Journal of Chromatography, 173 (1979) 365–371
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 11,736

AFFINITY CHROMATOGRAPHY ON CONCANAVALIN A–SEPHAROSE OF ANTIGENIC FRACTIONS OF HUMAN SEMINAL PLASMA

MARÍA N. MAZZINI and ALBERTO S. CEREZO*

Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Pabellón 2, Ciudad Universitaria, Buenos Aires (Argentina)

and

JOSEFINA M. S. DE CEREZO

Catedra de Biología, Facultad de Farmacia y Bioquímica, Buenos Aires, and Centro de Investigaciones en Reproducción, Facultad de Medicina, Piso 10, Buenos Aires (Argentina)

(Received November 30th, 1978)

SUMMARY

Elution diagrams obtained on affinity chromatography show that the antigenic fractions of human seminal plasma studied, namely (a) the non-dialyzable components of human seminal plasma and (b) its trichloroacetic acid-soluble fraction, (c) the trichloroacetic acid-soluble fraction of whole human seminal plasma and (d) the pronase digested human seminal plasma, are complex mixtures of glycoproteins with minor amounts of polysaccharides. Some of these glycoproteins contain significant percentages of carbohydrates while others contain only trace amounts. Most of the glycoproteins carry non-reducing end-chain groups comprising α-D-glucopyranosyl, α-D-mannopyranosyl or sterically related residues.

INTRODUCTION

Concanavalin A (Con A)–Sepharose is known to interact specifically with branched polysaccharides and glycoproteins, the non-reducing end chains of which comprise α-D-glucopyranosyl, α-D-mannopyranosyl, β-D-fructofuranosyl and sterically related residues[1]. The fractionation of branched-chain polysaccharides adsorbed on Con A is thought to depend on the differences in the degree of branching. Restrictions imposed by the immobilization of the lectin on the matrix may modify this operation. Thus, in the immobilized case, molecules may be fractionated according to differences in the length of the branches or the distribution of branch points which are not apparent when the complexing is carried out in solution[2].

In previous studies[3,4] four antigenic substances were isolated by gel filtration through Sephadex G-100 of the trichloroacetic acid-soluble fraction of human seminal plasma. The substances were then examined by chemical and physicochemical methods

* To whom correspondence should be addressed.

and identified as three glycopeptides (G-1, G-2 and G-4) containing *ca.* 10% protein, and a polysaccharide (G-3). All contained glucose as the main sugar component and their rotatory powers suggested the presence of glycosidic linkages of the α-D-type[4]. They form insoluble complexes with Con A[4,5].

It was of interest to analyze the macromolecular components of human seminal plasma and some of its fractions possessing antigenic activity by use of affinity chromatography on Con A–Sepharose, and to study the distribution of the molecules according to the configurations of their non-reducing end chains and, in those cases which gave adequate complexing with Con A, according to the differences in the degree of branching.

MATERIALS AND METHODS

Seminal plasma was prepared from whole normal semen obtained from healthy donors, as described previously[3]. Pooled normal human seminal plasma was used as starting material. Con A, covalently bound to Sepharose 4B by the cyanogen bromide method[6], was supplied by Pharmacia (Uppsala, Sweden) as a suspension containing 10 mg/ml of Con A in 0.1 *M* sodium acetate buffer (pH 6.0) with 1 *M* sodium chloride, 1 m*M* calcium chloride, 1 m*M* magnesium chloride, 1 m*M* manganese chloride and 0.01% merthiolate.

Other chemicals and reagents used were as noted previously[3,4].

Non-dialyzable fraction of human seminal plasma

The human seminal plasma prepared as above was dialyzed against 0.15 *M* sodium chloride at 4° for 24 h, centrifuged, dialyzed against distilled water under the same conditions and then freeze-dried.

Trichloracetic acid-soluble fraction of human seminal plasma

This was prepared as described[3].

