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AFFINITY CHROMATOGRAPHY WITH AMNIOTIC FLUID SOMATOMEDIN BINDING PROTEIN IN THE PURIFICATION OF INSULIN-LIKE GROWTH FACTOR I

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Summary. A simplified procedure has been developed for the isolation of insulin-like growth factor I from human plasma by use of affinity chromatography with the somatomedin binding protein. After acidification of human plasma and separation of insulin-like growth factor I and endogenous binding protein by cation exchange chromatography on SP-Sephadex the material was passed through a column packed with pure human amniotic fluid binding protein covalently coupled to Sepharose. The bound insulin-like growth factors I and II were eluted by 1 M acetic acid and separated on a Mono S cation exchange column by use of a salt gradient. The 30 µg insulin-like growth factor I and 18 µg insulin-like growth factor II recovered from 1 liter plasma gave an overall recovery of 30 % for insulin-like growth factor I but only 2.5 % for insulin-like growth factor II.

Insulin-like growth factors I and II (IGF-I, IGF-II) are the two main somatomedin polypeptides in human serum (1-8). Unlike other polypeptide hormones IGF-I and II in serum circulate bound to binding proteins, with at least two different molecular masses (9-13). Somatomedins released from organs and cells are accompanied by binding proteins (14-16). Human amniotic fluid has been found to be a rich source of a binding protein with a MW of 35 k dalton (17-20). This somatomedin binding protein (SMBP) was previously isolated and characterized (21). It displays a high affinity for IGF-I and IGF-II (21). By covalently coupling this human amniotic fluid SMBP to Sepharose, we report here a procedure for the isolation of IGF-I from human plasma.

MATERIAL AND METHODS

Acidification of plasma

Outdated human plasma was mixed with an equal volume of 1 M acetic acid, giving a final pH around 3.7, and was left for at least 6 hours at 4°C before chromatography.

Abbreviations: IGF-I, insulin-like growth factor I; IGF-II, insulin-like growth factor II; SMBP, somatomedin binding protein; RIA, radioimmunoassay; RRA, radioreceptorassay.

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Cation exchange chromatography on SP-Sephadex C25

Acidified plasma (corresponding to 1 liter of original plasma) was applied to a column (2.5×30 cm) with SP-Sephadex C25 cation exchange gel (Pharmacia, Sweden) pre-equilibrated with 1M Na-acetate buffer, pH 3.7. After washing the column with the equilibrating buffer the bound material was eluted with two bed volumes (approximately 300 ml) of Tris buffer, 0.05M, pH 9.0, and pH adjusted to 7.5 with 0.1M HCl (Fig. 1).

Affinity chromatography on the somatomedin binding protein coupled to Sepharose

Preparation of the gel. The human amniotic fluid SMBP was purified as described elsewhere (21). The protein was covalently coupled to CNBr activated Sepharose 4B (Pharmacia, Sweden), according to directions supplied by the manufacturer. By this process, 5 mg of pure human amniotic fluid SMBP were coupled to 10 ml of hydrated gel, poured into a glass column and equilibrated with Tris buffer, 0.05M, pH 7.5.

Development of affinity chromatography. The material was slowly passed through the binding protein–Sepharose column and thereafter the column was washed with Tris buffer, 0.05M, pH 7.5 until $A_{280} = 0$. The bound material was eluted with 20 ml of 1M acetic acid and lyophilized (Fig. 2).

Cation exchange chromatography on Mono S

The material from the affinity column was resuspended in Na-acetate buffer, 0.05M, pH 5.0, containing 0.05M NaCl, centrifuged, filtered through a 0.20 µ filter (Gelman, MI, USA) and the pH adjusted to 5.0 with Na-acetate. The material was applied to a Mono S cation exchange column (HR 5/5, Pharmacia, Sweden), pre-equilibrated with Na-acetate buffer, pH 5.0, with 0.05 NaCl. The chromatogram was developed with a gradient from 0.05 to 1M NaCl (Fig. 3).

