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Immunochemical cross-reactivity between albumin and solid-phase adsorbed histamine

L. K. Poulsen[1], H. Nolte[2], I. Søndergaard[3] and P. Stahl Skov[1]

[1] Laboratory of Medical Allergology and The Biotechnological Center of Immunology Medical Department TTA 7542, State University Hospital, Tagensvej 20, DK-2200 Copenhagen N, Denmark
[2] Paediatric Department, Århus Community Hospital, DK-8000 Århus C
[3] Chemical Institute, Royal Veterinary and Agriculture University, Thorvaldsensvej 40, DK-1871 Frederiksberg C

Abstract

For production of an antibody against histamine, this was coupled to human serum albumin (HSA) and used for immunization of rabbits. To test the antiserum, an immunoradiometric assay was developed comprising solid-phase bound histamine, antisera and radiolabelled protein A. Titration and inhibition experiments revealed that histamine adsorbed onto a solid-phase could bind the antiserum. However, neither free histamine nor histamine coupled to unrelated carriers could inhibit the binding of antiserum to the solid-phase histamine. Cross-reactivity was demonstrated between HSA and solid-phase bound histamine, as the immunoradiometric assay was inhibited by HSA. This unexpected cross-reactivity was established, as a commercially available antiserum with specificity to HSA without histamine also bound to the solid-phase bound histamine. It is suggested that preparations of antibodies against histamine are tested for this possible cross-reactvity.

Introduction

Histamine is a potent mediator, which is involved in many physiological and pathological conditions. Several attempts have therefore been made to develop sensitive analyses for histamine. In this respect specific antibodies to histamine are promising, being applicable not only for detection of histamine in fluid-phases, culture media, etc., but also for histological investigations. The procedure of coupling a low-molecular hapten to a carrier in order to make it immunogenic has been known for many years, and several investigators have applied the procedure for production of antisera against histamine [1–6]. We present experimental results concerning the production of an antiserum against histamine and tests of the specificity of this preparation in an immunoradiometric assay.

Abbreviations: a-his, Rabbit antibody against histamine-albumin-conjugate; a-HSA, Rabbit antibody against human serum albumin; BGG, Bovine gammaglobulin; BSA, Bovine serum albumin; cpm, Counts per minute; HSA, Human serum albumin, NRS, Normal rabbit serum; OA, Ovalbumin; PBSP, hosphate buffered saline.

Correspondence to: Lars K. Poulsen at the above address.

Materials and methods

Histamine-protein conjugates and immunosorbents

Coupling of histamine to rabbit albumin by the use of 1-ethyl-3-(dimethylaminoproyl) carbodiimide*HCl has been described previously [7] although the reaction conditions were not explained in details. In short, 25 mg of histamine dihydrochloride (Merck) were added to 10 ml of a solution of human serum albumin, HSA, (Sigma) con-

taining 10 mg/ml in distilled water. Subsequently, fifty mg of 1-ethyl-3-(dimethylaminopropyl) carbodiimide hydrochloride (Sigma) were added and allowed to react for 24 h at room temperature. Finally, the solution was dialyzed against distilled water and lyophilized. Conjugates between histamine and bovine gamma globulin (BGG, Sigma), ovalbumin (OA, Sigma) or bovine serum albumin (BSA, Sigma) were produced in the same manner using one fifth of the above mentioned reagents.
Immunosorbents of HSA or OA coupled to cyanogenbromide activated Sepharose (Pharmacia, Uppsala, Sweden) were prepared according to the instructions of the manufacturer.

Immunization

Three rabbits were immunized with 50 µl of histamine-albumin-conjugate (4 mg/ml) mixed with 50 µl of Freund's incomplete adjuvant. The animals received injections every second week for two months, and subsequently every fourth week for 2½ years. After 2½ months of immunization blood samples were drawn monthly, and sera from the three rabbits were pooled (Dakopatts A/S, Glostrup, Denmark). In the present study, antisera (called a-his) from the last part of the immunization period were used.
Immunoglobulin fractions of normal rabbit serum (NRS) and rabbit antibody against human serum albumin (a-HSA) were obtained from DAKOPATTS (Code nos. X 903 and A 001).