Pronase-digested human seminal plasma

The enzyme was suspended in 1/15 *M* phosphate buffer, pH 7.0 (1 mg/ml), and a portion of this suspension was mixed with nine times its volume of human seminal plasma. The latter was obtained by centrifugation of whole semen in a Sorvall centrifuge (rotor SS-34 at 12,000 *g*) followed by dialysis against 1/15 *M* phosphate-buffered saline (pH 7.1). The mixture of the enzyme and plasma was incubated at 37° for 90 min with constant stirring. The enzyme was centrifuged off, and the solution that remained was dialyzed until free from salts and then freeze-dried.

Trichloroacetic acid-soluble fraction of non-dialyzable human seminal plasma

The human seminal plasma was dialyzed against 0.15 *M* sodium chloride for 24 h at 4°, centrifuged at 12,000 *g* for 10 min and the trichloroacetic acid-soluble fraction then prepared as above.

Affinity chromatography

An aqueous slurry of adsorbent (52 ml of swollen gel) containing *ca.* 520 mg of Con A was packed into a column (107 × 0.8 cm) and washed with 0.1 *M* sodium

phosphate buffer–1 *M* sodium chloride (pH 7.2) at 20–25° and 0.176 ml/min until a steady state was reached. Solutions (10 mg/ml) of the fractions described above were applied at the top of the column and eluted with the same buffer. When all the non-bound or weakly bound material had been eluted, the eluent was changed to 0.01 *M* sodium borate buffer (pH 6.0), followed by a 0.1 *M* solution of the same buffer. The strongly adsorbed carbohydrates were finally eluted with a 2.0% solution of methyl α-D-glucopyranoside in 0.1 *M* sodium phosphate buffer (pH 7.2).

The eluate was continuously monitored for carbohydrate by the phenol–sulphuric acid reaction[7], and for proteins by UV spectrophotometry at 280 nm[8]. Since the same amounts of products were passed through the column in all cases, the elution diagrams in Fig. 1 and 2 are directly comparable.

The elution volumes of the macromolecules which are not retained by the Con A–Sepharose were determined by chromatography of a polysaccharide (a galactomannan having a branched molecule, the non-reducing end-chain groups of which are α-D-galactopyranosidic residues[9], a protein (bovine serum albumin) and a glycoprotein (ovalbumin[10]). These products gave no precipitate with Con A in solution[4].

RESULTS

Fig. 1 shows the elution patterns of the galactomannan, the bovine serum albumin and the ovalbumin. All were eluted with phosphate buffer; the polysaccharide and the protein gave only one peak each but the ovalbumin showed more than one peak in agreement with its heterogeneity[10]. The galactomannan eluted first (elution

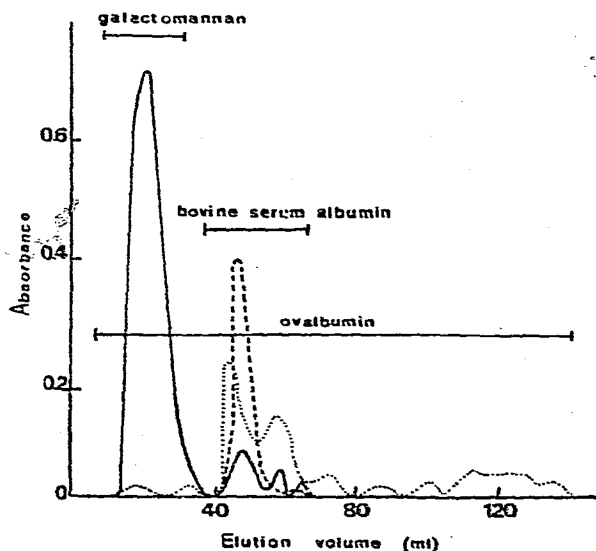

Fig. 1. Elution patterns on Con A–Sepharose of macromolecules (galactomannan, bovine serum albumin and ovalbumin) which do not interact with Con A. The elution was carried out with 0.1 *M* sodium phosphate buffer–1 *M* sodium chloride (pH 7.2) at 20–25° and 0.176 ml/min. Galactomannan: — —, carbohydrate. Bovine serum albumin: – – –, protein. Ovalbumin: . . ., protein; ———, carbohydrate.

volume 17–34 ml) followed by the protein and the glycoprotein at the same elution volume (40–60 ml, carbohydrate peaks for the ovalbumin).

