.5 mM KCl; b) and d): C_o = 150 mM KCl.

Discussion

Our results indicate that the nuclear membrane is in fact freely permeable to small ions. The nuclear volume, which is very sensitive to the ionic strength of the medium, does not change significantly by removing the nuclear membrane (Fig 1c). therefore, the ion concentration in the water spaces sufficiently apart from the chromatin fibers should be the same as in the bathing solution. The ions in close association to the chromatin fibers display higher concentrations, and all these ions are operationally taken as bound. As a first order approximation, these will be those ions not further apart from the chromatin surface than the Debye-Hueckel length which amounts to less than 4 nm even in the most diluted KCl solution.

This value is also small enough to be negligible in the morphometrical estimation of the water space in nuclei. It will be overcompensated by overprojection of the chromatin fibers in the 40-50 nm thick sections. Moreover, even if we exclude volume changes during the first fixation step (see Fig 4), there are probably volumetric changes during the successive processing steps. Therefore, the volume densities measured should be taken as a very first approximation only. However, even rather large changes in the volume fraction of nuclear water would not disturb the principal result of this investigation, namely the independence of the bound ion fraction on the bulk concentration.

This result is in full accordance with the predictions of the counterion condensation theory. Binding of ions to the nuclear chromatin must be an essentially cooperative phenomenon, therefore.

However, there are also important

Table 1

Measured ion concentrations (mean ± SEM, ions/phosphorus) in nuclei subjected to KCl or $MgCl_2$ solutions of varying ionic strength. In 75 mM KCl, both homogeneous and transformed nuclei were found. n ...number of measurements.

KCL solution:

C_o (mM)	State of cromatin	K/P	Cl/P	n
7.5	homogeneous	1.38 ± 0.11	0.47 ± 0.08	15
25	homogeneous	1.02 ± 0.13	0.49 ± 0.10	10
75	homogeneous	0.88 ± 0.18	0.52 ± 0.10	10
75	decondensed	1.11 ± 0.30	0.58 ± 0.16	10
75	condensed	0.74 ± 0.06	0.38 ± 0.05	10
150	decondensed	1.59 ± 0.34	0.77 ± 0.13	14
150	condensed	1.03 ± 0.13	0.42 ± 0.06	14

$MgCl_2$ solution:

C_o (mM)	State of cromatin	Mg/P	Cl/P	n
0.25	homogeneous	0.17 ± 0.04	0.09 ± 0.02	10
4.0	decondensed	0.20 ± 0.02	0.06 ± 0.01	16
4.0	condensed	0.20 ± 0.03	0.03 ± 0.01	16

Table 2

Concentration of Mg and (Na + K) (in ions/phosphorus) and of P (in mmol/kg dry weight) as measured in rat liver nuclei under in-vivo conditions. Values (mean ± SEM) were recalculated from von Zglinicki and Bimmler (1987). n ... number of measurements. No significant differences were found for the Mg concentrations in condensed and decondensed chromatin. Data were pooled together, therefore.

State of chromatin	Mg/P	(Na + K)/P	P (mmol/kg dry weight)	n
decondensed	0.09 ± 0.04	1.40 ± 0.11	357 ± 31	24
condensed		0.71 ± 0.04	697 ± 57	24

differences from the classical counterion condensation theory (Manning 1978): In this theory, the polyelectrolyte is modelled by a linear chain of univalent fixed charges of spacing b, b being small against the Debye-Hueckel length. Therefore, only univalent counterions can be bound. Contrary to this, we found experimentally cooperative binding of both cations and anions to the chromatin. That means the appropriate model of chromatin consists of clusters of net positive and net negative charges interspersed in such a way that the spacing between charges within every cluster is smaller than the Debye-Hueckel length, but the spacing between charge centers of clusters is larger.

In other words, the proposed mechanism of ion binding to chromatin is condensation of counterions to charge clusters of both signs with the cooperativity limited to the single cluster.

That means ion binding to the chromatin cannot be completely delocalized as in the case of pure DNA-cation binding (Manning 1978). Instead, bound ions have to be localized to their respective charge cluster on the macromolecular assembly and can be free to move only within the dimensions given by the size of that cluster.

