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Glycine and other amino compounds prevent chromatin precipitation at physiological ionic strength

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Glycine, proline and taurine, when present in the range 0.1–0.60 M, inhibit chromatin precipitation by sodium chloride. Histone gel electrophoresis revealed that the linker histones H1 and H5 were largely depleted from the supernatant chromatin fraction at 0.2 M NaCl, while this depletion was absent in the presence of glycine. These observations are discussed in relation with the various factors which may be involved in the precipitation process.

Chromatin precipitation; Glycine; Proline; Taurine; Trimethylamine *N*-oxide

1. INTRODUCTION

Amino compounds are used as major osmotic effectors with the inorganic ions K^+, Na^+ and Cl^-, and some other organic substances in cell volume and osmolarity control (review [1]). As recently pointed out, these amino compounds also play an essential role in protecting intracellular macromolecules against the disrupting effects of the changes in concentration of inorganic ions that occur in cells in the course of volume modifications or adaptations to changes in extracellular osmolarity [2–5]. They can indeed act: (i) as 'substituent osmolytes', allowing in some way, during long-term adaptation to anisosmotic media, a regulation of the intracellular level of inorganic ions; this should decrease the disrupting effect of the changes in these ions' levels on macromolecular structures; (ii) as 'compensatory solutes', directly inhibiting the effect on macromolecular architectures. The molecular basis of these interactions between macromolecules, inorganic ions and amino compounds is only poorly understood at the moment [6,7].

We have therefore initiated a study of the effects of the simultaneous presence of amino compounds and monovalent salts on the organization of macromolecular structures considering chromatin as a first model. It has indeed been shown recently that osmotic shocks induce significant modifications in the organization of this nucleoprotein in different vertebrate cell types [8,9]. Chromatin appears highly condensed at high NaCl concentrations and unfolded in low salt medium. Such changes are not seen in cells of euryhaline invertebrates which contain large amounts of 'compensatory' amino compounds [2–4]. Furthermore, progressive adaptation of a mammalian cultured cell type (PC12) to hyperosmotic media is concomitant with an increase in the intracellular level of amino compounds. In this situation, there is no significant change in the ultrastructural organization of chromatin (Delpire and Gilles, unpublished).

This preliminary report deals with the effect on chromatin condensation and precipitation by NaCl, of the amino acids glycine and proline, the parent zwitterionic sulfonate compound ethyl-

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aminosulfonate ($^{+}H_3N$-$(CH_2)_2$-SO_3^-) or taurine, and the uncharged polar compound trimethylamine *N*-oxide ($(CH_3)_3$-N=O) or TMAO.

2. MATERIALS AND METHODS

Chromatin samples from two different origins were used here. High-M_r chicken erythrocyte chromatin (CE chromatin) of length 50–70 nucleosomes, was prepared from isolated nuclei by mild micrococcal nuclease digestion (less than 3% acid-soluble residues for the suspension of nuclei, after digestion) using standard procedures described elsewhere [10,11]. Chromatin from cultured PC12 cells (rat adrenal pheochromocytoma [12]) was prepared similarly after extraction of nuclei according to [13].

The stock chromatin solutions were dialyzed vs 1 mM Tris-HCl, 0.2 mM EDTA buffer at pH 7.2 (for PC12 chromatin) and pH 7.9 (for CE chromatin).

The chromatin precipitation curves were determined at concentrations of 0.025 mg/ml (absorbance at 260 nm of the order of 0.5 for a 10 mm path length for PC12 chromatin) and 0.35 mg/ml ($A_{1\ mm}$(260 nm) = 0.7 for CE chromatin). Microvolumes of a concentrated NaCl solution were added to the chromatin solutions, which were left for about 20 min at 4°C; after centrifugation at 5000 × *g* for 15 min, the absorbance of the supernatant was measured at 260 nm. When required, the amino compound was added prior to the NaCl addition.

Gel electrophoresis of the histones was performed on 17.5% polyacrylamide, according to Wyns et al. [14].

3. RESULTS

Fig.1A and B shows the precipitation curves of CE and PC12 chromatins, with the well-known characteristic maximum at about 0.2 M NaCl [15]. Resolubilisation of chromatin above 0.2 M NaCl occurs concomitantly with progressive release of the histones, starting with histone H1 (and H5) [16]. The maximum percent precipitation varied among various preparations, depending on the amount of short fragments obtained (related to the percent acid-soluble residues reached at the end of the micrococcal digestion). It also increased by 10–20% for a factor of about 10 in chromatin concentration on the same preparation (not shown; cf. also fig.1A,B). Addition of glycine in the range 0.2–0.8 M produced an inhibition of chromatin precipitation.

Redissolution of precipitated chromatin in 0.2 M NaCl could also be achieved by glycine addition, total redissolution being attained at 0.55–0.6 M glycine (fig.2). Similar behavior was observed with proline and taurine, but not with TMAO. No precipitation occurred when any of the amino-organic compound was added to the chromatin solutions at 0.6 M.