Fig. 2 shows the elution patterns of (a) the non-dialyzable fraction of human seminal plasma, (b) the trichloroacetic acid-soluble fraction of the non-dialyzable part of the human seminal plasma, (c) the trichloroacetic acid-soluble fraction of human seminal plasma and (d) the pronase-digested human seminal plasma. The four elution diagrams are similar in that they indicate the existence of three different types of substances in the mixtures analysed, according to the eluents used, namely those which were eluted with phosphate buffer, compounds which were not eluted by phosphate buffer but are eluted by borate buffer and finally those which interact strongly with the lectin and are eluted only by the use of a molecular hapten inhibitor of low molecular weight (methyl α-D-glucopyranoside).

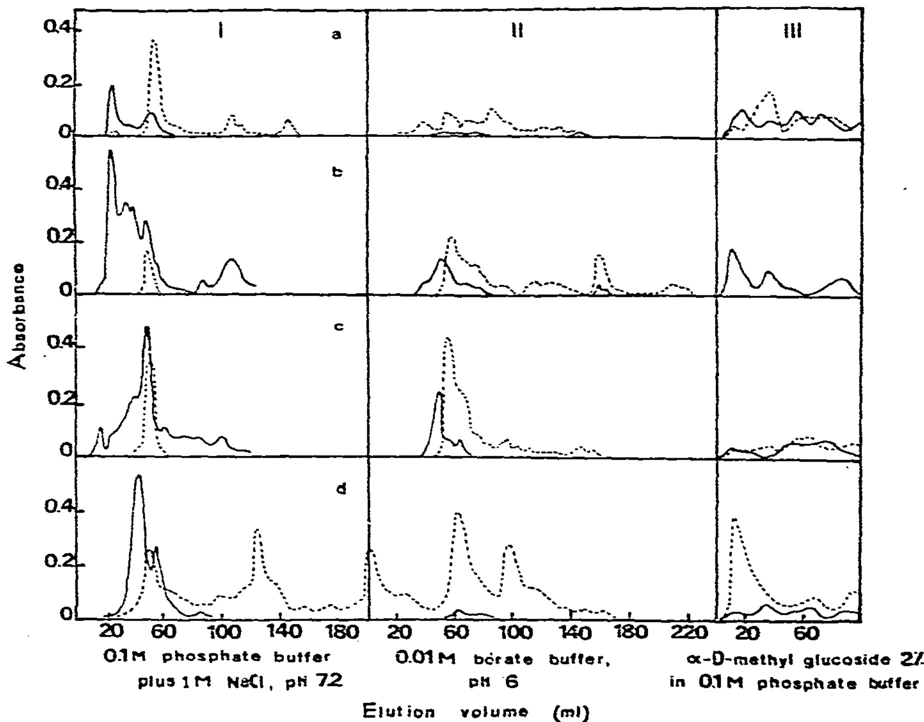

Fig. 2. Elution patterns on Con A–Sepharose of some antigenic fractions of human seminal plasma. a = Non-dializable fraction of human seminal plasma; b = its trichloroacetic acid-soluble fraction (in III the proteins were not determined); c = trichloroacetic acid-soluble fraction of whole seminal plasma; d = pronase-digested human seminal plasma. I = Elution with 0.1 *M* sodium phosphate buffer–1 *M* sodium chloride (pH 7.2); II = elution with 0.01 *M* sodium borate buffer (pH 6.0); III = elution with 2% methyl α-D-glucopyranoside in 0.1 *M* sodium phosphate buffer (pH 7.2). – – –, Protein; ———, carbohydrate.

