A COMPETITIVE RADIOIMMUNOASSAY ON A MAGNETIC PHASE FOR ACTIN DETECTION

Y. BENYAMIN, M. ROGER, Y. ROBIN* and N. V. THOAI

Centre de Recherches de Biochimie Macromoléculaire du CNRS, BP 5051, 34033-Montpellier Cedex and **Laboratoire de Biologie Marine de l'Ecole Pratique des Hautes Etudes. Collège de France, Place Marcellin-Berthelot, 75231 Paris Cedex 05, France*

Received 26 November 1979

1. Introduction

The understanding of the role of actin in cellular mechanisms [1] in which contractile proteins and membranous systems are implicated, such as endocytotic and exocytotic process [2] requires a discriminatory test able to detect this protein. Such a test should be sensitive enough to monitor actin in subcellular structures, its activity should be independent of extraction conditions of membranous proteins and its specificity large enough to be applied at different cellular types or animal origins.

Here we report an efficient radioimmunoassay able to detect actin in crude extracts, through only one step, in the ng range. The large specificity of anti unfolded actin antibodies [3] related to the conservative structure of this antigen [4], gives large investigation possibilities to this radioimmunoassay.

2. Materials and methods

Rabbit muscle and chicken gizzard actins, native, *S*-carboxymethylated or oxidized by performic acid were obtained as in [3]. Rabbit skeletal muscle was sliced up, washed with 0.15 M NaCl and homogenized at 4°C using a polytron 20 SM (Bioblock) homogenizer. Human platelets provided by the Blood Centre (Montpellier) were freed of plasma by centrifugation (5 min at 2500 × *g*) and washed 3 times in 0.01 M phosphate-buffered saline (PBS), pH 6.6. Proteins were extracted from muscle homogenates or platelet pellets in Thomas tissues homogenizers during 30 min at 4°C using 5 vol. extraction buffer (2 mM Tris–HCl, 2 mM dithiothreitol, 0.5% Triton X-100 (pH 7.4) then supplemented by 1% sodium dodecyl sulfate (SDS) and heated to 100°C during 15 min for unfolding. The mixture was carboxymethylated by iodoacetic acid [3] and centrifuged (15 min at 20 000 × *g*) before protein [5] and actin determinations. Purified anti-actin antibodies [3] were insolubilized on Magnogel 44 (Industrie Biologique Française) using a cyanogen bromide activation [6].

S-Carboxymethylated actin (10 μg) from rabbit muscle was labelled by the chloramine T method [7] with ^{125}INa (0.5 mCi) (Radiochemical Centre, Amersham). The labelled actin (20 μCi/μg) was stored at −30°C in PBS, bovine serum albumin 1% (pH 7.4). Assays were carried out in duplicate in this buffer. For a standard experiment, the following procedure was used: to 800 μl buffer was added successively 50 μl (1 ng) labelled actin, 100 μl unlabelled antigen or buffer-diluted extract and 50 μl insolubilized antibodies sampled from a magnet-decanted [8] solid phase. The mixture was incubated at 20°C in a Kahn agitator, washed 3 times on a magnet-rack [8] by 2 ml PBS, 0.05% Tween 20, and the solid phase counted in a γ counter.

3. Results and discussion

Preliminary studies showed that the optimal sensitivity of this radioimmunoassay is obtained with 50 μl (3.4 μg antibodies) of insolubilized antibodies diluted (50X) in inert Magnogel 44. In these conditions 30% of the ^{125}I-labelled actin present (1 ng) is bound to particles with a very good reproductibility (±2%). The optimal incubation time (fig.1A) is restricted to 1 h. After this period no important increase in the radioactivity bound has occurred.

A typical calibration curve is shown in fig.1B. The sensitivity of this radioimmunoassay permits the detection of 5 ng unfolded actin and the linear