Control experiments on chromatin structure at low NaCl concentration but in the presence of high concentrations (up to 0.4 M) of glycine, proline, taurine and TMAO were also performed using thermal denaturation and circular dichroism (CD) techniques. The melting temperature remained in the range 75–78°C, and no significant alterations in CD spectra were detected (not shown). Simultaneous addition of 0.2 M NaCl, and a concentration of any of the four selected compounds maintaining chromatin totally in solution, did not affect the CD spectra (not shown). This indicates that no dissociation of the histones from the DNA had occurred.

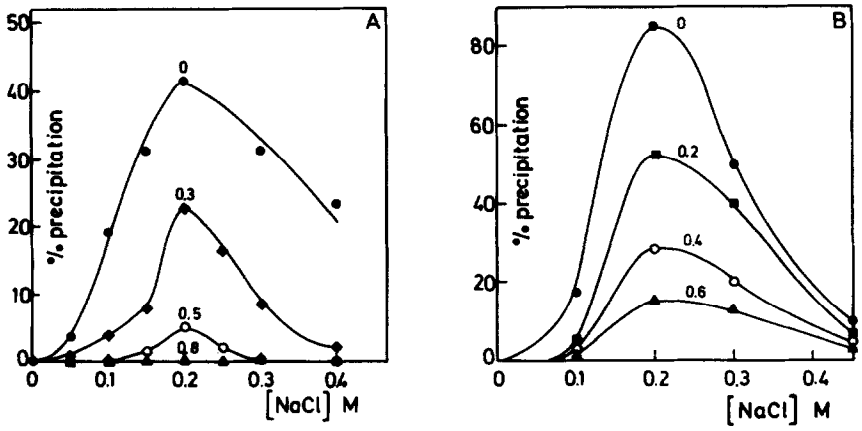

Fig.1. Precipitation profiles of chromatin by NaCl in the presence of glycine. Molar concentrations of glycine given as labels on the curves. (A) PC12 chromatin, (B) CE chromatin.

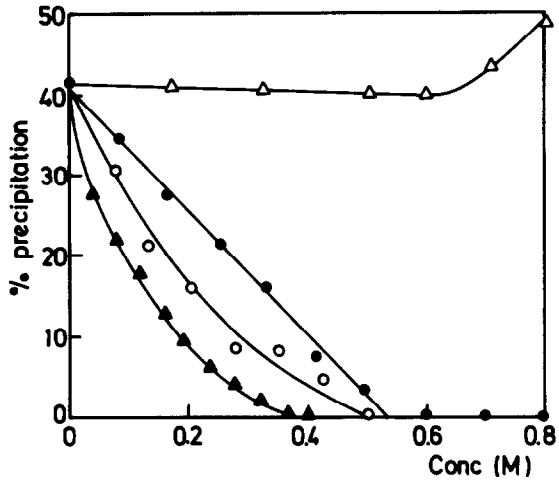

Fig.2. Redissolution of 0.2 M NaCl-precipitated PC12 chromatin upon addition of glycine (●), proline (○), taurine (▲) and TMAO (△).

It was also interesting to look at the distribution of the histone and non-histone proteins in the precipitate and in the supernatant at 0.2 M NaCl,

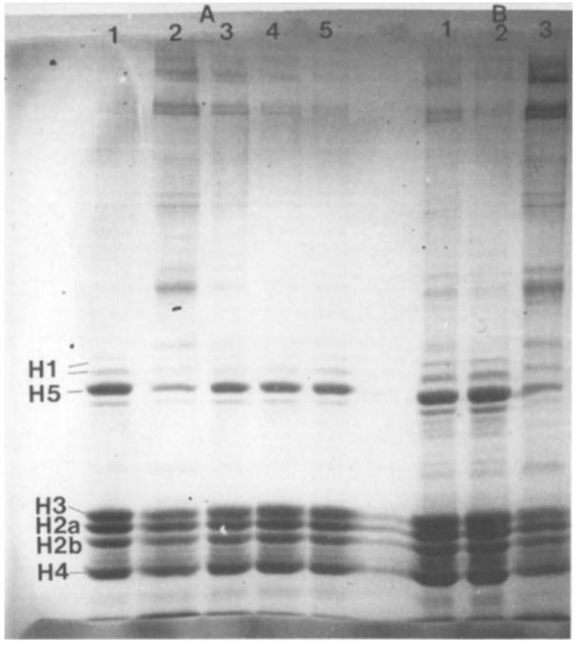

Fig.3. Histone gel electrophoresis of native CE chromatin (lane 1) and of soluble chromatin fractions in 0.2 M NaCl, in the absence (lane 2) and presence of glycine at various concentrations: 0.2, 0.6 and 1 M Gly for lanes 3–5, respectively. (B) Corresponds to loading of the gels with a 2-fold greater sample volume compared to (A).

in the presence of varying amounts of amino compound. Fig.3 indicates that the soluble chromatin fraction at 0.2 M NaCl contains low amounts of extranucleosomal histones (particularly H5) but is enriched in non-histone proteins. Addition of glycine at increasing concentrations progressively eliminates this depletion process which yielded an accumulation of H1/H5 in the precipitates. Glycine is thus able to attenuate or to impede the action of NaCl on the interactions between DNA and histone H1/H5 and non-histone proteins. However, we do not know at present if the H1/H5 histones found in the supernatant are really bound to the chromatin. Preliminary X-ray scattering measurements (Koch et al., in preparation) in the presence of glycine and NaCl indicated that the chromatin was not degraded in mononucleosomes or smaller particles, and condensed normally in the range of 0–0.1 M NaCl while remaining in solution up to 0.2 M NaCl in 0.6 M glycine.