Radiolabelled protein A

Protein A was iodinated by means of a modification of the Chloramin T method [8]: To 100 µg of protein A (Pharmacia) in 100 µl of PBS, 700 µCi of 125-I (sodium iodide, Amersham, England, Code no. IMS 30) and 50 µl (100 µg) of Chloramin T were added. The reaction was stopped after 60 seconds by addition of 50 µl (100 µg) of sodium metabisulphite. Desalting was made on a Pharmacia PD-10 column (Pharmacia) in PBS containing 10 mg of HSA/ml. In the assay the 125-I-protein A was diluted 1:600 in Pipes. More than 97% of the radiolabelled preparation was precipitable by trichloroacetic acid.
The iodinated protein A was tested with polystyrene tubes (see below) coated with human IgG (Sigma) 0.1 mg/ml or HSA 0.1 mg/ml as described below. The IgG-coated tubes bound 94.5% of the radioactivity added, whereas the latter bound less than 0.2%.

Immunoradiometric assays for antibody specificity

Two sandwich radioimmunoassays for specificity of antibodies against histamine and albumin were developed. Both were based on high adsorption polystyrene tubes ('Maxisorp' test tubes, 70 × 11 mm, Nunc, DK-4000, Roskilde, Denmark).

Histamine coated tubes were prepared by incubation overnight of the test tubes with histamine 10 mg/ml in Pipes buffer (10 µ*M* of piperazine-N, *n*-(bis-ethane sulphonic acid), analytical grade). The next day the tubes were washed 4 times in PBS (sodium phosphate 10 m*M*, sodium chloride 150 m*M*, sodium azide 100 mg/l, pH 7.4) + 0.1% Tween.

Albumin coated tubes were made according to the same procedure by incubation of the tubes with HSA (1 mg/ml) in Pipes buffer.
Blocking of the tubes with an irrelevant carrier (ovalbumin, 10 mg/ml), did not change the binding compared to washing 4 times with 0.1% tween. Thus only wash with tween-containing buffer was used as blocking.
The antibodies were diluted in Pipes buffer and 300 µl were added to the tubes and incubated at 37 °C for 2 h. The tubes were washed 4 times in PBS, and incubated overnight with 300 µl of radiolabelled protein A. Finally the tubes were washed and counted in a gammascintillation counter (LKB Clinigamma 1272) for 1 min. All determinations were done in duplicate and results are given as means. In figures are depicted the range.

Results

Dose-response experiments

Based on previous experiences with assays in which proteins are coated onto the walls of polystyrene tubes, we made an assay for antibody specificity to histamine. Antiserum (a-his, diluted 1:100 to 1:10000) raised against histamine-albumin-conjugate was added to histamine coated

