TRITIUM LABELLING OF THE LYMPHOCYTE - STIMULATING LEUCOAGGLUTININ FROM KIDNEY BEANS (PHASEOLUS VULGARIS)

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SUMMARY

Lymphocyte-stimulating leucoagglutinin isolated from extract of kidney beans (Phaseolus vulgaris) *was labelled with tritium by treatment with acetic anhydride-^3H in aqueous solution. The labelling procedure used, which introduced 1.7 acetyl groups per leucoagglutinin molecule (molecular weight:140 000) had no detectable effect upon the lymphocyte-stimulating or agglutinating activity of the leucoagglutinin. The label was found to be stable after 2 months' storage at 4°C or -20°C.*

INTRODUCTION

Extracts of kidney beans contain glycoproteins with lymphocyte-stimulating as well as erythro- and leucoagglutinating activities (1-4). Some authors have labelled crude bean extracts with radioisotopes (5), sodium isothiocyanate (6,7) or mercury (8). However, there has been no evidence that the labelling is specific to the lymphocyte-stimulating principle, and it is erroneous to claim that the distribution of the label in lymphocyte cultures was identical with that of the stimulating factors. Conard and Demoise labelled kidney beans by growing them in a nutrient medium containing tritiated water (9). From these beans they isolated a lymphocyte-stimulating protein in highly purified form. Unfortunately, the specific activity of their protein was only 0.1 - 0.5 μCi/mg,

and their labelling procedure must be considered very complicated and tedious. It has previously been shown that antibodies can be acetylated with acetic anhydride without losing their ability to combine with antigen (10,11). Agrawal et al. have acetylated concanavalin A, the lectin from jack beans (Canavalia ensiformis), without inactivating it (12). However, it has previously been reported that kidney bean extracts lose their agglutinating activities upon acetyaltion (13). Nevertheless, it appeared worth while to try to elaborate a method for the acetylation of the kidney bean leucoagglutinin, using tritiated acetic anhydride.

MATERIAL AND METHODS

Leucoagglutinin, prepared as previously described (3,4), was acetylated essentially according to Nisonoff and Pressman (11). 10 - 12 mg leucoagglutinin

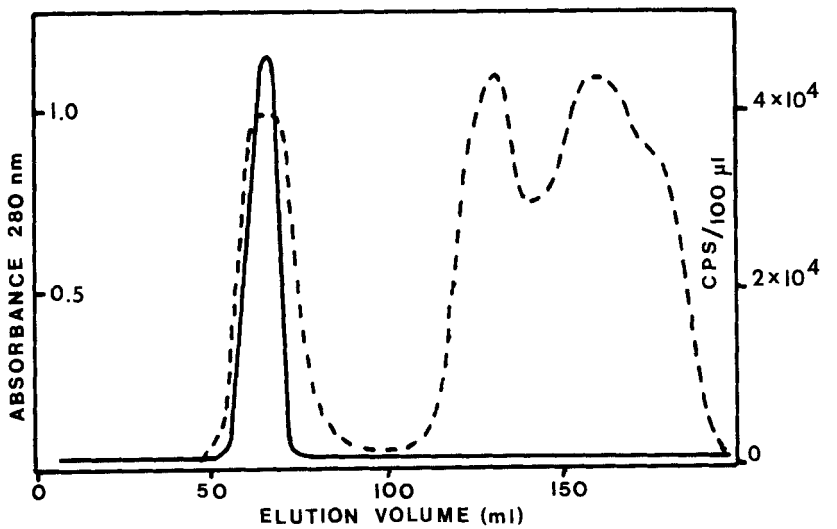

Fig. 1. Exclusion chromatography of the reaction mixture after acetylation of kidney bean leucoagglutinin. Sephadex G-25, Medium, column size 35.5 cm x 2.5 cm, fraction volume 3 ml, flow rate 10 - 12 ml/hour, elution buffer: saline (pH 7.2). The interrupted line indicates the radioactivity and the continuous line the protein concentration.

was dissolved in 4 ml of phosphate-buffered saline (pH 7.2). Immediately before the acetylation the pH was brought to 9.0 by addition of some drops of 1 M Na_2CO_3. The solution was chilled to 0 °C in an ice bath and added to 25 mCi precooled acetic anhydride -^3H (The Radiochemical Centre, Amersham, specific activity 5.8 μCi/mmol). The reaction was allowed to proceed for 30 min at 0 °C, after which time 20 mg lysine-HCl in 0.5 ml distilled water was added to stop the acetylation of protein. The solution was subsequently submitted to exclusion chromatography on Sephadex G-25, Medium (Pharmacia) at 4 °C in order to remove non-protein reactants (Fig. 1). After rechromatography on Sephadex G-25, the labelled leucoagglutinin was sterilized by Millipore^R filtration and stored at 4 °C or -20 °C.