In the case of the pronase-digested human seminal plasma, small amounts of compounds, which absorb at 280 nm as do proteins but do not react as carbohydrates, were not eluted with phosphate or 0.01 *M* borate buffers, but were displaced by borate 0.1 *M* buffer (these compounds are not shown in Fig. 2).

Fig. 2 also shows that in all cases the major quantities of carbohydrates were eluted by phosphate buffer I, while those products containing minor amounts of sugars were separated from the lectin by the borate buffer II. Proteins of the non-dialyzable (a) and the pronase-treated (d) human seminal plasma were eluted in similar quantities by the three eluents, but those of the trichloroacetic acid-soluble fractions (b and c) were mainly eluted by borate buffer and only very little by phosphate.

The superposition of sugars and proteins in the elution diagrams of Fig. 2 suggests the presence of three types of compounds. First, those formed by carbohydrates (I; b and c), which may contain a little protein (I; a). They are displaced by phosphate buffer and their elution volumes (15–40 ml) are similar to that of the galactomannan or higher (60–120 ml) (I; b and c). Secondly, glycoproteins which are eluted by both buffers, those displaced by phosphate having elution volumes similar to that of ovalbumin or bovine serum albumin. All the material eluted by the methyl α-D-glucopyranoside solution belongs to this type of compounds. Finally, there are peaks (I; a and d) (II; d) and zones (II; b and c) which react only as proteins. No carbohydrates were detected in these.

DISCUSSION

Human seminal plasma is a very complex mixture of macromolecules which has been analyzed by several procedures[11–17]. The immunological methods[12,13,18–20] are based on the interaction of the antigens with their specific antibodies, although the chemistry of these interactions is often unknown.

The use of immobilized Con A permits an analysis of the high-molecular-weight components of human seminal plasma or of fractions derived from it on the basis of specific interactions, the chemistry and structural aspects of which are known (see Introduction).

It is noteworthy that only carbohydrates (with a very small amount of protein in fraction a) were eluted at the elution volume of the galactomannan, while superposition of peaks of protein and carbohydrates was found at the elution volume of the ovalbumin or bovine serum albumin (Fig. 2). It was concluded that human seminal plasma contains polysaccharides (with a very small amount of protein in fraction a) (I, Fig. 2) and glycoproteins (I, Fig. 2) which are linear molecules or, if branched, their non-reducing end-chains do not contain α-D-glucopyranosyl, α-D-mannopyranosyl or its 2-amino-2-deoxy derivatives and β-D-fructofuranosyl residues.

Since the immobilization of the lectin on the matrix introduces a geometrical factor in its reaction with carbohydrates[2] it is possible that the above macromolecules contain adequate end-chain residues for interaction with Con A but that the distance between them precludes the reaction.

Only non-interacting glycoproteins (or mixtures of polysaccharides and proteins) were found in the non-dialyzable fraction of human seminal plasma treated with pronase (d, Fig. 2). Further elution with phosphate buffer (I, Fig. 2) eluted some weakly adsorbed products. These react as carbohydrates in fractions b and c and as proteins in fractions a and d. Since proteins do not interact with Con A[21] it is supposed that they contain a very small amount (not detectable) of carbohydrates which are responsible for the interaction.

Compounds which interacted with Con A were eluted with 0.01 *M* borate

buffer (II, Fig. 2). Superposition of protein and carbohydrate peaks occurred in the four cases, and also compounds were eluted which react only as proteins. It is concluded that two types of glycoproteins which contained major amounts of protein were eluted in this step, one type containing only traces of carbohydrates, the other containing measurable amounts of sugars.

After elution with 0.01 *M* borate, a more concentrated borate buffer (0.1 *M*)[2] was passed through the column. Only a small amount of substances appeared on chromatography of fraction d, and none with the other fractions. These substances are glycoproteins which must contain traces of carbohydrates.

Only glycoproteins were eluted by the methyl α-D-glycopyranoside.

In summary, the results of affinity chromatography on Con A–Sepharose of different fractions of human seminal plasma suggest the following.

