PRECIPITATION OF POLYRIBOSOMES WITH PEPSIN DIGESTED ANTIBODIES

G. Holme, Susan L. Boyd, and A. H. Sehon

Department of Immunology, Faculty of Medicine, University of Manitoba

Winnipeg 3, Manitoba, Canada.

Received August 2, 1971

Summary

Polyribosomes prepared from rabbit reticulocytes were precipitated with a "γG" fraction prepared from goat antiserum to rabbit hemoglobin (specific). A similar preparation from rabbit antiserum to rat hemoglobin (control) precipitated the same proportion of the polyribosomes. The non-specificity of the precipitation was significantly reduced by pre-digestion of the antibodies with pepsin. After pre-digestion of either whole antiserum or of the "γG" fraction, the precipitation of polyribosomes by the specific antibodies was increased slightly while precipitation of polyribosomes by the control antibodies was virtually eliminated.

In numerous studies specific antibodies to a given protein have been used to precipitate polyribosomes synthesizing the protein. For example, this technique has been employed for the identification of polyribosomes producing a given peptide (1) and for the isolation of the unique mRNA coding for α-glucosidase (2). However, experiments in this laboratory demonstrated that this selective precipitation has a low specificity, probably due to the binding of IgG to ribosomes (3). Kern et al (4,5) had shown that the Fc portion of IgG was responsible for the binding to ribosomes. Therefore, it was decided to investigate the effect of removing the Fc portion of the IgG with pepsin on the specificity of precipitation. The results presented here demonstrate that the removal of the Fc' portion of IgG enables polyribosomes to be precipitated with a high degree of specificity.

Experimental

For the preparation of antisera, the isolation of ^{32}P-labelled rabbit reticulocyte polyribosomes and the subsequent counting procedures, established methods were used (6). Antisera were centrifuged at 40,000g for one hour and the lipids and sedimented material were removed. A crude preparation of antibodies, called "γG", was prepared from antisera by precipitation at 4°C in 40% saturated ammonium sulphate.

Pepsin digestion of "γG" or of whole antiserum, using a 2% ratio of pepsin/protein, was carried out at 37°C for 20 hours in 0.2 M acetate buffer, pH 4.0. The reactions were terminated by adjusting the pH to 8.0 with 2M NaOH. The $F(ab')_2$ fragment was not purified; the complete mixture of digestion products was used as the antibody preparation. The pepsin digests were stored in PBS (0.14M NaCl, 0.02M phosphate buffer, pH 7.4) and were dialysed against TMK (0.01M Tris, HCl, pH 7.4, 0.0015M $MgCl_2$, 0.01M KCl) before use. Quantitative precipitin reactions were carried out in TMK to determine the optimum ratio of antibody, or antibody fragment, and its respective antigen that gave maximum precipitation. While maintaining the optimum ratio, the amounts of antibody and antigen were adjusted to yield about 0.5 mg of protein.

The contribution of the nascent peptide to the total amount of antigen was estimated to be, at most, 0.5% of the weight of the polyribosomes (6). Consequently, by limiting the polyribosomes to 1 mg and reducing the added hemoglobin by 5 µg from the optimum amount that was required to cause maximum precipitation with the added antibody, the actual precipitation was performed in slight antibody excess. This ensured that all antigen, including that on polyribosomes in the form of nascent peptides, was precipitated. To limit degradation by RNase, yeast RNA (0.5 mg) was added to each portion of the polyribosome suspension that was used in the precipitation reaction.

The immunoprecipitation of polyribosomes was carried out in TMK in a total volume of 1.5 ml. The required amount of either the specific antibody (i.e. goat anti-rabbit hemoglobin) or the control antibody (i.e. rabbit anti-rat hemoglobin) was added to the polyribosome suspension (50 µl) and allowed to react at 4°C for 5 minutes. The appropriate amount of either rabbit hemoglobin or rat hemoglobin was added followed by incubation at 37°C for 10 minutes and then 1 hour at 0°C. After incubation, a portion of each suspension was used to determine the total TCA precipitable radioactivity. The remainder of the suspension was centrifuged at 1000g for 30 minutes. The pellets were washed twice with TMK buffer, dissolved in 0.1M NaOH and their radioactivity was measured.

Results

The total polyribosomes were collected from rabbit reticulocytes that had been labelled in vivo with ^{32}P-sodium phosphate. Suspensions of these polyribosomes were treated with a "γG" preparation of either the specific antiserum or the control antiserum. The majority of the polyribosomes were co-precipitated by both these preparations (Table 1) indicating, as previously reported (3,6), that the reaction lacked specificity. However, the specificity was significantly enhanced by digesting the "γG" preparations with pepsin. The results (Table 1) showed that after such a treatment, the specific antibodies brought down an increased proportion of the polyribosomes while the extent of precipitation by the control antibodies was greatly reduced.

The advantage of using a pepsin digest was confirmed when a suspension of the ^{32}P-labelled reticulocyte polyribosomes was separated by sucrose density gradient centrifugation and then each fraction was treated with the digested "γG". The profiles of precipitated radioactivity (Fig. 1) demonstrated that the reaction was highly selective. Thus, whereas the digest of specific

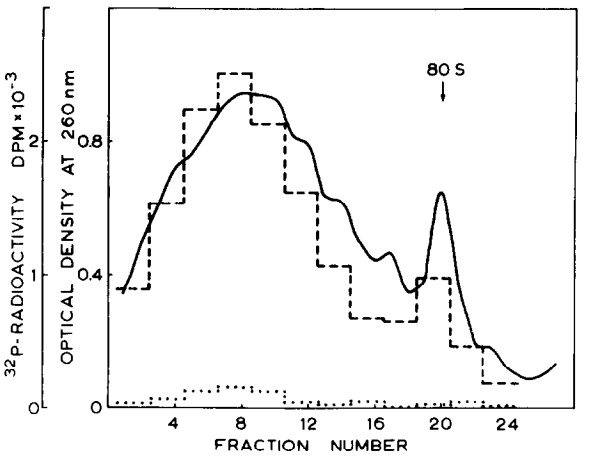

Fig. 1: Sucrose density gradient separation and immunoprecipitation of reticulocyte polyribosomes.

