Vol. 82, No. 3, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
June 14, 1978 Pages 887-892

MOLECULAR LOCALIZATION OF TWO CATTLE ALLOTYPIC SPECIFICITIES

Domenico Iannelli[1,2] and Giovanna de Benedictis[2]

[1]Institute of Animal Production, University of Naples, Portici, 80055, Naples (Italy)

and

[2]Department of Cellular Biology, University of Calabria, Cosenza (Italy)

Received January 12, 1978

SUMMARY: McA1 and McA2 are two cattle allotypic specificities carried on high molecular weight glycoproteins and controlled by allelic genes. Results derived from two independent experimental approaches (digestion of McA1 and McA2 antigen preparations with specific glycosidases and inhibition tests of anti McA1 and anti McA2 with simple sugars) showed that mannose is essential in determining the serological specificity of McA1 and glucose of McA2.

INTRODUCTION: At the present moment great interest is being shown in the phenomenon of allotypy since allotypes have proved useful in a number of immunological and genetic studies (1,2). Allotypes have already been described in various species, including cattle (3-6). McA1 - or Ci(a) as it was named when first described (4) - and McA2 are two cattle allotypic determinants carried on high molecular weight serum glycoproteins and controlled by allelic genes*. The system (two immunological specificities sharply differentiating one type of glycoprotein from the other and controlled by allelic genes) offered a valuable opportunity to study gene action at a biochemical level. In order to do so, it seemed appropriate to begin by ascertaining whether the isoantigenic difference was associated with differences in the amino acid portion of the molecule - as, for example, in the case of human Inv(a+) and Inv(a-) (7) - or in the prosthetic groups (carbohydrates) - as in the case of human ABH, Lewis and MN antigens (8). The latter possibility seemed equally likely a priori since the molecules on which McA1 and McA2 reside have the sugar residues necessary for interaction with ConA and WGL* and, consequently, contain carbohydrates which are known to be highly antigenic (9).

* Iannelli, D., unpublished results.

0006-291X/78/0823-0887$01.00/0

This paper presents evidence that one single sugar plays a dominant role in determining the serological specificity of each of the two allotypes: mannose is an essential component of the antigenic structure McA1 and glucose of McA2.

MATERIAL AND METHODS: Identification of McA1 and McA2. Originally these two specificities (referred to as A1 and A2, for brevity) were detected respectively by DD and SRD in agar. These techniques however proved unsatisfactory for studying the problems considered in the present paper. An alternative method for identifying the two allotypes was therefore developed, based on a passive hemagglutination reaction of SRC coated with preparations carrying the relevant allotype.

Sensitization of SRC. A1 and A2 antigen preparations used for SRC sensitization were obtained by ConA-Sepharose affinity chromatography from the serum of cows homozygous respectively for the A1 or A2 gene. A1 and A2, which bound to the column, were eluted with 1.0M α-methyl-D-glucoside concentrated by ultrafiltration (Amicon XM100 membranes) to 3.0 mg of protein per ml and linked on the surface of SRC with ConA (400 μg/ml) according to the method described by Leon and Young (10).

Hemagglutination test. Equal volumes (0.025 ml) of sensitized SRC and appropriate antiserum (diluted 1/50; 1/100; 1/200) were mixed in "V" type Microtiter trays (Cooke Engineering Co, Alexandria, Va) and allowed to stand for 30 min. at r.t. This incubation time was sufficient for agglutination to develop clearly. A control, where antiserum was replaced by diluent, was routinely included in the test.

Hemagglutination inhibition test. The sugar N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, 2-Deoxy-D-glucose, 2-Deoxy-D-galactose, D-mannose, D-glucose, D-glucosamine, D-fucose, L-fucose, D-galactose were tested as inhibitors of anti A1 and anti A2 antisera. Each sugar was tested at different molar concentrations (see Fig. 1) against three different dilutions of appropriate antiserum (1/50; 1/100; 1/200). Equal volumes (0.025 ml) of sugar solution and antiserum were mixed in "V" type Microtiter trays and incubated for 30 min. at r.t. Then 0.025 ml of sensitized SRC were added to each cup. Trays were read after 30 min. incubation and again after 1 and 2 hrs from the first reading. Controls, where either the antiserum or the inhibitor was replaced by diluent, were included in each test.

Enzymatic treatment of A1 and A2 antigen preparations. All enzymes were purchased from Boehringer Mannheim, GmH, Germany.
Incubation with αmannosidase (from the jack bean) was carried out in duplicate at 30°C for 12 hrs in 0.05M citrate buffer, pH 4.6 using E/AG ratios ranging from 0 to 0.5 (v/v). Incubation with α or βglucosidase (from yeast and sweet almonds respectively) was carried out in duplicate at 37°C for 24 hrs in 0.15M acetate buffer, pH 5.5 using E/AG ratios ranging from 0 to 0.15 (v/v). Incubation with βgalactosidase (from E. coli) was carried out in duplicate at 37°C for 24 hrs in 0.05M potassium

phosphate, pH 7.4 containing 0.01M $MgSO_4$ and using E/AG ratios ranging from 0 to 0.15 (v/v).
Enzyme action was tested by measuring mannose, glucose and galactose release according to Schachter (11).