(a) The use of protein-removal techniques, such as treatment with a broad spectrum protease (pronase) or precipitation with trichloroacetic acid solution, does not produce mixtures of macromolecules simpler than the original human seminal plasma. The precipitation of human seminal plasma or its non-dialyzable fraction with trichloroacetic acid solution increases the relative content of carbohydrates, but, unexpectedly, treatment of the non-dialyzable fraction with pronase reduces the carbohydrate content.

(b) The polysaccharides and some glycoproteins do not interact (or do so weakly) with Con A and so they do not contain the sugar residues and/or the geometrical requirements for the interaction. On the other hand, most of the glycoproteins interact, some very strongly, with Con A–Sepharose. These results are in accord with the composition of some of the antigens isolated from the trichloroacetic acid-soluble fraction of human seminal plasma[4].

(c) In terms of the strength of the interaction with the insoluble Con A, compounds might be divided into those which do not interact or weakly interact and are eluted with phosphate buffer, those which bind to the protein and are not eluted with phosphate but are eluted with borate buffer and finally those which are strongly adsorbed on the lectin and are displaced only by a solution of methyl α-D-glucopyranoside. This fractionation reflects the differences in the structures of the polysaccharides or in the structures of the carbohydrate portion of the glycoproteins, namely in the identity of the end-chain residues, the amount of branching and the length and distribution of the branches. Further research is necessary to elucidate these structural features.

(d) The elution diagrams show that the fractions of human seminal plasma studied are complex mixtures of glycoproteins with lesser amounts of polysaccharides. Some of these glycoproteins contain significant amounts of carbohydrates while others contain only trace amounts.

ACKNOWLEDGEMENT

This work was supported by a grant 74.136.1.77. from the Programa Latinoamericano de Investigación en Reproducción Humana, "PLAMIRH".

REFERENCES

1 I. J. Goldstein and L. L. So, *Arch. Biochem. Biophys.*, 111 (1965) 407.
2 J. F. Kennedy and A. Rosevear, *J. Chem. Soc., Perkin Trans I*, (1973) 2041.
3 J. M. S. Cerezo, *Int. J. Fertil.*, 19 (1974) 211.
4 J. M. S. Cerezo and A. S. Cerezo, *Int. J. Fertil.*, 22 (1977) 162.
5 J. M. S. Cerezo and A. S. Cerezo, unpublished results.
6 K. Aspberg and J. Porath, *Acta Chem. Scand.*, 24 (1970) 1893.
7 M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith, *Anal. Chem.*, 28 (1956) 350.
8 B. Warburg and W. Christian, *Methods Enzymol.*, 111 (1957) 451.
9 C. Leschziner and A. S. Cerezo, *Carbohyd. Res.*, 15 (1970) 291.
10 R. Montgomery, in W. Pigman and D. Horton (Editors), *The Carbohydrates, Chemistry and Biochemistry*, Vol. 11B, Academic Press, New York, 1970, p. 647.
11 S. Shulman and P. Bronson, *Fertil. Steril.*, 19 (1968) 549.
12 S. Shulman and P. Bronson, *J. Reprod. Fertil.*, 18 (1969) 481.
13 T. S. Li and C. G. Beling, *Fertil. Steril.*, 24 (1973) 134.
14 S. Shulman and T. S. Li, *Int. J. Fertil.*, 19 (1974) 19.
15 T. S. Li and S. Shulman, *Int. J. Fertil.*, 16 (1971) 87.
16 W. P. Herrman and G. Herrman, *Fertil. Steril.*, 20 (1969) 521.
17 S. Katsh, *Int. J. Fertil.*, 6 (1961) 53.
18 A. Hekman and P. Runkel, *Fertil. Steril.*, 20 (1969) 312.
19 S. Defagio, B. K. Lewis and M. M. Ketchel, *J. Reprod. Fertil.*, 20 (1969) 29.
20 W. P. Herrman, *Andrologie*, 1 (1969) 11.
21 I. J. Goldstein and R. N. Iyer, *Biochim. Biophys. Acta*, 121 (1966) 197.