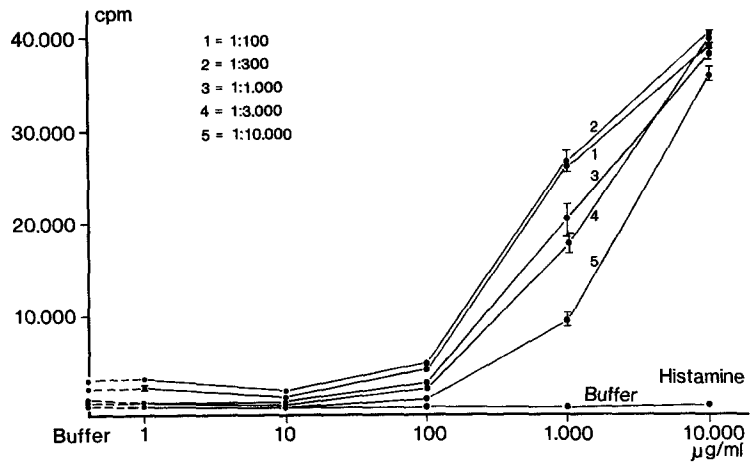
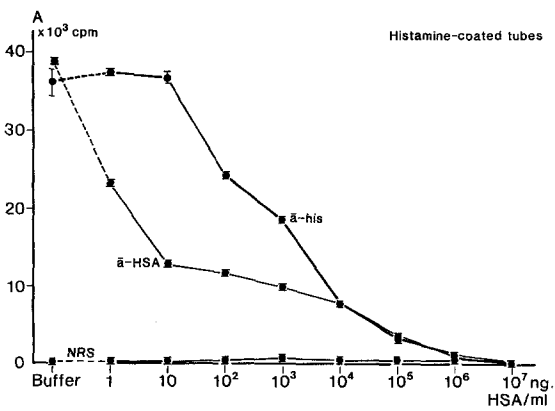
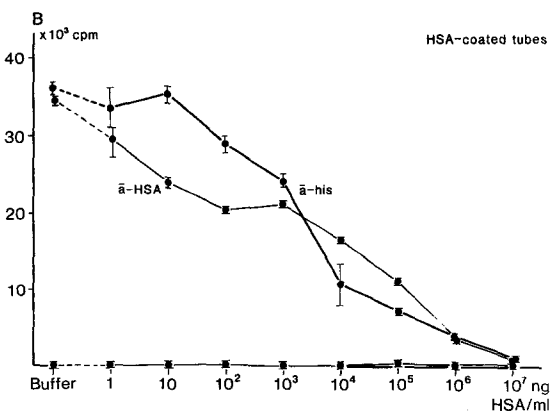


Figure 1
Antiserum titration curves. Histamine in varying concentrations (1 µg/ml to 10 mg/ml) was coated to test tubes and incubated with varying dilutions (1:100 to 1:100 000 = 1–5) of antiserum raised against histamine-HSA-conjugate. Buffer: Coating without histamine and/or incubation without antiserum. Bound antibody was detected by addition of radiolabelled protein A (90 000 cpm).





tubes and finally detected with radiolabelled protein A. Titration curves for solid-phase bound histamine and antiserum concentrations (concentrations of histamine used for coating: 1 µg/ml to 10 mg/ml) are shown in Fig. 1. It appears that a-his binds to the tubes in a way dose-dependant of both histamine and antiserum. Incubation of buffer instead of histamine and/or antiserum was included as a control. When either histamine or a-his are omitted, radioactivity comparable to the lowest doses of these reagents were detected in the tubes. Neither did normal rabbit serum (NRS) or hyperimmune serum of unrelated specificity, cause any binding to the histamine-coated tubes as demonstrated for NRS in Fig. 2A.

Inhibition experiments with free histamine

To check the specificity of the histamine-antibody binding, histamine in concentrations from 100 ng/ml to 1 mg/ml was preincubated with a-his before

Figure 2
Inhibition experiments in which antisera preincubated with HSA in varying concentrations (0–10^7 ng of HSA/ml) were added to test tubes. The binding of antibody was detected by additions of radiolabelled protein A (100 000 cpm). a-his: antiserum raised against histamine-HSA-conjugate. a-HSA: antiserum raised against HSA. NRS: Normal rabbit serum. A: Histamine-coated tubes (10 mg/ml). B: HSA-coated tubes (1 mg/ml).

Table 1
Affinity chromatography of a-his antiserum. The antiserum was applied to either of the two columns and incubated for 2 h at room temperature. Elution was performed as described in Materials and methods. Fractions were diluted 100 times and tested in tubes coated with histamine. Results (±standard deviation) in c.p.m.