RESULTS AND DISCUSSION: It is known that low molecular weight molecules having structures similar to, or identical with, the antigenic determinants of a complex antigen can combine with the corresponding antibodies and thus inhibit the successive antigen-antibody reaction (12). Application of this principle revealed that mannose is an important part of the A1 determinant. This sugar in fact, alone among those tested, inhibited the agglutination of A1 sensitized SRC by anti A1 antibodies (Fig. 1a). As has been said, a number of sugars (see Material and Methods), when tested under the same experimental conditions as those used for mannose, failed to inhibit anti A1 antibodies. This suggested that the inhibition displayed by mannose was specific.
The same sugars were also tested for their inhibiting capacity towards anti A2 antibodies. Again, only one of them - glucose - could prevent anti A2 antibodies from agglutinating A2-coated SRC (Fig. 1b).
In interpreting the results obtained it should be remembered that ConA, used to bind A1 and A2 to SRC, can interact with glucose and mannose. Hence the absence of agglutination observed when using these sugars could be due to their affinity for ConA, which would cause the antigen to be released from the surface of the SRC, rather than to their neutralization of antibodies. This possibility however was excluded for the following reasons: firstly, N-acetyl-D-glucosamine, which, besides glucose and mannose also interacts with ConA (13) failed to inhibit either anti A1 or anti A2 antiserum; secondly, glucose and mannose inhibited respectively anti A1 and anti A2 antibodies even when ConA was replaced by bis-diazotized benzidine to link A1 and A2 on the surface of SRC; thirdly, if the observed inhibition was indeed due to the release of the antigen preparation from the surface of SRC, then both

Abbreviations: ConA, concanavalin A; WGL, wheat germ lectin; A1, antigen McA1; A2, antigen McA2; A1, gene synthesizing antigen A1; A2, gene synthesizing antigen A2; DD, double diffusion; SRD, single radial diffusion; SRC, sheep red cells; E/AG, enzyme/antigen preparation ratio.

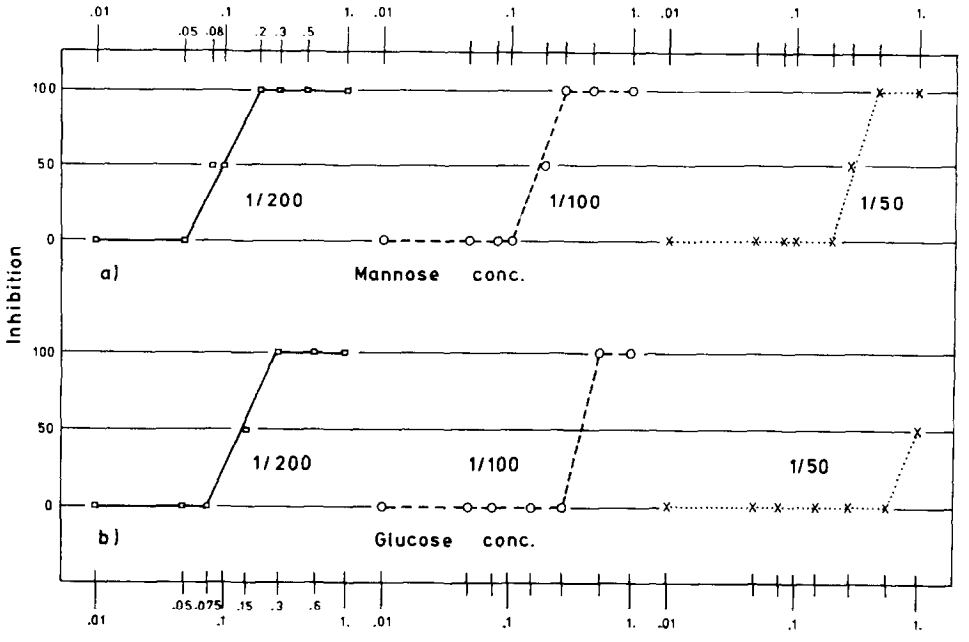

Figure 1a. Agglutination inhibition by mannose of A1 sensitized SRC-anti A1 antibody system.
Experimental conditions: 0.025 ml of anti A1 (diluted 1/200, 1/100, 1/50) plus an equal volume of mannose solution in phosphate buffer, pH 7.4 (at the M concentrations reported in abscissa) were incubated for 30 min at r.t. and then 0.025 ml of A1 sensitized SRC were added. 100% inhibition refers to replacement of mannose by A1; 0% replacement by diluent.