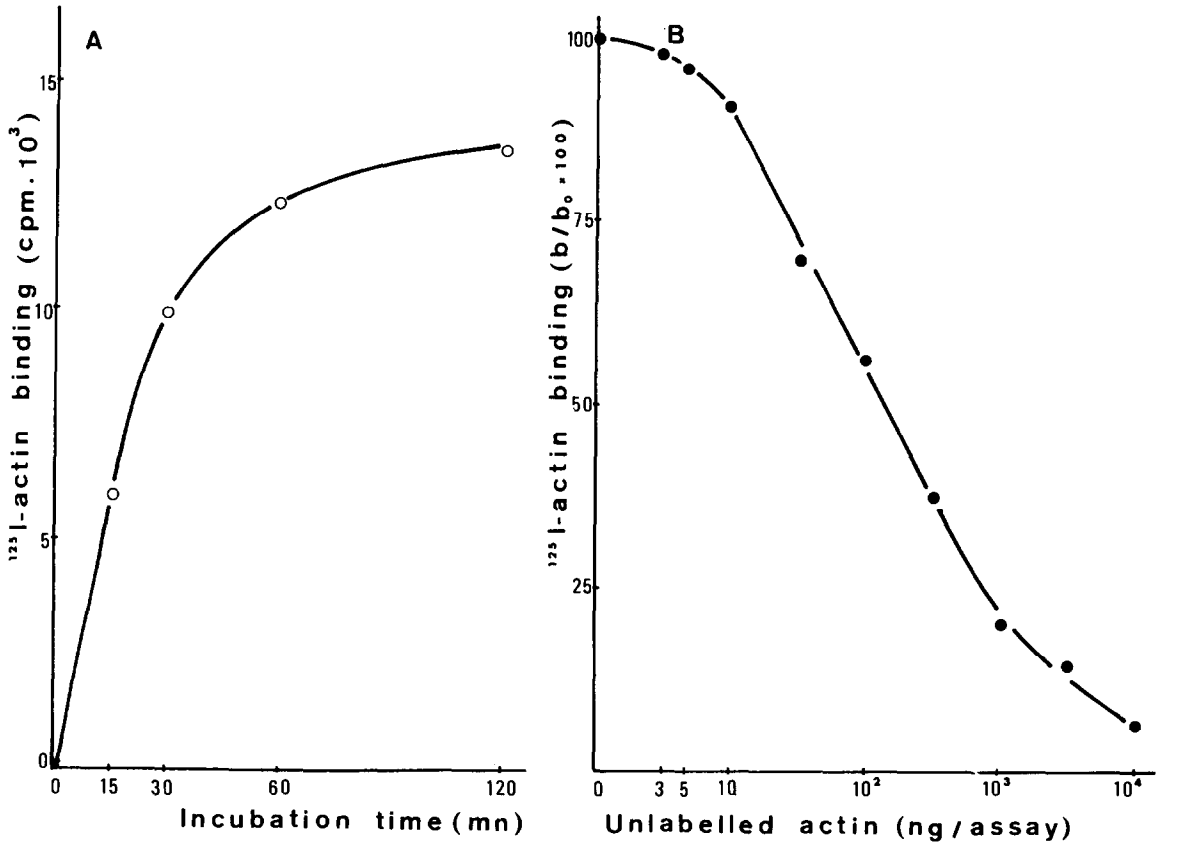

Fig.1. ^{125}I-Labelled actin (1 ng) binding on Magnogel 44-insolubilized antibodies (3.4 μg) according to (A) incubation time (○——○) and (B) unlabelled actin present (●——●).

experimental range in this assay is about 2 orders of magnitude, between 10 ng of 1 μg.

The specificity and the discriminatory properties of this radioimmunoassay were checked (fig.2) by using actins from rabbit muscle and chicken gizzard in native or unfolded state. Performic acid-oxidized bovine serum albumin was used as a non-related protein. It appears that rabbit oxidized-actin which was the immunogen used [3] gives a better sensitivity (1 ng) and a similar linear range response than the *S*-carboxymethylated one. On the other hand native

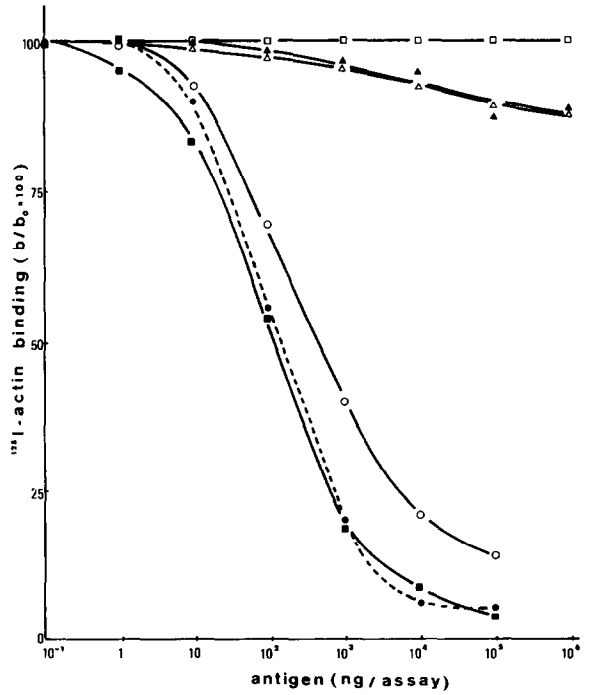

Fig.2. Rabbit muscle *S*-carboxymethylated ^{125}I-labelled actin (1 ng) binding in presence of increased amounts of rabbit muscle oxidized actin (■——■), rabbit muscle *S*-carboxymethylated actin (●- - -●), chicken gizzard *S*-carboxymethylated actin (○——○), oxidized bovine serum albumin (□——□), rabbit muscle (▲——▲) and chicken gizzard native actins (△——△).