A second difference from the classical counterion condensation theory is that chromatin cannot be modelled as a linear chain; the diameter of the fiber is considerably larger than the Debye-Hueckel length. A more appropriate geometric model would be a rod with the charge clusters distributed at its surface.

The consequence is that the contribution of the electrostatic repulsion/attraction to the overall free energy of the system has to be reformulated. It is expected that this reformulation will solve the second contradiction between the

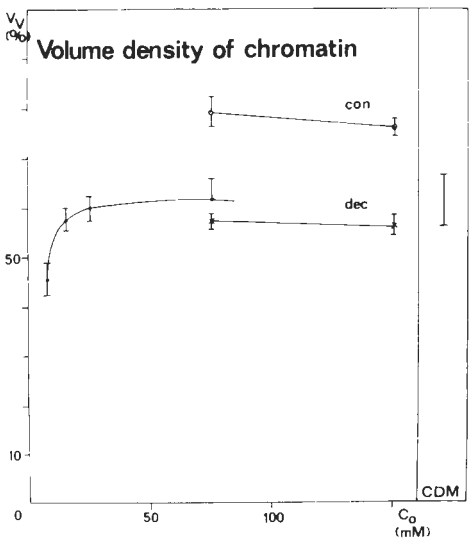

Fig 3: Volume density of chromatin $V_{v,c}$ (mean ± SEM, in %) vs KCl concentration in the medium. The critical difference between means (CDM) at the 5% significance level is indicated.

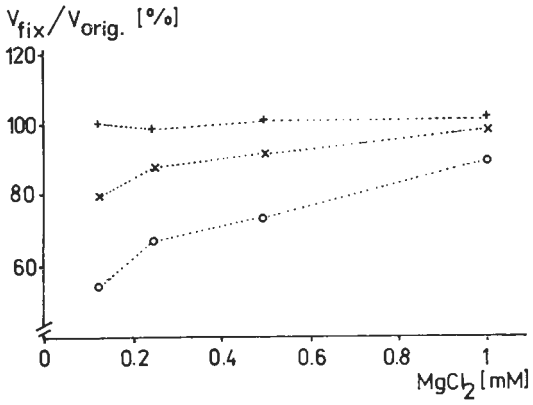

Fig 4: Relative volume of nuclei after fixation in CCP-buffered glutaraldehyde (GA) as estimated by phase contrast microscopy.
+ ...30 mM CCP, 2.5% GA
x ...40 mM CCP, 2.0% GA
o ...80 mM CCP, 1.2% GA

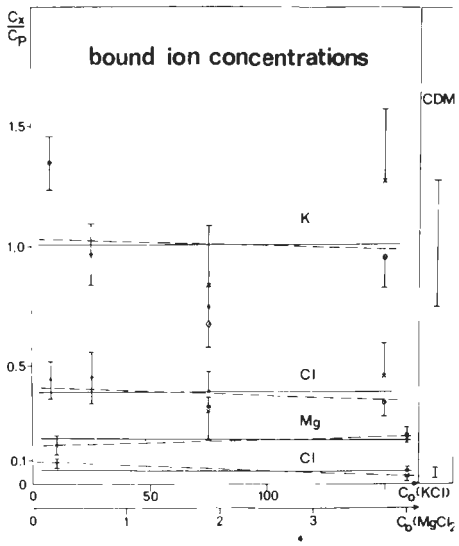

Fig 5: Concentrations of bound ions in KCl and $MgCl_2$ solutions. The upper two curves belong to the KCl solutions. Values are mean ± SEM. The CDM is indicated, if there are significant differences at the 5% level. Full lines indicate the means, dashed lines give the linear regression. The slopes of the regression lines are not significantly different from zero. Filled circles: homogeneous chromatin; crosses: decondensed chromatin; open circles: condensed chromatin.

expected and the experimental results, namely the fact that we found the concentration of bound monovalent ions to be about 5 times higher than that of divalent ones (see Fig 5), while they should be bound roughly in inverse proportion to their charge number according to the classical theory (Manning 1978).