Figure 1b. Agglutination inhibition by glucose of A2 sensitized SRC-anti A2 antibody system.
Experimental conditions as in Figure 1a.

sugars should have inhibited both A1 and A2.

Additional evidence for the specificity of the glucose-mannose inhibition was obtained from an independent source: digestion experiments of A1 and A2 with αmannosidase, α and βglucosidase, βgalactosidase. In agreement with data from inhibition studies with simple sugars, α mannosidase destroyed A1 (but not A2) while αglucosidase destroyed A2 (but not A1) (Fig. 2). The remaining enzymes were entirely inactive on both antigens.

Having shown that A1 and A2 determinants are localized on the prostethic (carbohydrate) portion of the molecule, it seemed worth finding out

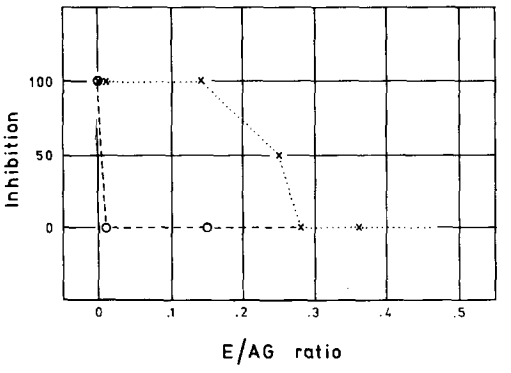

Figure 2. Agglutination inhibition activity of A1 and A2 antigen preparations upon treatment with αmannosidase and αglucosidase respectively. Experimental conditions: 0.025 ml of antiserum (anti A1 or anti A2, used at the same titer) plus an equal volume of antigen preparation (A1 or A2), digested respectively with αmannosidase or αglucosidase at the E/AG ratio reported in abscissa, were incubated for 30 min. at r.t. and then 0.025 ml of A1 or A2 sensitized SRC were added. 100% inhibition refers to replacement of digested by undigested A1 or A2; 0% to replacement of A1 or A2 by diluent.
o---o = digestion of A2 by αglucosidase; x...x = digestion of A1 by αmannosidase.

whether in heterozygous individuals they are both present on the same molecule or, alternatively, on different ones. Anti A2 antiserum is not reactive in double diffusion tests. This technique, consequently, could not be used to obtain evidence for the existence of macromolecules with simple or double specificities. Thus anti A1 and anti A2 IgG preparations isolated from the corresponding antisera by recycling G-200 gel filtration were used. Addition of either anti A1 or anti A2 to the serum of a heterozygous animal precipitated both A1 and A2 specificities: the serum became negative in both cases. When the same procedure was applied to an artificial mixture of A1 and A2 homozygous sera, only one specificity was found to be carried down in the precipitate.

Finally, the exact mechanism whereby A1 and A2 genes act on the biosynthesis of the macromolecules (whether their function is to mediate the addition of glycosyl units as in the case of ABH antigens in man (8) or a different one) remains to be clarified.

ACKNOWLEDGEMENTS: The project was financed by a grant from the Consiglio Nazionale delle Ricerche (contract n° 76.00927.80).

REFERENCES:

1. Oudin, J. (1960). J. Exp. Med., 112, 107-124.
2. Mage, R., Lieberman, R., Potter, M. and Terry, W.D. (1973) in The Antigens (M. Sela, ed.) vol. I, 299-376, Academic Press, New York
3. Rapacz, J., Korda, N. and Stone, W.H. (1968) Genetics, 58, 378-398.
4. Iannelli, D. (1969) Experientia, 25, 423.
5. Blakeslee, D., Butler, J.E. and Stone, W.H. (1971) J. Immunol., 107, 227-235.
6. Iannelli, D., Oreste, U., Masina, P., De Benedictis, G. and Cianfrigl M. (1977) Anim. Blood Grps Biochem. Genet. (in press).
7. Baglioni, C., Zonta, L.A., Cioli, D. and Carbonara, A. (1966) Science 152, 1517-1519.
8. Watkins, W.M. (1966) Science, 152, 172-181.
9. Giblett, E.R. (1969) Genetic markers in human blood, Blackwell, Oxfor
10. Leon, M.A. and Young, N.M. (1970) J. Immunol., 104, 1556-1557.
11. Schachter, H. (1975) in Methods in Enzymology (W.A. Wood, ed.), 41, 3-10, Academic Press, New York.
12. Landsteiner, K. (1962) The specificity of serological reactions, Dove Publications, New York.
13. Golstein, I.J., Hammarstrom, S. and Sundblad, G. (1975) Biochim. Biopl Acta, 405, 56-61.