RESULTS

Properties of the labelled leucoagglutinin

The labelled leucoagglutinin had biological properties identical with the starting material, i.e. maximum lymphocyte stimulation at a concentration of 1 - 3 μg/ml and detectable leucoagglutination at 5 - 10 μg/ml (3,4). The radioactivity of the labelled leucoagglutinin was measured in a liquid scintillation counter (Wallac Inc., Turku, Finland), using external and internal standards. The specific activity of the leucoagglutinin was 70 μCi/mg protein, which means that the labelling procedure used introduced 1.7 acetyl groups per protein molecule. The molecular weight of the leucoagglutinin is 140 000 (3). The conditions chosen ensured that only the free amino groups were labelled (14).

Stability of the labelled leucoagglutinin

The stability of the label was followed over a period of 2 months. Samples of 100 μl diluted 1:5 with non-radioactive carrier leucoagglutinin were submitted to exclusion chromatography on Sephadex G-25, Fine (column size 26 cm x 0.7 cm). The amount of radioactivity released from the protein was checked at certain time intervals (Fig. 2). Less than 5 per cent of the label was released during two months of storage at 4 °C. Storage at -20 °C significantly increased the stability of the label.

DISCUSSION

The procedure used for labelling the kidney bean leucoagglutinin thus yields a product of good stability and high specific activity. The labelled leucoagglutinin has been used in short-term in vitro lymphocyte cultures in

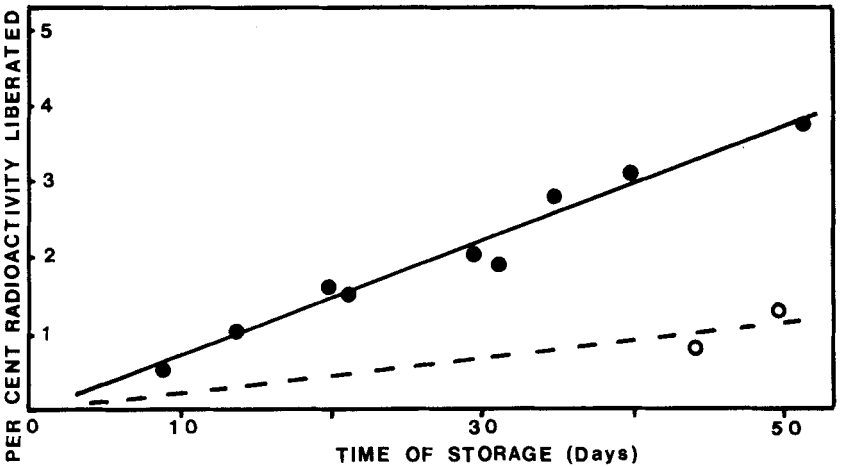

Fig. 2. Stability of label during storage of leucoagglutinin at 4 °C (continuous line) and -20 °C (interrupted line).

order to elucidate the site of the lymphocyte-stimulating factors in the cells. The stability of the label during lymphocyte culture is likely to be good as it has been shown that the lymphocyte-stimulating factors are not consumed by the cells (15,16) and enzymatic deacetylation of an intact protein molecule is not to be expected. In contradiction to previous opinions (5-9), the stimulating leucoagglutinin was found to be situated predominantly near or on the outer cell membrane of the lymphocytes during in vitro cell culture (17,18). This discrepancy is probably due to the fact that, in earlier studies, pure and homogenous stimulating factors have not been used or that the specific activity of the labelled substances has been too low to allow the use of adequate concentrations. The tritium-labelled leucoagglutinin provides an excellent tool for the study of the mechanism of lymphocyte stimulation in vitro and for the study of the interactions between lymphocytes and the kidney bean leucoagglutinin.

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