Table 1
Quantitation of actin in striated muscle and platelets by the radioimmunoassay

Tissue	Protein (mg/ml)	Actin (mg/ml)	Actin (%)	Previous actin estimations (%)
Skeletal muscle	18	4.86	27	25 (14)
Platelets	18	4.14	23	16.2 ± 3.27 (15) 21 ± 3

Equal amounts (500 mg) of material were homogenized and extracted. After carboxymethylation the volume was adjusted to 5 ml with extraction buffer. The data given are means of 5 expt.

actins give flattened responses shifted to higher doses. Oxidized bovine serum albumin in 0.01% SDS is unable to compete with ^{125}I-labelled actin. Thus no residual interaction linked to oxidized residues or SDS carried by the immunogen can be detected at this sensitivity level. The discriminatory properties of this radioimmunoassay to actins issued from different genes are illustrated by the ability of gizzard actin to compete with rabbit ^{125}I-labelled actin (fig.2). A similar sensitivity is obtained and the response is somewhat shifted to higher dose but the gizzard actin is unable to displace the whole labelled rabbit actin. This result which underlines the genetic divergence of these two actins is in good accord with the comparative study [3].

The detection of actin in muscle and platelets extracts was carried out using as standards *S*-carboxymethylated actin purified [3], respectively, from rabbit skeletal muscle and chicken gizzard.

It appears (table 1) that 27.5% of proteins extracted from muscle and 23.1% in the platelets case interact with anti-actin antibodies. The identification of this antigenic activity as the actin one is supported by the specificity of antibodies [2,3] and by the resolution in only one band at actin position on SDS gels [9] of the material desorbed [10] after a batchwise procedure between the insolubilized antibodies and the rabbit muscle extract. Moreover estimations (table 1) using quantitative electrophoretic analysis on SDS gels [11] are compatible with our results, and in [12] the intracellular actin concentration in platelets, the more studied cytoplasmic model, was estimated through a DNase inhibition assay to 40 mg/ml, that is close to 41.6 mg actin/g platelets calculated from our results (table 1).

In conclusion this competitive radioimmunoassay which is able to detect actin concentration in the ng range, comparative to the assays in [3,11–13] which characterize this protein in the μg range, could be adapted to investigations in motile systems not easily available. Moreover the specificity of this radioimmunoassay to unfolded actin makes it possible to monitor actin in subcellular fractions containing bound, free, polymerized or monomenic actins, after a denaturing treatment which fully releases actin from its contractile or cytoskeletal complexes.

Acknowledgements

The authors are grateful to Dr D. Richard for platelets and to Jacques Martin for his expert technical assistance. This work was supported by a grant from DGRST (AC 76 71 185).

References

[1] Pollard, T. D. (1976) J. Supramol. Struct. 5, 317–334.
[2] Gabrion, J., Travers, F., Benyamin, Y., Sentein, P. and Thoai, N. V. (1980) Cell Biol. Int. Rep. in press.
[3] Benyamin, Y., Roger, M., Gabrion, J., Robin, Y. and Thoai, N. V. (1979) FEBS Lett. 102, 69–74.
[4] Vandekerkhove, J. and Weber, K. (1978) Eur. J. Biochem. 90, 451–462.
[5] Lowry, O. H., Rosebrough, N. J., Fall, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[6] Wilchek, M., Bocchini, U., Becker, M. and Givol, D. (1971) Biochemistry 10, 2828–2835.
[7] Greenwood, F. C. and Hunker, W. M. (1963) Biochem. J. 89, 114–122.
[8] Guesdon, J. L. and Avrameas, S. (1977) Immunochemistry 14, 443–447.
[9] Porzio, M. A. and Pearson, A. M. (1977) Biochim. Biophys. Acta 490, 27–34.
[10] Andersson, K., Benyamin, Y., Douzou, P. and Balny, C. (1978) J. Immunol. Methods 23, 17–21.
[11] Bray, D. and Thomas, C. (1975) Biochem. J. 147, 221–228.
[12] Blikstad, I., Markey, F., Carlsson, L., Persson, T. and Lindberg, U. (1978) Cell 15, 935–943.
[13] Lessard, J. L., Carlton, D., Rein, D. C. and Akeson, R. (1979) Anal. Biochem. 94, 140–149.
[14] Lowey, S. (1976) in: Handbook of Biochemistry and Molecular Biology, 3rd edn, Proteins (Fasman, G. D. ed) vol. 2, p.306, CRC Press, Cleveland.
[15] Muhlrad, A., Eldor, A. and Kahane, I. (1978) FEBS Lett. 92, 85–88.
[16] Harris, H. E. and Weeds, A. G. (1978) FEBS Lett. 90, 84–88.