Monitoring of Active Groups of Cyanogen-Bromide-Activated Paper Discs Used as Allergosorbent

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Abstract

The amount of active groups of cyanate esters and imidocarbamates present on cyanogen-bromide-activated paper discs were estimated. The [protein-binding](#) capacity of activated discs was found to be dependent on the amount of these active groups. The results suggested that monitoring of active groups on activated discs can help in preparing consistent allergosorbent for immunoassays.

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Cyanogen bromide (CNBr)-activated paper discs are widely used as allergosorbent in the radioallergo-sorbent test (RAST) for estimating allergen-specific immunoglobulin E [1]. The CNBr activation of paper discs depends on experimental conditions such as pH, temperature and duration of activation which in turn affect the binding of allergen proteins to the discs. The degree of activation of paper discs could be one of the reasons for variation in the RAST results observed in different laboratories. Therefore, it is necessary to standardize the CNBr-activated paper discs before allowing coupling with allergen proteins. The activation of paper discs with CNBr results in the formation of active groups of cyanate esters and imidocarbamates and inactive carbamate groups on activated paper [2, 3]. The cyanate esters are reported to be predominantly more active in binding protein, while imidocarbamates are less reactive [3]. Earlier workers standardized CNBr-activated discs on the basis of increase in total nitrogen content following activation [3], but this does not distinguish between the nitrogen incorporated in active/inactive groups on activated discs. The estimation of cyanate esters is often recommended while preparing affinity chromatography media from CNBr-activated Sepharose, Sephadex or cellulose [4]. In the present study, a similar approach has been adopted for CNBr-activated paper discs.

The paper discs were activated with CNBr according to the method described by Gleich and Yunginger [5] with slight modifications. Briefly, 1 g of discs punched from Whatman paper were activated with 2 g of CNBr dissolved in dimethylformamide. The discs were tumbled on a horizontal shaker for 10 min at 4°C, and pH was maintained between 10 and 11. The discs were washed at least 5 times with 200 ml of cold 0.1 M NaHCO₃ and 4 times with cold acetone. The activated discs were dried over anhydrous calcium chloride in a vacuum desiccator. Two hundred milligrams of lyophilized pollen extract was allowed to react with 1 g of CNBr-activated paper discs (approx. 1,000 discs) as described by others [5]. The weed pollen extracts (*Xanthium strumarium*, *Artemisia scoparia* and *Ageratum conyzoides*) utilized in the present study bind with CNBr-activated paper discs in the range of 50–80%. It was

believed that 200 mg pollen extract was sufficient to react with all the free cyanate ester and imidocarbamate groups on 1 g CNBr-activated paper discs.

The comparison of three types of Whatman papers studied showed that the CNBr-activated paper discs punched from Whatman 50 had more active groups (cyanate esters 200 $\mu\text{mol/g}$ discs; imidocarbamates 0.414 mg N/g discs) as compared to Whatman 1 and Whatman 40 discs (table 1). The activation of discs resulted in a 3.38-fold increase in protein-binding capacity of Whatman 50 discs, a 2.20-fold increase in Whatman 42 discs and a 3.42-fold increase in Whatman 1 discs as compared to that of corresponding plain discs (table 2). The protein-binding capacity was also maximum in the discs punched from Whatman 50 paper (table 2). This indicated that protein

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Table 1. Estimation of cyanate esters and imidocarbamates content on CNBr-activated discs

Table 3. Increase in total nitrogen after activation of paper discs with CNBr (mg N/g discs)

König reagent was eluted with dimethylformamide and absorbance read at 590 nm.

Imidocarbamates were estimated by acid hydrolysis of imidocarbamates to NH_4^+ [6]. The nitrogen in the NH_4^+ was then estimated by the micro-Kjeldahl method [7].

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Table 2. Protein-binding capacity of activated paper discs (mg protein/g discs)

Paper

1.875 4.125 2.250

3.250 11.000

7.750

Whatman 1 Whatman 42 Whatman 50

Plain and CNBr-activated paper discs were exposed to 1 % BSA at 4°C for 24 h on a horizontal shaker followed by 7–8 washings with borate-saline buffer (pH 8). The coupled protein was estimated by measuring the increase in nitrogen incorporated in the paper discs.

7a 5-

M

J? 0

30

3

Patients

Fig. 1. Comparison of RAST results of allergosorbent prepared from Whatman 1 (□) and Whatman 50 (■) discs. Allergen-specific IgE expressed as PRU/ml in sera of 5 patients sensitive to weed pollen was estimated by Phadebas RAST kit (Pharmacia Diagnostics, Sweden).

binding was directly proportional to the amount of active groups present on the discs. The small amount of protein bound by plain paper discs (table 2) was not simply due to adsorption, as treatment with 1 M NaCl did not remove the bound protein. It could be due to the presence of certain other reactive groups present in cellulose chains of Whatman paper.

As evident from table 3, the increase in total nitrogen following CNBr-activation was maximum in Whatman 50 discs; they also showed a corresponding increase in protein-binding capacity

(table 2). However, it is very inconvenient and laborious to estimate the nitrogen content in discs by the micro-Kjeldahl method after digestion of the activated discs with sulphuric acid and selenium dioxide, as done by Brighton et al. [3]. In comparison, it is easier to estimate the cyanate esters on the CNBr-activated paper discs. The qualitative or quantitative test for cyanate esters [6] can readily give an idea of the extent of activation of paper discs. On the other hand, the estimation of increase in total nitrogen of activated discs cannot distinguish between the nitrogen incorporated in active groups (cyanate esters, imidocarbamates) or inactive carbamate groups.

In the present study, very reproducible results on RAST were obtained with different batches of allergosorbent prepared from CNBr-activated Whatman 50 discs, while poor or low RAST results were obtained with allergosorbent prepared from paper discs having low amounts of cyanate esters (fig. 1). Phadebas RAST units (PRU), estimated by RAST in sera of 5 patients sensitive to weed pollen, showed lower PRU with allergosorbent prepared from activated Whatman 1 discs as compared to Whatman 50 discs

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(fig. 1). From these results, it can be concluded that RAST results are significantly affected by the degree of activation of paper discs. Therefore, the estimation of cyanate esters on CNBr-activated paper discs prior to coupling with pollen allergen extracts can help in preparing a good allergosorbent and in maintaining the consistency of RAST results.

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