	Effluent	Eluent
HSA-sepharose column	1415±105	16543±116
OA-sepharose column	12745±237	2560± 32
Buffers	1899± 8	889± 1

addition to the tubes (Table 2). Surprisingly, no inhibition occurred. Also unsuccesfull was an attempt to bind radiolabelled histamine with a-his adsorbed onto sepharose-protein A particles (results not shown). These data indicate that specificity of a-his to a histamine-epitope is expressed only when histamine is coupled to a solid-phase or a macromolecule like HSA.

Cross-reactivity between solid-phase bound histamine and HSA

The binding between a-his and solid-phase bound histamine could readily be inhibited by HSA (Fig. 2A). Furthermore, it was shown that antiserum against pure HSA would also react with solidphase bound histamine, and this reaction was also inhibited by HSA in a dose-dependent way (Fig. 2A). As could be expected, both antibodies bound to HSA-coated tubes (Fig. 2B). The binding of antisera to both solid-phase bound histamine and HSA, was inhibited in a similar way as shown by the shape of the curves in Fig.2A and 2B. NRS bound neither to histamine nor to HSA when tested against them. The experiments depicted in Fig. 2 were repeated on several occasions.
To further investigate whether the histamine binding activity in the antiserum could be dissociated from the albumin reactivity, affinity chromatography was performed. Table 1 gives the binding to histamine-coated tubes in an experiment in which the a-his antiserum was fractionated on either HSA-sepharose or OA-sepharose. This experiment clearly demonstrated that the eluent from the HSA-column also contained the histamine reactive part. In the control experiment all the histamine binding activity was recovered in the effluent.

Table 2
Inhibition (in %) of the binding of a-his antiserum to histamine coated tubes.
Free histamine and native proteins:

Concentration of inhibitors (μg/ml)	Histamine	HSA	OA	BGG
1000	30	87	−20	−14
100	9	99	− 2	21
10	27	85	21	15
1	11	47	22	9
0.1	17	21	6	−12

Histamine-conjugated proteins:

Concentration of inhibitors (μg/ml)	his-HSA	his-BSA	his-OA	his-BGG
1000	88	−1	−5	−5
300	77	−3	−3	−4
100	not done	4	0	−5
10	26	−4	−1	−7
1	−12	−5	−6	−6

his = histamine
HSA = human serum albumin
BSA = bovine serum albumin
OA = ovalbumin
BGG = bovine gamma globulin

A further indication of a common epitope between solid-phase bound histamine and HSA was provided by the time course of immunization depicted in Fig. 3. A close correlation (Spearman's rank coefficient of correlation, $r=0.88$, $p<0.001$) was observed between the histamine- and HSA-reactivity in the different blood samples drawn during the immunization. The half-yearly fluctuations probably stem from the immunization protocol, which initiates a new batch of histamine-albumin conjugate every 6 months. In order to examine the cross-reactivity between albumin and other carrier molecules, an inhibition test was set up. In the first experiment the anti histamine serum was preincubated with varying concentrations of histamine, human serum albumin, ovalbumin (OA) or bovine gamma globulin (BGG) (Table 2). Neither free histamine nor unrelated carrier molecules demonstrated any inhibition of the antiserum binding to solid-phase adsorbed histamine. This was also true when carriers conjugated with histamine was used for inhibition. Of the proteins HSA, bovine serum albumin (BSA), OA and BGG only the first