Reversed phase chromatography

Material eluted from the Mono S column was further chromatographed in a reversed phase column (Pro PAC, HR 5/10, FPLC system, Pharmacia, Sweden) using a water/methanol gradient from 40 % to 80 % methanol.

Radioreceptor assay (RRA) and radioimmunoassay (RIA)

The purification process was guided by an RRA for somatomedin A with human placenta membrane as matrix as described elsewhere (22,23). Final recovery was based on RIA for IGF-I and IGF-II (10,24). As standard and ligands IGF-I and IGF-II purified in our laboratory according to Enberg et al. (8) were used. The crossreactivity of IGF-II in the IGF-I RIA is only 1 % and of IGF-I in the IGF-II RIA is 10 %.

Amino acid analyses

Amino acid analyses were performed with a Beckman 121 M instrument after hydrolysis in evacuated tubes for 24 h at 110°C with 6M HCl, 0.5 % phenol.

N-terminal sequence analyses

Liquid-phase sequencer degradations were performed as previously described (8) in a Beckman 890 D sequencer using a 0.1M quadrol peptide program in the presence of glycine-precycled Polybrene (25). Phenylthiohydantoin derivatives were identified by reverse-phase high-performance liquid chromatography (26). For the sequence analyses 2 nmol protein was used.

RESULTS

Most of the somatomedins present in acidified plasma bound to a column with SP-Sephadex, equilibrated with Na-acetate, pH 3.7, whereas the low molecular form of binding protein and the bulk of the proteins passed through. Almost all adsorbed IGF-I was eluted with Tris buffer, 0.05M, pH 9.0 (Fig. 1). Further

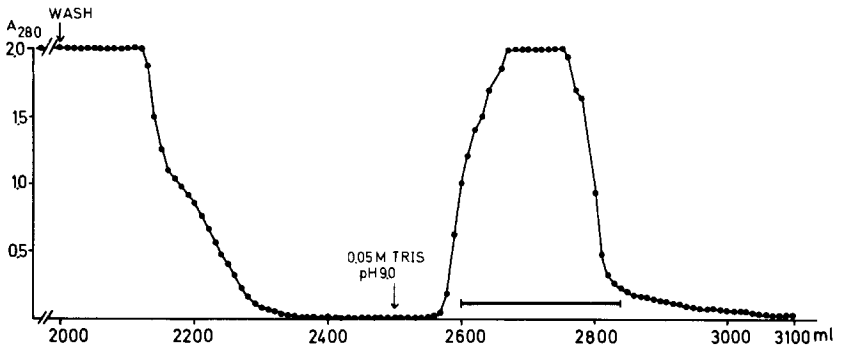

Fig. 1. Chromatography on SP-Sephadex cation exchange. The acidified plasma (2 l) was applied to a column of SP-Sephadex (2.5×30 cm) equilibrated with 1M Na-acetate buffer, pH 3.7. As indicated by the arrows the column was washed with equilibrating buffer until $A_{280} = 0$ and then eluted with two bed volumes of 0.05 Tris, pH 9.0. The horizontal bar indicates the fractions containing somatomedins as determined by RRA.

elution with 1M NaCl in Tris buffer 0.05M, pH 9,0, only gave a small amount of somatomedins as determined by RRA. The Tris pH 9.0 eluate was adjusted to pH 7.5 and applied to the affinity column with somatomedin binding protein. Nearly all the applied IGF-I could be eluted in a single peak with 1M acetic acid (Fig. 2). Further purification and separation of IGF-I and IGF-II was achieved on a Mono S column. As showed in Fig. 3, IGF-I eluted in a sharp peak at ± 25 % of the gradient used and IGF-II at ± 65 % of the gradient. To confirm the purity of IGF-I it was further chromatographed on a reversed phase column (Pro PAC, HR 5/10, FPLC system, Pharmacia, Sweden) by using a water/methanol gradient. Only one peak could be detected at 55 % of the gradient. The recovery of IGF-I at this step was only 50 %.