It is not clear at present why the amount of bound anions is so much smaller in the $MgCl_2$ as compared to the KCl case. Anyway, the measured ratio of bound anions/cations of about 0.3 to 0.4 gives some idea about the relative size and/or frequency of positively and negatively charged clusters on the surface of the chromatin fibers.

Ion concentrations as measured here in isolated nuclei in KCl solutions are remarkably similar to the (Na+K) concentrations found in liver nuclei under in-vivo conditions (compare Table 1 with Table 2 in von Zglinicki and Bimmler 1987). The results might be expected, therefore, to have some physiological significance. Moreover, they might be used to obtain some estimation of the free ion concentration in nuclei in vivo. From K^+/Rb^+ exchange experiments done on amphibian oocytes, Cameron (1985) concluded that the chemical activity of $(Na^+ + K^+)$ in this oocyte nuclei might be considerably smaller than 120 mM. This is in accordance with results of Hazlewood and Kellermayer (1988).

The ion concentrations we measured in the nuclei of rat liver (von Zglinicki and Bimmler 1987) are recomputed in Table 2. These values are in agreement with those obtained by Somlyo et al. (1985). Assuming all the Mg to be bound and synergistic

binding of (Na + K) with a ratio (Na + K)/Mg = 5.3:1 up to a level corresponding to 0.19 Mg/P or 1.01 (Na + K)/P, we end up with a free ($Na^+ + K^+$) concentration of 70±80 mM in the condensed regions and of 80±20 mM in the decondensed regions of rat liver nuclei. Although this estimate is rather rough, it supports the conclusions drawn by Hazlewood and Kellermayer (1988) and Cameron et al. (1988).

From the constancy of bound ion concentrations in homogeneous, condensed, and decondensed chromatin it can be concluded that the bound ions are not involved in the regulation of the condensed / decondensed state transition. This regulation is evidently done by long-range shielding of the remaining unsaturated charges by the ions free in solution. The bound ions are responsible for the maintenance of the stability of the 20...30 nm chromatin fiber. If the bound ions are titrated away from the chromatin at extreme low-salt conditions, unfolding of that fiber into the so-called beads-on-the-string configuration occurs (Brasch et al. 1971).

References

Brasch K, Selegy VL, Setterfield G (1971) Effects of low salt concentration on structural organization and template activity of chromatin in chick erythrocyte nuclei. Exp Cell Res 65, 61-72.

Cameron IL (1985) Electron probe X-ray microanalysis studies on the ionic environment of nuclei and the maintenance of chromatin structure. In: Advances in Microscopy. Cowden RR, Harrison FW (eds), Alan R Liss Inc, New York, pp 223-239.

Cameron IL, Fullerton GD, Smith NKR (1988) Influence of cytomatrix proteins on water and on ions in cells. Scanning Microsc 2, 275-288.

Edelmann L (1984) Subcellular distribution of potassium in striated muscle. Scanning Electron Microsc 1984; II: 875-888.

Hazlewood CF, Kellermayer M (1988) Ion and water retention by permeabilized cells. Scanning Microsc 2, 267-273.

Katchalski A (1971) Polyelectrolytes. Pure Appl Chem 26, 327-373.

Kellermayer M, Ludany A, Jobst K, Szucs G, Trombitas K, Hazlewood CF (1986) Cocompartmentation of proteins and K within the living cell. Proc Natl Acad Sci USA 83, 1011-1015.

Ling GN (1984) In Search for the Physical Basis of Life. Plenum, New York, 1-791.

Manning GS (1978) The molecular theory of polyelectrolyte solutions with applications to the electrostatic properties of polynucleotides. Quart Rev Biophys 11, 179-246.

Negendank W (1984) The permeability of human lymphocytes to chloride. Biochem Biophys Res Comm 122, 522-528.

Palmer LG, Civan MM (1977) Distribution of Na, K and Cl between nucleus and cytoplasm in Chironomous salivary gland cells. J Membr Biol 33, 41-61.

