IMMUNOLOGICAL CROSS REACTIVITY BETWEEN BOVINE FIBRINOGEN AND BOVINE K-CASEIN*

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1. Introduction

Two coagulation processes occur in nature: the clotting of blood and the clotting of milk. The enzymic digestions of κ-casein and fibrinogen, the natural substrates of chymosin (EC 3.4.23.4) and thrombin (EC 3.4.4.13), respectively, present some common features which have been summarized by P. Jollès [1] in a recent review article: high specificity of the enzymes, conservation of the rennin- and thrombin-sensitive sequences in the course of the evolution, liberation of hydrophilic acid peptides (caseinoglycopeptides or fibrinopeptides) devoid of aromatic amino acids, cyst(e)ine and arginine. Furthermore homology in the amino acid sequences between cow and sheep κ-caseins and the β and γ chains of human fibrinogen has been suggested by J. Jollès et al. [2].

These observations constituted the starting point of a first series of immunological studies concerning a possible cross reactivity between fibrinogen and κ-casein.

2. Materials and methods

Cow whole casein, cow α_S-casein, cow κ_B-casein and sheep κ_B-casein were prepared according to Alais and Jollès [3,4]. Bovine fibrinogen was a gift from Hoffmann-La Roche (Basle).

Anti-human fibrinogen antisera were commercial samples (Centre Régional de Transfusion Sanguine et de Génétique humaine, F-76230 Rouen Bois-Guillaume). Anti-cow κ_B-casein antisera were prepared by subcutaneous injections of 1 mg κ_B-casein in 0.5 ml of saline solution emulsified with 0.5 ml complete Freund's adjuvant (Calbiochem) into two male rabbits (breed: Les Fauves de Bourgogne). A second injection of 1 mg protein was administered after one week followed by boosts every fortnight. Antisera were collected after the sixth boost by cardiac punction. The presence of precipitating antibodies against cow κ_B-casein was tested by immunoelectrophoresis. Each antiserum was active until 1/16 against homologous antigens. The protein concentrations were 3 mg/ml.

Immunodiffusion was performed in 1.5% agarose gel in 0.04 M veronal buffer pH 8.6 [5] for 48 h in a damp room. The precipitin lines were colored with amido-schwartz.

Immunoelectrophoresis was achieved in 0.01 M veronal buffer pH 8.6 [6] during 1 h (6.5 V/cm). After electrophoresis in 1.5% agarose gel, the antiserum was added and allowed to diffuse during 24 h. The proteins were stained with amido-schwartz.

3. Results and discussion

An immunological cross reactivity was observed between bovine fibrinogen and anti-cow κ_B-casein antiserum by immunoelectrophoresis and immunodiffusion.

* 37th communication on caseins; 36th communication, ref. [1].

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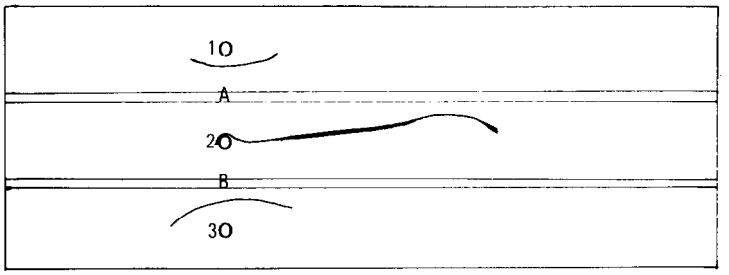

Fig.1. Immunoelectrophoresis concerning the reaction of two antisera: A (anti-cow κ_B-casein antiserum) and B (anti-fibrinogen antiserum) with: 1 and 3: bovine fibrinogen. 2: cow κ_B-casein.

3.1. *Immunoelectrophoresis*

We observed a single precipitation band between bovine fibrinogen and anti-fibrinogen antiserum and also between bovine fibrinogen and anti-κ_B-casein antiserum. The precipitin line between anti-fibrinogen antiserum and bovine fibrinogen was exactly at the same place as the one observed between bovine fibrinogen and anti-κ_B-casein antiserum (fig.1). Furthermore cow κ_B-casein and bovine fibrinogen had a different electrophoretic behaviour. No cross reactivity was observed between κ_B-casein and anti-fibrinogen antiserum. No reaction was noticed between non-immunized rabbit antiserum and bovine fibrinogen. The precipitin line between κ_B-casein and anti-κ_B-casein antiserum was complex and seemed to indicate either several antigenic sites in κ_B-casein or a micro-heterogeneity in the carbohydrate moity as previously mentioned [7].

3.2. *Immunodiffusion*

Ouchterlony tests corroborated the results obtained by immunoelectrophoresis and allowed an additional observation. When cow κ_B-casein and bovine fibrinogen were put in the presence of anti-cow κ_B-casein antiserum a spur of partial antigenic identity was observed (fig.2). Thus these two antigens seem to possess a common antigenic determinant. α_S-casein, a casein fraction devoid of the κ-component, did react neither with anti-cow κ-casein antiserum nor with anti-fibrinogen antiserum.

This first series of experiments using immunological techniques seem to corroborate our observation arisen from structural studies [2] concerning some homology between bovine fibrinogen and bovine κ-casein.

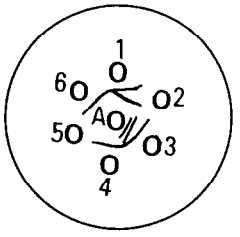

Fig.2. Ouchterlony patterns concerning the reaction of anti-cow κ_B-casein antiserum (A) with: (1) Cow whole casein. (2) Cow α_S-casein. (3) Cow κ_B-casein. (4) Bovine fibrinogen. (5) Sheep κ_B-casein. (6) Bovine fibrinogen.

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