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Purification of the Pregnancy Zone Protein by Affinity Chromatography

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A method for purification of the pregnancy zone protein (PZP) by affinity chromatography was developed. A monospecific immunoglobulin fraction, covalently coupled to Sepharose 4B, was used as binding agent and the elution conditions for PZP are described. The purified protein was shown to have identical properties compared to native PZP with regard to molecular weight, immunodiffusion precipitation and immunosuppressive activity.

Techniques for purification of proteins by immunadsorption chromatography are used in increasing frequency, because of the rapid and comparatively specific separation pattern that can be obtained. Some proteins are difficult to obtain in pure form with conventional purification techniques. One of these proteins is the "pregnancy zone protein" (PZP)[1,2] also named pregnancy associated α_2-globulin, which is believed to have immunosuppressive properties.[3,4] This high molecular weight glycoprotein has been obtained in pure form using combinations of precipitations with salts, organic solvents, ion exchangers, gel filtration and preparative polyacrylamide gel electrophoresis.[5–7] All the present techniqus are complicated, time-consuming and give a rather low yield of purified protein. Lack of material has hampered the studies of the biological role of PZP.

The present communication describes an affinity chromatography which is rapid, simple with a high yield of native immunosuppressive protein.

METHODS AND EXPERIMENTAL

Antibodies against PZP [6] were prepared in goat. The antiserum was made monospecific by adsorption with 10 % normal male serum. The immunoglobulin fraction was obtained by precipitation with an equal volume of 4 M ammonium sulfate pH 6.8. The precipitate formed was washed, dialyzed against 50 mM sodium acetate of pH 5.70. The immunoglobulin fraction was further purified on a $DEAE^-$ Sephadex A-50 medium column in the same buffer, and the immunoglobulins were eluted in the first fractions.

CNBr-activated Sepharose® 4B, (22 g, Pharmacia, Sweden) was washed with 0.001 M HCl. Purified immunoglobulin corresponding to 30 ml of goat antiserum were dialyzed against 0.1 M $NaHCO_3$, 0.5 M NaCl, pH 7.8. Coupling was performed under slow rotation for 2 h at room temperature. Unbound material, which failed to give visible precipitates in double diffusion tests, was washed away with coupling buffer, and any remaining active groups were reacted with 1 M ethanolamine at pH 8 for 2 h. The slurry was washed three times with 0.1 M sodium acetate, 1 M NaCl pH 4.0, and 0.1 M sodium borate, 1 M NaCl pH 8.0 according to Pharmacias instructions. Finally, the material was equilibrated with 0.1 M NaCl, 0.05 M sodium phosphate, pH 7.8 (starting buffer) and packed in a 35 × 70 mm column.

Protein fractions from the column, 8 ml, were measured spectrophotometrically at 280 nm and PZP concentration was measured by either an electroimmunoassay or a radioimmunoassay.[8]

Analytical polyacrylamide gel electrophoresis was performed according to Davis.[9]

Molecular weight determinations were performed by sodium dodecylsulfate electrophoresis according to Weber and Osborn,[10] with IgG (mw 160 000), transferrin (mw 90 000), albumin (mw 68 000), ovalbumin (mw 43 000), myoglobin (mw 17 800). Dimers of all reference proteins were used for the calibration curve.

Double diffusion test was performed in 1 % agarose as earlier described.[11]

Mixed lymphocyte cultures were prepared

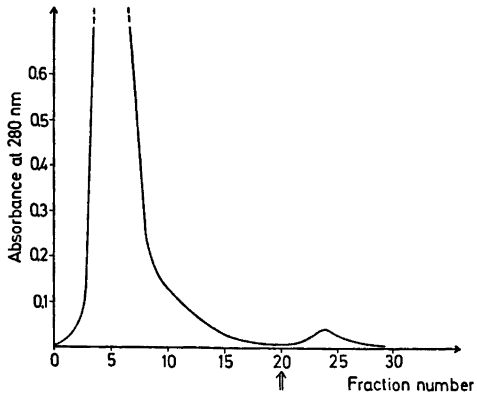

Fig. 1. Affinity chromatography of pregnancy serum on a Sepharose-4B column coupled with goat antihuman PZP. Arrow indicates change of buffer. A_{280} was measured spectrophotometrically.

with lymphocytes from healthy non-pregnant unrelated blood donors as previously described.[4] Cultures with a volume of 200 μl and a final lymphocyte concentration of 1.5×10^6/ml were prepared. Cultures contained: synthetic culture medium (RPMI 1640 containing 20 mM HEPES buffer (Flow laboratories), saline or affinity-purified PZP in saline. Lymphocyte transformation was measured after six days using ^{14}C-thymidine and values were expressed as counts per min.