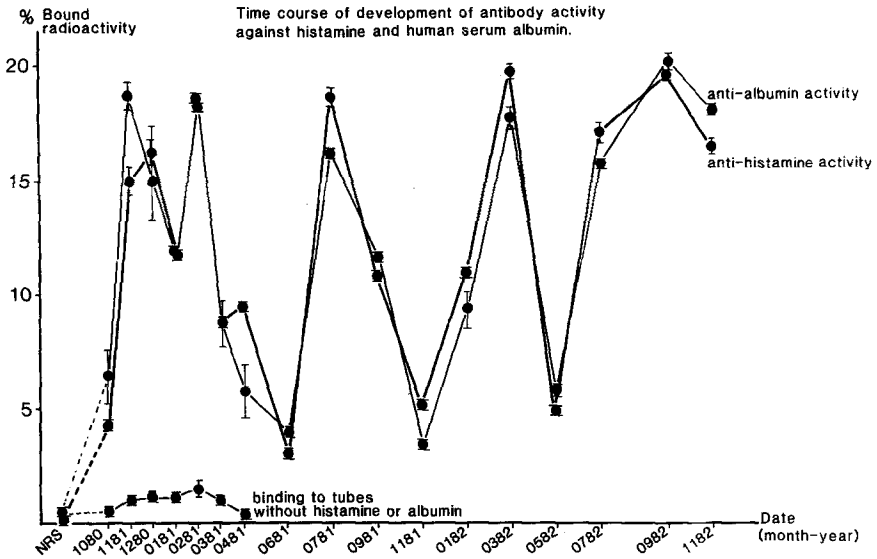


Figure 3
Time course of immunization of rabbits with histamine-HSA-conjugate. Antisera from blood drawn every second month during a three-year immunization period were added to test tubes coated with histamine (10 mg/ml), HSA (1 mg/ml) or buffer. Bound antibody was detected by addition of radiolabelled protein A (150000 cpm). Results are expressed as % bound radioactivity. Spearman's rank coefficient of correlation between anti-histamine- and anti-HSA-activity was 0.88 ($p<0.001$).

showed inhibition when coupled covalently to histamine (Table 2). This, togehter with the fact that free histamine does not cause inhibition, indicates that the epitope involved in antibody binding to solid-phase adsorbed histamine is a part of the HSA-molecule. The trivial error, i.e. that histamine or buffer are polluted with HSA, can be excluded, because the batch of histamine (and the buffer) used for coating and inhibition is the same.

Discussion

Histamine is known to bind to different solid-phase surfaces [9], thus it is not surprising that it will bind to these polystyrene tubes, which have been activated in order to make them highly adherent to proteins [10]. Dose-response experiments show, however, that higher histamine concentrations are necessary to produce a response equal to the one obtained with albumin coated onto the solid-phase. From the present data we cannot discriminate whether this is caused by a lower binding of histamine to the solid-phase or by a lower avidity of the antibodies. The binding of antiserum to histamine-coated tubes shows dose-dependency, and control experiments indicate that no unspecific binding of neither immunoglobulin nor 125-I-protein A to tubes with or without histamine occur. We therefore conclude that the assay does in fact measure the binding between antiserum and solid-phase bound histamine.

We were unable to inhibit a-his and a-HSA by the use of free histamine, whereas the antisera readily bound to solid-phase adsorbed histamine. Several investigators have attempted to produce antisera against histamine [1–6], but not all have been successfull. Some antisera with documented specificity against histamine have a much lower affinity to the free molecule than to histamine coupled to a carrier [3, 6]. This seems to be in accordance with systems in which low molecular weight substances act like haptens, such as ethylene oxide or acid anhydrides which have been shown to elicit IgE-mediated allergic reactions in man. Thorough investigations of patient sera have shown that in some cases the hapten itself or hapten-lysin conjugates completely lack inhibitory activity against the binding between antibody and hapten-HSA complex [11, 12].

We have no efficient explanation of the unexpected cross-reactivity between solid-phase bound histamine and HSA. The possibility that solid-phase bound histamine produces a "sticky" surface binding immunoglobulins in a non-specific way, can be excluded due to the facts that NRS and unrelated hyperimmune serum does not bind to the tubes, and further that preincubation of the antiserum with HSA inhibits the binding. The cross-reactivity is likely to be caused rather by a common determinant in which the imidazol-group of histamine and histidine is dominating. Little is known about the conformational changes of the histamine molecule imposed by its binding to solid-phase polystyrene. In the light of the findings described, we suggest that future preparations of antisera against histamine should be checked for this form of cross-reactivity.

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