Adjacent fractions from the gradient were pooled and 0.2 ml portions were treated with the pepsin digest of "γG" from either specific (- - - - - - - -) or control (. . . . . .) antisera. Optical density at 260 mμ (_____).

antibodies gave a profile of precipitated radioactivity closely following the distribution of polyribosomes throughout the gradient, the digest of control antibodies brought down an insignificant amount of material.

To simplify the procedure, the feasibility of using a pepsin digest of the whole antiserum was investigated. When such a preparation of the specific or control antiserum was used, the reaction was found to be as specific as when peptic digests of "γG" were used (Table 1).

Table 1

The Co-Precipitation of Reticulocyte Polyribosomes by Various Preparations of Antibodies

Antibody Preparation	Radioactivity precipitated by antibodies, % of total	
	Specific	Control
"γG"	77	61
Pepsin digest of "γG"	88	2
Whole antiserum	55	53
Pepsin digest of whole antiserum	85	3

Specific antibodies were goat anti-rabbit hemoglobin and Control antibodies were rabbit anti-rat hemoglobin.

The precipitation of polyribosomes using two antisera in sequence has also been demonstrated to lack specificity (3). In further experiments the possibility was investigated that pepsin digestion of each antibody may lead to increased specificity. The comparison was made using the whole digest of the "γG" fraction of each serum. The peptic digest of goat anti-rabbit hemoglobin was incubated at 37°C for 2 minutes with a suspension of ^{32}P-labelled reticulocyte polyribosomes. The optimum amount of a peptic digest of rabbit anti-goat IgG was added, followed by incubation first at 37°C for 5 minutes then at 0°C for 1 hour. As a control, parallel reactions were performed

using a peptic digest of "γG" from normal goat serum in the first stage of the reaction The results showed that the specific peptic digests precipitated 42% of the available radioactivity compared with 24% brought down by the control antibodies and suggested that purification of $F(ab')_2$ from the digests was required.

Discussion

The separation of polyribosomes synthesizing a single protein by co-precipitation with antibodies to the antigenic determinants on their nascent peptide chains has important implications. Not only would it allow the identification of polyribosomes synthesizing a given protein but it would provide a means towards isolating the mRNA that directs the synthesis of that protein. However, it had been demonstrated that the reaction was not sufficiently specific when whole antisera were used in a medium which retained the integrity of the ribosomes (3,6) One cause for this non-specificity was ascribed to the binding of IgG to ribosomes through the Fc portion (4,5,7). In an attempt to reduce this binding antibodies were digested with pepsin. The $F(ab')_2$ fragment was not purified from the digest for it was considered sufficient for these exporatory experiments to simply "detach" the Fc' portion from the antibody combining sites.

The results clearly demonstrated that the procedure leads to a more specific precipitation of polyribosomes. It is not necessary to purify the antibodies from the antisera before digestion with pepsin, for digests of whole antisera were highly specific in precipitating polyribosomes. The specificity of precipitation that is obtained using the peptic digests of the antibodies is more dramatically demonstrated by the results shown in Figure 1. The reaction exhibits a high degree of selectivity, with only the digests of the specific antibodies precipitating significant amounts of the polyribosomes along the gradient. This result may be constrasted with previous results, in which both specific and control antisera precipitated similar amounts of the polyribosomes throughout the gradient (3,6).

In contrast, the results of the experiment using the combined digests of two antisera were characterized by their low specificity and this finding is receiving further attention. Its likely cause is that the Fc' fragments in the digest of the first "γG" solution bind to ribosomes and then the $F(ab')_2$ fragments in the second digest react with them and hence cause the inclusion of polyribosomes in the precipitate. Alternatively, a portion of the IgG may be resistant to digestion with pepsin (8). To carry out the reaction using two antibodies. and also achieve high selectivity, it will be necessary to separate the $F(ab')_2$ from the digest.

Acknowledgements

Financial support from the Medical Research Council of Canada and the Banting Research Foundation is acknowledged. S.L.B. was the holder of a National Research Council of Canada Postgraduate Scholarship.

References

1. Williamson, A. R., and Askonas, B. A., J. Mol. Biol. 23, 201, (1967).
2. Hartlief, R.,and Koningsberger, V. V., Biochim. Biophys. Acta 166, 512, (1968).
3. Holme, G., Delovitch, T. L., Boyd, S. L., and Sehon, A. H., Biochim. Biophys. Acta (in press).
4. Kern, M., Helmreich, E., and Eisen, H. N., Proc. Natl. Acad. Sci. U.S. 47, 767, (1961).
5. Swenson, R. M., Cohen, H. J., D'Amico, R. P., and Kern, M., J. Biol. Chem. 244, 440, (1969).
6. Boyd, S. L., Holme, G., and Sehon, A. H., Arch. Biochem. Biophys. (submitted)
7. Moav, B.,and Harris, T. N., J. Immunol. 104, 957, (1970).
8. Leslie, G. A., and Benedict, A. A., J. Immunol. 104, 810, (1970).