RESULTS

Pregnancy serum (1 – 2 ml) which contains PZP in average concentrations of 1000 μg/ml was diluted to approx. 30 ml with starting buffer and applied to the column. The flow was then stopped for 1 h allowing antigen and antibody to equilibrate. Thereafter the column was washed for approx. 3 h with the starting buffer until A_{280} was below 0.01. The elution buffer (0.1 M acetic acid, 3 M NaCl pH 4.5) was thereafter applied and PZP was eluted as a distinct peak (Fig. 1). PZP-fractions were pooled and immediately dialyzed against 30 mM sodium phosphate buffer pH 7.4. The average yield from repeated elutions was between 30 and 50 %. The yield increased from the first cycles and was rather stable after ten cycles. The column was used for at least fifty cycles. Most probably only a minor part of the immunoglobulin molecules participates in the chromatography. During the first cycles high affinity antibodies were saturated with PZP and functionally blocked. Reversely low affinity antibodies might bind PZP more weakly because of the comparatively high ionic strength (5 mM Tris HCl 0.1 M NaCl pH 7.8) in the starting buffer. The washing buffer was adjusted to avoid unspecific adsorption to the column (albumin) and to maintain high specific binding properties.

Fractions from the column were analyzed by polyacrylamide gel electrophoresis. As shown in Fig. 2, the main protein peak (B) contains no apparent PZP band, which is found as a single band in the minor fraction (C).

Double diffusion tests between purified PZP fractions from the affinity chromatography and the original technique,[6] revealed complete fusion reaction. Furthermore, the molecular weight as determined by sodium dodecylsulfate polyacrylamide gel electrophoresis was determined to 360 000, which is the value for the native protein. Thus, the covalently coupled dimer of PZP [13] can be reversely bound and released from the antibody without loosing parts of its structure.

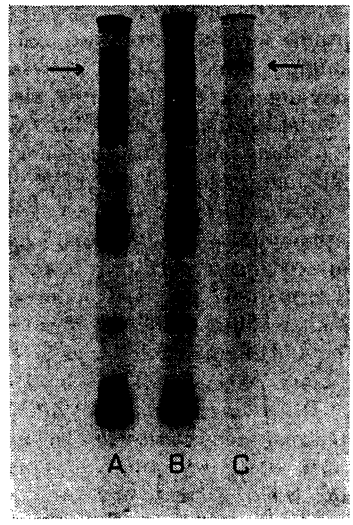

Fig. 2. Analytical polyacrylamide gel electrophoresis of (A) pregnancy serum, (B) main protein fraction, and (C) isolated PZP (arrow) from the column.

Table 1. Influence of affinity purified PZP on the mixed lymphocyte reaction.

Culture	*n*	C.P.M. (mean ± S.D.)
Medium	8	6383 ± 1641
Saline	8	8186 ± 2972
PZP (280 μg/ml) in saline	6	859 ± 576

DISCUSSION

Affinity techniques using Sepharose-coupled immunoglobulin as ligand make a careful selection of the procedure to elute the protein necessary. When the strong binding between antigen-antibody is broken, the antigen can easily be denatured and lose biological properties. This has recently been reported especially for pregnancy-associated globulins.[12] The present elution procedure was set up after studies of the stability of PZP at different pH and salt concentrations. At an elution pH below 4 or above 10, significant changes were found in double diffusion precipitation pattern against monospecific antibodies. The precipitates were broad, diffuse and irregular, indicating a partial denaturation of PZP. The elution buffer used, does not disturb the diffusion pattern.

Another control of the eluted PZP-fraction was made on its effect in the mixed lymphocyte culture. Previously PZP purified by conventional techniques has been found to exert an inhibitory effect on the blastoid transformation.[4] Table 1 shows that PZP purified by affinity technique, in a concentration of 280 μg/ml has the same pronounced inhibitory effect.

The affinity technique described above is rapid, and the yield is high. It is not necessary to use haptoglobin 1 – 1 sera as starting material. As the properties of the protein seem to remain unchanged with regard to molecular weight, immunodiffusion pattern and immunosuppressive activity, the method could be beneficial in further studies of the biological role of this protein, where larger amounts of the purified fraction are necessary.

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