Somlyo AP, Bond M, Somlyo AV (1985) Calcium content in mitochondria and endoplasmic reticulum in liver frozen rapidly in vivo. Nature 314, 622-625.

von Zglinicki T (1983) Quantitative Röntgenmikroanalyse biologischer Ultraschnitte mit Aluminium-Kohle Aufdampfschichten als Standards. (Quantitative X-ray microanalysis of biological thin sections with evaporated Al/C layers as standards.) Acta Histochem 72, 195-203.

von Zglinicki T (1988) Monovalent ions are spatially bound within the sarcomere. Gen Physiol Biophys 7, 495-504.

von Zglinicki T, Bimmler M (1987) The intracellular distribution of ions and water in rat liver and heart muscle. J Microsc 146, 77-85.

Weibel ER (1979) Stereological methods. Vol 1. Practical methods for biological morphometry. Academic Press London, New York, pp 101-160.

Ziervogel H (1989) Elektrorotation kernhaltiger Zellen und isolierter Zellkerne. (Electrorotation of nucleated cells and isolated nuclei.) D.Sc. Thesis, Berlin, Humboldt University, 88-103.

Discussion with Reviewers

L. Edelmann: Do you have experimental data with bulk ion concentrations lower than 7.5 mM K^+?
Authors: No. Swelling of nuclei at very low ion concentrations leads to excessive rupture of the nuclear membrane.

I.L. Cameron: Might the pelleting of fixed nuclei have changed the volume density of the chromatin fractions?
Authors: Pelleting of nuclei was done at rather low speed (400 g). We would therefore not expect volumetric changes at that step (see also Hazlewood and Kellermayer 1988).

L. Edelmann: You mentioned that volumetric changes of nuclei during conventional dehydration and embedding procedures are conceivable. Do you consider the possibility of using freeze-substituted and embedded material for the morphometrical estimation of water spaces or do you propose other simple methods which avoid conceivable artifacts?
Authors: Dehydration is the step which introduces the largest volume change (shrinkage) in biological material, both in conventional preparations and during freeze-drying. Freeze-substitution might be advantageous in this respect, however, we do not know whether this has been examined.

L. Edelmann: How long did you equilibrate the nuclei with the different KCl solutions? Is the uptake of K^+ by nuclear chromatin a fast event or did you observe a difference in K^+ binding when incubating the nuclei for different lengths of time?
Authors: Equilibration was done for 30 min including two washing steps in the final solution. Concentrations should therefore be at equilibrium, (compare Negendank 1988).

I.L. Cameron: Do you worry about a differential extraction of phosphorus containing material due to higher KCl treatment of the isolated nuclei? It seems likely to me that increasing the KCl concentration to 150 mM would extract a certain set of chromatin proteins. If so expression of data as a ratio to P does not seem wise.
Authors: In earlier studies (Fenske et al. 1983) we found that depending on the type of cell studied between 6 and 20% of chromatin was extracted at moderate ionic strength. In addition, proteins might be specifically extracted. Both effects will change the mass ratio of chromatin to structural proteins. Because nucleic acids are by far the main source of P in nuclei, expression of the data as ratio to P seems to us the best way to describe the interaction of ions with the chromatin.

C.F. Hazlewood: In our experience with Triton X-100 (Scanning Microsc 2, 267-273, 1988) there was an immediate loss of cytoplasmic and nuclear K^+. And, the associated K^+ was what one might expect with a Donnan equilibrium. On the other hand, when we used Brij 58, there was significant retention of K^+ for some minutes. In the latter case, most of the K^+ appears to be co-compartmented with the proteins. This association, however, is loose or certainly does not appear to be tightly bound. How does one translate 1 mM K^+/unit phosphorus into the percentage of the total K^+ that is bound.
Authors: In your experiments, the K^+ lost from the nuclei was, at least partially, exchanged against Na and Ca from the bathing medium. In our measurements, K^+ was the only cation present in the medium and no detergents were used. This makes it difficult to compare our results.

Depending on the degree of hydration, 1 mM K^+/ mM phosphorus corresponds to between 200 and 400 mmol/kg dry weight bound K^+. Bound in this respect means simply that there is some interaction which increases the local concentration of K^+ in the vicinity of the macromolecules, but does not imply any assumption about the type or strength of binding. It is assumed, that the binding forces are determined by an interplay of electrostatic and solvation energy (Manning 1978).