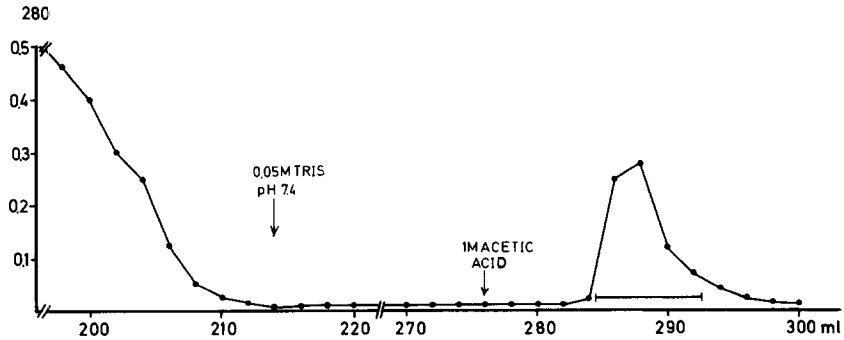

Fig. 2. Affinity chromatography on the SMBP Sepharose column. The column (10 ml) was equilibrated with 0.05M Tris, pH 7.5. The material from the SP-Sephadex column was passed through the column. After washing with equilibrating buffer until $A_{280} = 0$ the bound material was eluted with 20 ml of 1M acetic acid. The horizontal bar shows the elution volume of somatomedins determined by RRA.

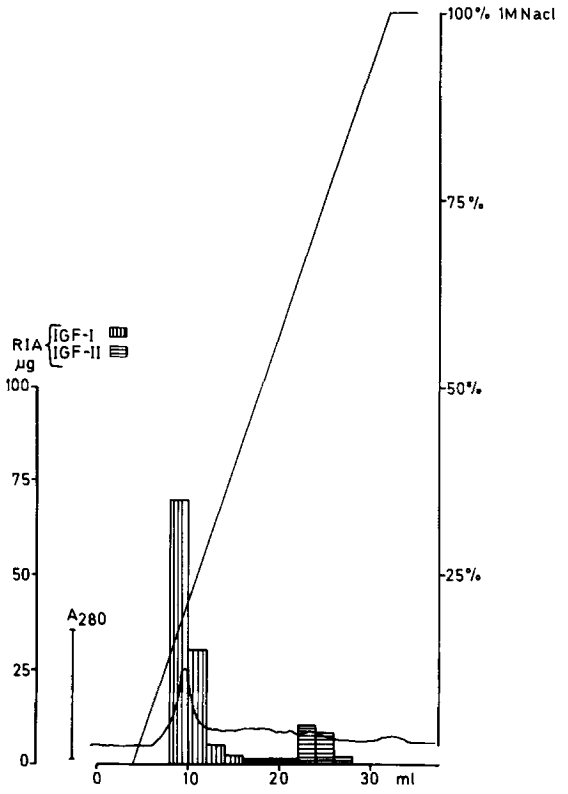

Fig. 3. Cation exchange chromatography. This was made on a column of Mono S (FPLC system, Pharmacia, Sweden) developed with a linear gradient of NaCl from 0.05 to 1M. The straight line indicates the gradient and the curve the absorbance at 280 nm. The bars indicate the profile of elution of IGF-I and IGF-II by RIA.

The amino acid composition of the isolated polypeptide was identical to that for IGF-I apart from the presence of some histidine which probably was a contamination. The N-terminal amino acid sequence was Gly-Pro-Glu-Thr-Leu-Cys-Gly-Ala-Glu-Leu. In the RIA for IGF-I, the dose-response curve of the purified IGF-I was superimposible on the curve of IGF-I isolated by Rinderknecht and Humbel (2) (Fig. 4). About 30-40 µg of immunoreactive IGF-I was obtained from 1 liter of outdated plasma, containing about 100 ng IGF-I per ml, which gave a final recovery of 30 %. Before the last step of reversed phase chromatography the yield was about 60 %. The yield of immunoreactive IGF-II was only 18 µg from 1 liter outdated plasma.

Apart from IGF-I and IGF-II an additional peptide was eluted from the Mono S cation exchange column at 40 % of the salt gradient, a position between the elution of IGF-I and IGF-II. This peptide was further separated from IGF-I and IGF-II in the reversed phase column using a water/methanol gradient where it was eluted at 45 % methanol, but is not yet analysed.

