BLOOD-GROUP ACTIVITY OF HUMAN CHORIONIC GONADOTROPIN

L. März, O. P. Bahl[1]
Department of Biochemistry

and

J. F. Mohn[1]
Department of Microbiology, Blood-Group Research Unit

School of Medicine
State University of New York at Buffalo
Buffalo, N. Y. 14214

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SUMMARY

Human Chorionic Gonadotropin has been found to have blood-group A-like activity as shown by the hemagglutination inhibition technique. All of the activity is located in the α subunit of the hormone although it does not contain any N-acetylgalactosamine, a component necessary for the blood-group A activity.

Human Chorionic Gonadotropin (hCG) is a glycoprotein hormone comprised of two noncovalently bonded subunits (hCG-α and hCG-β) with approximate molecular weights of 15,000 and 23,000, respectively and each containing about 30% carbohydrate. The monosaccharide and amino acid sequences in both subunits have been recently established (1-4). The carbohydrate moiety of hCG-α is made up of D-galactose, D-mannose, N-acetyl-D-glucosamine and sialic acid while that of hCG-β contains N-acetyl-D-galactosamine

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and L-fucose, in addition to the above monosaccharides. Furthermore, the carbohydrate part of the hCG-β molecule has some unusual structural features inasmuch as two types of carbohydrate-protein linkages, N-acetylglucosaminyl asparagine and N-acetylgalactosaminyl serine are present in the same molecule. In this respect the hormone resembles mucins on the one hand and the serum glycoproteins on the other. These structural considerations prompted an examination of the molecule for various blood-group activities. This communication presents the results of such an investigation and their possible physiological significance.

MATERIALS AND METHODS

Highly purified samples of hCG prepared as previously described (5) were employed in these studies. The subunits of hCG were prepared by dissociation of the hormone with 8 M urea followed by chromatography on DEAE-Sephadex A-50 and Sephadex G-100 (8,2). Ovine luteinizing hormone (LH) was kindly supplied by Dr. Papkoff. Fetuin and α_1-acid glycoprotein were purified by published procedures (6,7).

Determination of ABH and Lewis blood group activities (9): Serial twofold serologic dilutions in phosphate buffered saline solution, pH 7.35 (PBS) of 1% solutions (w/v) of each substance under examination were mixed with an equal volume of one pre-selected dilution of antiserum or lectin just causing maximum agglutination of appropriate 3% PBS cell suspensions in the preliminary titration carried out immediately prior to each assay. The sub-

stance-antibody mixtures were incubated at room temperature for 15 minutes in tests for ABH inhibitions and 30 minutes for Lewis, and then appropriate detector cells were added. After mixing their contents, the tubes were incubated for an additional 15 or 30 minutes at room temperature accordingly and, following centrifugation at 600 g for 3 min, were read for macroscopic agglutination.

In the examinations for H antigen-like inhibitory properties, a lectin with anti-H specificity prepared as a simple saline extract of the seeds of Ulex europaeus (common gorse) as described previously (10) was used with group O cells. The controls consisted of single samples of human group O secretor and nonsecretor salivas inactivated and clarified as reported by Mohn et al. (9). Any A-like activity was detected using a natural (nonimmune) human anti-A serum and group A_2 erythrocytes with 1% solutions of porcine A substances and human group A_1 and group A_2 secretor and nonsecretor salivas as controls. Similarly any B-like property was determined with a natural (nonimmune) human anti-B serum and group B cells with controls of human secretor and nonsecretor group B salivas. Lewis blood-group activity was examined with a human anti-Le^a serum and Le(a+b-) cells using as controls human salivas of the phenotypes Le(a+b-), Le(a-b+) and Le(a-b-).

RESULTS AND DISCUSSION

Human Chorionic Gonadotropin was examined for A,B,H and Lewis blood-group activities by the hemagglutination inhibition technique. The hormone showed a significant cross-reac-

Table I

Inhibition of Agglutination of Human Anti-A Serum by hCG and its Subunits

	Salivas				Porcine A Substances		hCG		
	Group A_1		Group A_2						
	Se	se	Se	se	GS-009	GS-147	Intact	alpha	beta
Undiluted	-	++++	-	++++	-	-	-	-	(+)
1:2	-	++++	-	++++	-	-	(+)	-	+
1:4	-	++++	-	++++	-	-	+	-	++
1:8	-	++++	-	++++	-	-	++	-	++
1:16	-	++++	(+)	++++	-	-	++	(+)	+++
1:32	-	++++	+	++++	-	-	+++	+	+++
1:64	(+)	++++	++	++++	-	-	++++	++	++++
1:128	+	++++	+++	++++	-	-	++++	++	++++
1:256	++	++++	+++	++++	-	-	++++	+++	++++
1:512	+++	++++	++++	++++	-	-	++++	++++	++++
1:1024	+++	++++	++++	++++	-	-	++++	++++	++++
Saline	++++	++++	++++	++++	++++	++++	++++	++++	++++

Se = secretor

se = nonsecretor

tivity with blood-group A substance (Table I). Furthermore, almost all of the A-like activity resided in the α subunit and, on a weight basis, was much greater in the α subunit than in the intact hormone (Table I). The activity in the β subunit was negligible. Under similar assay conditions ovine LH, fetuin from bovine fetal serum, and human α_1-acid glycoprotein did not show any activity.

This activity could not be due to any contaminant of blood-group A substance present in hCG preparations since hCG used in these studies was highly purified. Furthermore, all of the activity was located in the α subunit which does not contain any detectable N-acetylgalactosamine, a necessary component of blood-group A substance. Blood-group A activity is generally associated with non-reducing terminal N-acetylgalactosamine (11), but there seems to be exceptions to this rule according to a recent report on carcinoembryonic antigen (CEA) of the human digestive system. The antigen was found to possess A-like activity and did not contain any N-acetylgalactosamine (12). The monosaccharide components of the antigen were sialic acid, N-acetylglucosamine, mannose and L-fucose which, with the possible exception of L-fucose, are also present in hCG-α. Since these monosaccharides individually do not inhibit the hemagglutination reaction, it appears that the overall structure of the whole or part of the carbohydrate unit must impart the conformation necessary for this cross-reactivity with blood-group A substance.

Although more work is needed to determine precisely the structural components required for the A-like activity,

the carbohydrate of hCG-α does seem to be associated with the serological activity. It is interesting to note that in the hCG-anti-hCG reaction the antigenic determinants are not located in the carbohydrate part of the molecule, since the sequential removal of the monosaccharides from hCG with specific glycosidases does not very much alter its binding with the antibody[2] as determined by radio-immunoassay. Also, it appears that the carbohydrate, at least a major part of it, is not necessary for the binding of the hormone with its testicular and ovarian plasma membrane receptors[2].

The precise physiological significance of the present findings is not yet clear, but it is fascinating in view of the possible role of hCG in the suppression of lymphocyte function during the implantation of the fetus on maternal endometrium (13). The question which has been puzzling transplantation biologists is the acceptance of the fetus by the mother since the fetal antigens are potentially different from those of the mother. It has been postulated that hCG might be involved in the suppression of the immunoresponse by binding with maternal lymphocytes (13). It may be noted that hCG is the first hormone which has been found to exhibit blood-group activity.

2. Manuscript in preparation.

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