SHORT COMMUNICATIONS

Precipitating Insulin-Antiinsulin Complexes*

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Summary. Beef insulin was chelated with glutaraldehyde into soluble aggregates possessing molecular weights of approximately 280 000. Antibodies to normal insulin which form soluble complexes with normal insulins can be precipitated with glutaraldehyde-insulin.

Complexes précipitants insuline-anti-insuline

Résumé. L'insuline de boeuf a été chélatée avec de la glutaraldéhyde dans des aggrégats solubles possédant des poids moléculaires de 280 000 approximativement. Les anticorps à l'insuline normale, qui forment des complexes solubles avec les insuline normales, peuvent précipiter avec l'insuline-glutaraldéhyde.

Präzipitierende Insulin-Antiinsulin-Komplexe

Zusammenfassung. Rinderinsulin wurde mit Glutaraldehyd zu löslichen Aggregaten vernetzt, deren Molekulargewicht etwa 280 000 betrug. Solche Aggregate präzipitieren Antikörper, die gegen normales Insulin gerichtet sind und die mit normalem Insulin lösliche Komplexe bilden.

Key-words: Insulin-glutaraldehyde aggregates, glutaraldehyde-insulin-chelates, insulin-antiinsulin precipitates.

Antigen-antibody complexes are thought to be insoluble because a large three dimensional arrangement of the reactants is formed (for literature references Steffen [6], Hansen and Werner [3]). A precondition for precipitation is that the antibody molecule should have two binding sites and the antigen molecule should have at least three determinant groups. When the antigen has only two determinant groups, then only linear soluble complexes between antigen and antibody will be formed.

Some investigators have reported conditions under which insulin and antiinsulin will form immunoprecipitates [2]. However, normally the insulin-antiinsulin complexes remain soluble, a fact which might be explained in two ways. First it is possible that antiinsulin is a univalent antibody or a fragment of a bivalent antibody which has lost one of its haptophore groups. Or secondly, the insulin molecule might not be able to bind three different antibody molecules, either because it has less than three determinant groups or because of its small size.

In order to test the second of these possibilities, aggregates of insulin which should have more determinant groups were made by treatment of insulin with glutaraldehyde [1, 5]. The results reported here show that such aggregates of insulin will indeed form insoluble precipitates with antibodies elicited both by normal insulin and by glutaraldehyde-insulin (GA-insulin).

Insulin aggregates were prepared by mixing 0.08 ml of a solution of glutaraldehyde (25%, w/v) and 2 ml of a solution of crystalline beef insulin¹ (2%, w/v in 0.05 M imidazole buffer pH 7.0). The pH was immediately readjusted to 7.0 by further addition of imidazole in order to prevent precipitation of insulin. The mixture was kept at 4°C for 48 h during which the colour gradually changed to yellow. Then the remaining aldehyde groups were destroyed by dialysis for 24 h against a 0.1% w/v solution of hydroxylam-

Fig. 1. Rechromatography on Sephadex G 150 of glutaraldehyde-treated insulin (front fraction of G 100 chromatographic run, —), bovine gamma globulin (dimer and monomer forms, - - -) and human serum albumin (.....) in 0.05 imidazole buffer pH 7.0

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¹ Generously supplied by the Farbwerke Hoechst

monium chloride (Merck, Darmstadt). The pH was then readjusted to 7.0 with imidazole and the remaining hydroxylamine was removed by dialysis against imidazole buffer pH 7.0. Finally, the solution was concentrated by pressure dialysis and buffer was added to obtain a solution of 2.0% insulin. This solution was subjected to gel filtration on Sephadex® G 100. The fraction eluting in the void volume was used for the following experiments and had a molecular weight of about 280 000 as estimated by filtration on Sephadex® G 150 (Fig. 1).

The effect of the glutaraldehyde-insulin (GA-insulin) on the concentration of blood sugar was tested in two insulin-responsive rabbits. The GA-insulin did not reduce the concentration of blood sugar

even though one hundred times the normal dose was used.

Guinea pigs were then immunized against GA-insulin. Each animal received a total dose of 14 mg GA-insulin within two weeks; the doses were injected intraperitoneally every second day and were increased from 1.0 mg at the start to 2.5 mg at the end. Ouchterlony diffusion tests were performed on glass slides with a solution of antigen (0.07%, w/v) and solutions of gamma globulins obtained from antisera to either regular beef insulin or GA-insulin, by fractionation with $(\text{NH}_4)_2\text{SO}_4$ [4]. A distinct precipitation line was observed between GA-insulin and its homologous antibody (Fig. 2a). No precipitation occurred, however, with a solution of regular beef insulin as antigen. Precipitation of GA-insulin could be prevented by adding regular beef insulin to the anti-GA-insulin antibody (Fig. 2b). This fact demonstrates that regular insulin and GA-insulin are immunologically similar. GA-insulin reacts not only with its homologous antibody but also with ordinary antiinsulin (Fig. 2c). The different intensity of the two precipitation lines is probably due to different antibody concentrations in the two gamma globulin preparations.

These results demonstrate that a chemical aggregation of the insulin molecule enables it to form insoluble immunoprecipitates. Thus the absence of an insoluble immunoprecipitate in the reaction between insulin and antiinsulin appears to be due to molecular size or other properties of insulin rather than to the antibodies which insulin elicits.

References

1. Avrameas, St., Ternynck, Th.: The cross-linking of proteins with glutaraldehyde and its use for the preparation of immunoadsorbents. *Immunochemistry* **6**, 53–66 (1969).
2. Ceska, M.: Insulin-anti-insulin precipitates in the presence of various dextrans. *Immunology* **15**, 837–843 (1968).
3. Hansen, K., Werner, M.: *Lehrbuch der klinischen Allergie*. Stuttgart: Thieme 1967.
4. Kallee, E., Lang, G., Heinzel, W.: Neuer quantitativer, immunologischer Nachweis der leichtreversiblen Adsorption an Proteine. *Acta isotop.* **3**, 303–321 (1963).
5. Sachs, D.H., Winn, H.J.: The use of glutaraldehyde as a coupling agent for ribonuclease and bovine serum albumin. *Immunochemistry* **7**, 581–585 (1970).
6. Steffen, C.: *Allgemeine und experimentelle Immunologie und Immunpathologie*. Stuttgart: Thieme 1968.

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Fig. 2. Immunodiffusion tests with different insulin antibodies and insulin preparations

- a) (1) anti-GA-insulin-[guinea pig gamma globulin]
(2) regular beef insulin (0.07%, w/v)
(3) normal guinea pig gamma globulin
(4) GA-insulin (0.07%, w/v)
- b) (5) anti-GA-insulin-[guinea pig gamma globulin], absorbed by addition of regular beef insulin (0.07%, w/v)
(6) GA-insulin (0.07%, w/v)
(7) anti-GA-insulin-[guinea pig gamma globulin], not absorbed
- c) (8) anti-GA-insulin-[guinea pig gamma globulin]
(9) GA-insulin (0.07%, w/v)
(10) ordinary antiinsulin-[guinea pig gamma globulin]