4. CONCLUSIONS

The above effects may be due to particular electrostatic interactions with the DNA phosphate groups, with the concomitant implication of the molecular dipoles of the zwitterionic molecules used, and may also reflect alteration of the hydration layers of the DNA and of the histone molecules. Effects of hydrophobic interactions could also be involved. Zwitterionic molecules such as glycine, proline and taurine would bind electrostatically to the DNA phosphate groups and also to the basic and acid side chains of the histones in chromatin, maintaining a high surface charge and keeping the sodium counterions further away from the surface, which would reduce the intermolecular interactions and inhibit precipitation upon salt addition. Recent studies on the interaction of glycine with DNA using thermal denaturation [17] revealed the presence of a stabilizing electrostatic contribution and a destabilizing specific interaction with the DNA base pairs. Competition with salt ions was also noted.

We believe that the most important factor which should be taken into account is the effect of these amino compounds on the water structure around macromolecules. Timasheff et al. [6,7,18–20] have shown that organic osmolytes produce a better structuration of the water layer around the protein

macromolecules with a subsequent conformational change to a more compact, native structure. This situation could be encountered in the case of DNA and histones in chromatin. The addition of amino compounds could prevent the modulation of the histones' H1 and/or H5 structures which seems to be required for interfiber aggregation of chromatin upon NaCl addition [15]. It is possible that such interactions are essential to keep chromatin 'soluble' in the (intracellular) nuclear medium.

Further detailed studies on these particular phenomena are now in progress in our laboratory and hopefully will help us to understand the complex mechanism of these interactions. Searches to rationalize the present findings on structural and molecular interaction grounds are being made.

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REFERENCES

[1] Gilles, R., Kleinzeller, A. and Bolis, L. (1987) Curr. Top. Membranes Transp. 30, 289.
[2] Gilles, R. (1989) Renal Physiol. Biochem., in press.
[3] Gilles, R. (1987) in: Comparative Physiology: Life in Water and on Land (Dejours, P. et al. eds) pp. 485–502, Springer, Berlin.
[4] Gilles, R. (1987) Curr. Top. Membranes Transp. 30, 205–247.
[5] Yancey, P.H., Clark, M.E., Hand, S.C., Bowlus, R.D. and Somero, E.N. (1982) Science 217, 1214–1222.
[6] Arakawa, T. and Timasheff, S.N. (1983) Arch. Biochem. Biophys. 224, 169–177.
[7] Arakawa, T. and Timasheff, S.N. (1985) Biophys. J. 47, 411–414.
[8] Delpire, E., Duchêne, C., Goessens, G. and Gilles, R. (1985) Exp. Cell Res. 160, 106–116.
[9] Delpire, E., Gilles, R., Duchêne, C. and Goessens, G. (1985) Mol. Physiol. 8, 293–306.
[10] Bordas, J., Perez-Grau, L., Koch, M.H.J., Nave, C. and Vega, M.C. (1986) Eur. Biophys. J. 13, 157–174.
[11] Marquet, R., Colson, P., Matton, A.M., Houssier, C., Thiry, M. and Goessens, G. (1988) J. Biomol. Struct. Dyn. 5, 839–857.
[12] Greene, L.A. and Tischler, A.S. (1976) Proc. Natl. Acad. Sci. USA 73, 2424–2428.
[13] Lerner, M.R. and Steitz, J.A. (1979) Proc. Natl. Acad. Sci. USA 76, 5495–5499.
[14] Wyns, L., Lasters, I. and Hamers, R. (1978) Nucleic Acids Res. 5, 2345–2358.
[15] Jin, Y.-J. and Cole, R.D. (1986) J. Biol. Chem. 261, 15805–15812.
[16] Burton, D.R., Butler, M.J., Hyde, J.E., Phillips, D., Skidmore, C.J. and Walker, I.O. (1979) NATO ASI Ser., Ser. A 21a, 137–165.
[17] Bruskov, V.I. and Smol'yaninova, T.I. (1987) Stud. Biophys. 121, 111–124.
[18] Inoue, H. and Timasheff, S.N. (1972) Biopolymers 11, 737–743.
[19] Lee, J.C. and Timasheff, S.N. (1974) Biochemistry 13, 257–265.
[20] Gekko, K. and Timasheff, S.N. (1981) Biochemistry 20, 4667–4676.