COMMENTS ON THE METHOD OF ANALYSIS OF THE PROTEINS FROM NUCLEAR PARTICLES

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Received 15 February 1974
Original figures received 14 March 1974

1. Introduction

A large discrepancy is observed between the results of various groups concerning the number of proteins associated with heterogenous DNA-like RNA in nuclear particles. In conditions where disulfide bonds were reduced, some groups detected either a unique polypeptide [1, 2] or a small number of polypeptides (up to 3) [3–5]; other groups [5–11] observed a much larger number of them (more than 10). Since this discrepancy was observed even between groups using the same system, i.e. rat liver [1–3 and 6–8] it could therefore not be related to tissue or species specificity. On the other hand, the method of analysis of proteins could account for the differences: the proteins were electrophoresed either [1–5] in the presence of urea at pH 4.5, a method where the charge of the proteins is of importance, or [5–11] in the presence of dodecylsulphate (SDS) where the major parameter for migration is the molecular weight.

We show here that the method of analysis of proteins is indeed critical since the phosphorylated proteins from the particles [10] do not enter the gels at pH 4.5. In all likelihood, other proteins perhaps because their isoelectric point (pI) is close to 4.5, behave similarly.

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2. Methods

Five rats were injected intracisternally with 1.5 mCi of $^{32}PO_4^{3-}$ per rat for 16 hr. Purified nuclei were prepared from the brains [5, 12]. They were lysed with 0.2% deoxycholate in buffer E (100 mM Tris–HCl, pH 8; 25 mM KCl; 2.5 mM $MgCl_2$; 0.14 M NaCl). After centrifugation for 5 min at 100 000 *g* [5, 12], the supernatant was layered on a 10–25% linear sucrose gradient and centrifuged for 195 min at 69 000 *g*. Fractions from 180 to 220 S were pooled and centrifuged for 2 hr at 100 000 *g*. The pellet containing the purified particles was suspended in 1 ml of 10 mM Tris–HCl, pH 8, containing 2 μg/ml of pancreatic RNAase and incubated at 4°C for 1 hr. Trichloroacetic acid (TCA) was added to a final concentration of 10%. After overnight precipitation the pellet was collected by centrifugation for 30 min at 88 500 *g* and suspended in 0.2 ml 6 M urea, 10 mM sodium acetate, pH 4.5, 0.1 mM dithiothreitol (DTT). After overnight dissociation, the concentration of DTT was adjusted to 1 mM. 100 μl were analysed in urea gels at pH 4.5 [13, 14], as described previously [5]. 50 μl aliquots were treated with SDS and electrophoresed in the presence of 1% SDS as described previously [5] with the following modifications: 0.4 ml of 12% acrylamide was polymerised at the bottom of the gel tube; 1.6 ml 10% acrylamide was layered on top. The stacking gel was unmodified. The samples were first electrophoresed at 0.35 mA/gel for 2 hr 30 min and then at 2.5 mA/gel until the tracking dye (Bromophenol blue) reached the end of the gel (about 2 hr 30

Urea and SDS gels were stained with Coomassie Brilliant Blue R. and protein peaks scanned with a Vernon recorder (Paris, France). One mm slices were cut from frozen gels and counted for ^{32}P radioactivity.

3. Results and discussion

It was observed previously [10] that several polypeptides from the nuclear particles were phosphorylated in vivo and remained bound to DEAE-cellulose at pH 7.6 in the presence of 6 M urea, together with non-phosphorylated species. Only few proteins showed a basic behaviour under these experimental conditions. This suggested that a large part of the proteins from the particles were relatively acidic, an assumption that was supported by a direct determination of their pI (C. Ducamp et P. Jeanteur, personal communication) and by their electrophoretic behaviour in other systems (J. P. Fuchs, personal communication).

The proteins from the same preparation of particles labelled with ^{32}P phosphates in vivo were analysed in polyacrylamide gels either in the presence of urea at pH 4.5 (fig. 1) or in the presence of SDS (fig. 2). In the urea gels some precipitated material was always present on top on the stacking gel, at the boundary between the pH 4.5 electrophoretic buffer and the pH 6.8 gel. A large amount of ^{32}P radioactivity was associated with the precipitated material. At the boundary between the stacking (pH 6.8) and the running (pH 4.5) gels, some ^{32}P radioactivity was again detected together with stained material. In the upper third of the running gel a major peak of absorbance with a shoulder and a few peaks of lower mobility were observed in agreement with previous experiments [5]. The radioactivity at this level was low, of the order of magnitude of the background. No peak of radioactivity could be attributed to the absorbance peaks.

Half the quantity of material used for the urea gels was analysed by SDS gels (fig. 2). Numerous species were found to be present. The use of longer gels (see Methods) permitted a much better resolution than previously [10]. This will be discussed in more details elsewhere. ^{32}P radioactivity was detected in several bands all along the gel.