I.L. Cameron: You say that the 20...30 nm diameter chromatin fiber remains unchanged over the whole ion concentration range studied but give no measurements in support of this statement. One might expect a change in fiber diameter. Further data on this point would be helpful to our understanding of chromatin structure and its regulation. We long ago reported a larger chromatin fiber diameter of euchromatin versus heterochromatin in fixed sections of several cell types and I now wonder if or how various ions might be involved in such chromatin fiber differences.
Authors: We demonstrated different mean fiber diameters in condensed and decondensed chromatin of rat liver nuclei mainly due to a larger fraction of 10 nm fibers in the decondensed regions (von Zglinicki et al. 1981) This distribution of fiber diameters as seen in fixed whole tissue could be restored in isolated nuclei if a mixture of inhibitors of endogenous protease and phosphatase (PMSF, Na-molybdate, NaF, papaverin) was applied throughout the preparation. Without these inhibitors, 20...30 nm fibers were the predominant fiber type found in isolated nuclei. This is in accordance with the present results: No inhibitors were used and we estimated the following mean diameters D of chromatin fibers by measuring 90 randomly selected fibers from 10 nuclei per group. C_o = 7.5 mM KCl: D = 23.3 ± 0.6 nm, C_o = 150 mM KCl, condensed chromatin: D = 23.6 ± 0.6 nm, decondensed chromatin: D = 22.0 ± 0.9 nm. Values are mean ± SEM. These values are means including around 10% of 10 nm fibers. We never attempted to measure small changes in the diameter of thick fibers as you did (Pool and Cameron 1978).

I.L. Cameron: Might the 'clusters' of negative and positive charges be different molecules like DNA and histones?
Authors: We do not know. However, one might speculate that most of the DNA charges are neutralized by histone binding and that the clusters available for K^+ binding are mainly on histones and/or nonhistone proteins. In muscle, the existence of those clusters of sufficient size within one protein molecule is highly probable (von Zglinicki 1988).

L. Edelmann: The amount of bound K^+ is determined under the assumption that the water of the nuclei has no exclusion properties. How do you rule out this possibility? I suggest that the concentration dependent uptake of K^+ by nuclei should be studied by an independent method. A flux study. for instance, may yield saturable and nonsaturable fractions. Additional information could be obtained by investigating Na^+ and other alkali cations. It would be interesting to

know whether there is an ion specific difference in ion uptake by nuclear chromatin in isolated intact nuclei, in Triton-treated nuclei and in intact lymphocytes.

Authors: In fact, our results demonstrate that the binding of K^+ in nuclei, at least under equilibrium conditions, is independent on the bulk K^+ concentration over a broad range. This holds true, even if significant amounts of the nuclear water would be structured because the assumption of a bulk water phase within nuclei is a worst-case assumption with respect to the amount of bound ions. If the nuclear water has exclusion properties, the bound fraction would turn out to be even higher.

Exchange experiments of the suggested type have been reported by Negendank (1988) by using whole lymphocytes and by Hazlewood and Kellermayer (1988) with detergent-treated cells. It would surely be interesting to compare these results with those from isolated nuclei in media with differing Na^+/K^+ ratio. However, we have not done those experiments yet.

Additional References

Fenske H, von Mickwitz CU, Von Zglinicki T, Heymann S, Boettger M, Eichhorn I, Lindigkeit R (1983) Characterization of salt soluble chromatin as decondensed chromatin. Stud Biophys 95, 193-203.

Negendank W (1988) A cooperative transition theory applied to the kinetics of ion exchange in cells. Cell Biophys 13, 93-117.

Pool TB, Cameron IL (1978) Chromatin fiber differences between condensed and dispersed chromatin: a comparative ultrastructure study. Cytobios 19, 93-100.

von Zglinicki T, Heymann S, Karawajew L, Lindigkeit R (1981) Dekondensiertes Chromatin in situ und in Lösung. (Decondensed chromatin in situ and in solution.) 10th Natl Congr Elect Microsc GDR. Luppa H, Heydenreich J (eds), pp 198-199. (Available from authors).