Taking into account the difference in the sample volumes, the same amount of ^{32}P material was

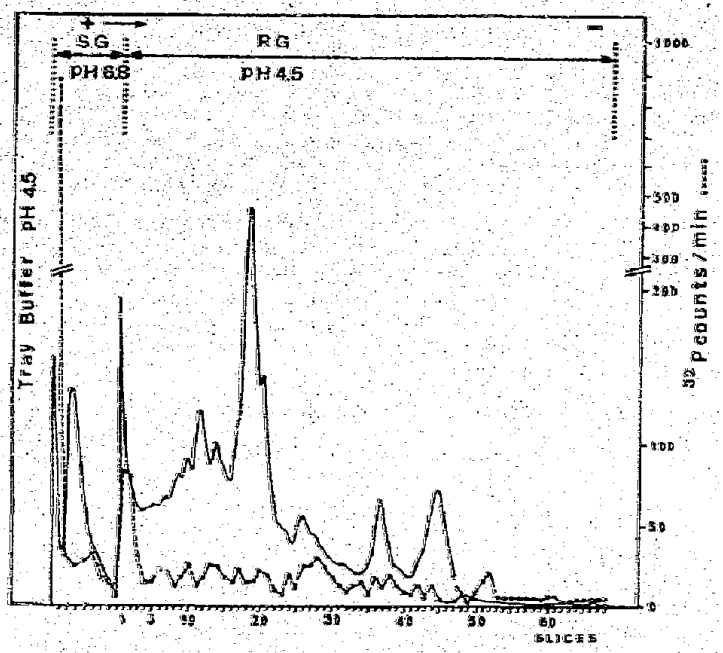

Fig. 1. 0.1 ml of a solution of proteins from particles were analysed in polyacrylamide gels in the presence of urea. Stacking gel (SG, 2.5% acrylamide) buffer was at pH 6.8. Running gel (RG, 7.5% acrylamide) as well as electrophoretic buffers were at pH 4.5. RNAase which was added to the samples for RNA digestion migrated in the high mobility region. 1905 cpm of ^{32}P were recovered in the gels. —— Absorbance; ——— ^{32}P radioactivity.

recovered in the SDS gels and at the boundaries of the stacking gel with the urea pH 4.5 method.

It is clear from these experiments (and from similar ones, not shown) that the phosphorylated proteins precipitated at pH 4.5 and did not enter the gels. It is likely that other proteins, perhaps because their pI is close to 4.5, do not enter the gels either.

Therefore, a large fraction of the proteins escape detection in pH 4.5, urea gels. This can explain the discrepancies between the results of the various groups [1–11]. Ultimately, it is obvious that the method of analysis at pH 4.5 in the presence of urea is not suitable for the analysis of the proteins from the nuclear particles.

Acknowledgements

This work was supported by the grant A 655 4303

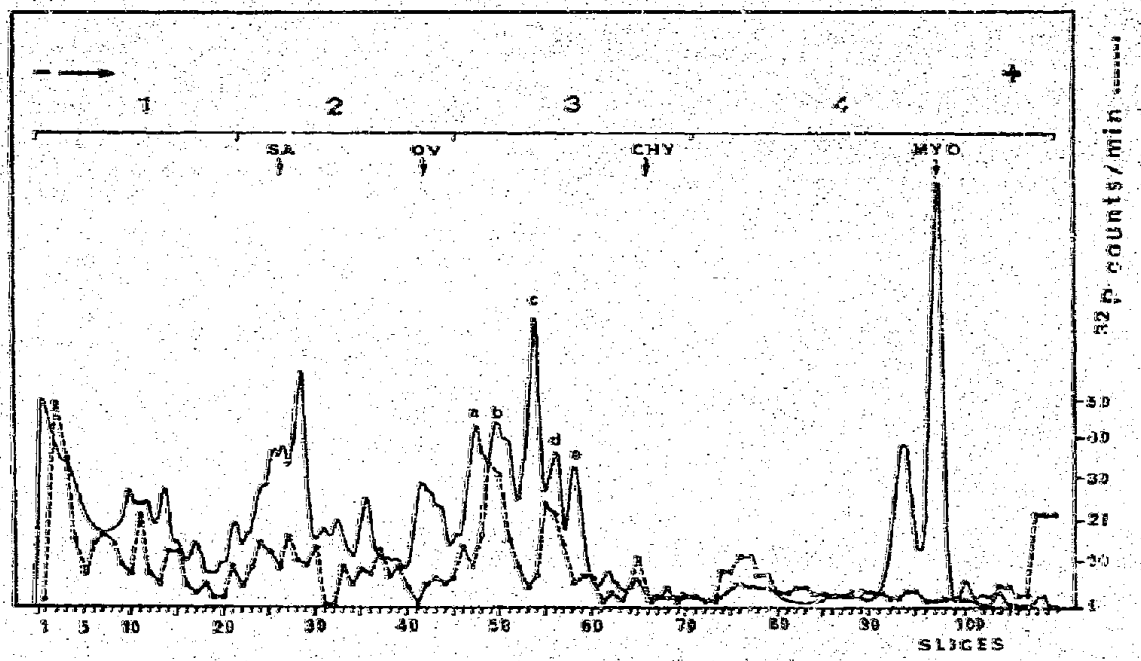

Fig. 2. 0.05 ml of the same solution of proteins as in fig. 1 were analysed in polyacrylamide gels in the presence of SDS. Myoglobin (Myo) was added as an internal marker. The migration of other molecular weight markers (SA : seramalbumin, Ov : ovalbumin, Chy : chymotrypsinogen) run in a parallel gel are indicated. Nomenclature of the peaks is identical to that used previously [5, 10] although more bands were resolved in these gels than in shorter ones. 950 cpm of ^{32}P were recovered in the gels. —— Absorbance; – – – – ^{32}P radioactivity.

(ATP) from the Centre National de la Recherche Scientifique. Radioactive material was supplied by the Commissariat de l'Energie Atomique (Département de Biologie), Saclay, France.

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