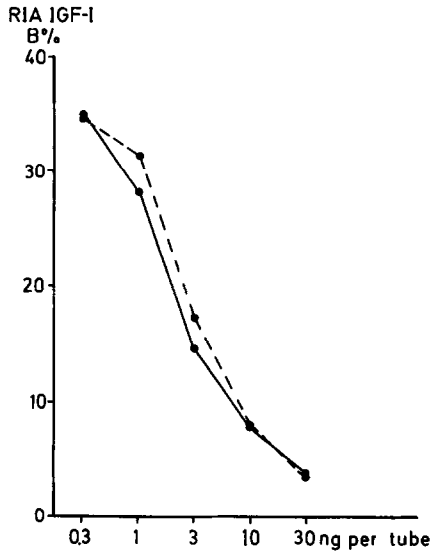

Fig. 4. Crossreaction of our purified IGF-I in the RIA for IGF-I. The dilution curve of our purified IGF-I (---) is shown in comparison to curve using IGF-I purified by Humbel (——).

DISCUSSION

A simple and efficient purification procedure of IGF-I from serum was developed by using its affinity to the SMBP, isolated from amniotic fluid. The advantages of this affinity chromatography step are the specificity for somatomedins and the high recovery of IGF-I. About 30-40 µg of immunoreactive IGF-I were recovered from 1 liter plasma. The yield of IGF-I was higher than the 16 % yield achieved in the purification procedure earlier developed (8). The present recovery is also higher compared with immunoaffinity chromatography using monoclonal antibodies (17-18 %) as reported by others (27,28). However, this yield in all these purifications were severalfold better than the 2-5 % achieved by methods previously described when starting from Cohn fraction IV (2, 29-32).

Before using the affinity chromatography step it was necessary to dissociate and separate IGF-I from endogenous binding proteins in plasma. This was achieved by acidification and cation exchange chromatography on SP-Sephadex as previously described (32,33). Unfortunately, this step led to a loss of IGF-II and we have not yet found the explanation or developed an alternative step. After the SMBP affinity chromatography step the IGF-I and IGF-II polypeptides were separated by ion exchange chromatography on a Mono S column with a salt gradient. Between IGF-I and IGF-II, we also found a variant of somatomedin/IGF which crossreacted in the radioreceptor assay but had lower crossreactivity than IGF-II in the RIA for IGF-II and did not crossreact in the RIA for IGF-I.

The developed purification procedure can be applied in the purification of endogenous somatomedins from plasma and acid extracts of different tissues but also of biosynthetic IGFs. In addition, not only IGF-I and IGF-II but also variants of these polypeptides could be expected to bind to SMBP.

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REFERENCES

1. Daughaday, W.H., Hall, K., Raben, M.S., Salmon, W.D., Van den Brande, J.L., and Van Wyk, J.J. (1972) Nature 235, 107.
2. Rinderknecht, E., and Humbel, R.E. (1976) Proc. Natl. Acad. Sci. USA 73, 2365-2370.
3. Rinderknecht, E., and Humbel, R.E. (1978) J. Biol. Chem. 253, 2769-2776.
4. Van Wyk, J.J., and Underwood, L.G. (1978) In: Litwack, G. (ed.) Biochemical Actions of Hormone, 5:102-142. Academic Press, New York.
5. Zapf, J., Walter, H., and Froesch, E.R. (1981) J. Clin. Invest. 68, 1321-1330.
6. Klapper, D.G., Svoboda, M.G., and Van Wyk, J.J. (1983) Endocrinology 112, 2215-2217.
7. Hall, K., and Sara, V. (1983) Vitam. Horm. 40:175-233.
8. Enberg, G., Carlquist, M., Jörnvall, H., and Hall, K. (1984) Eur. J. Biochem. 143, 117-124.
9. Hintz, R.L., and Liu, F. (1977). J. Clin. Endocrinol. Metab. 45, 988-995.
10. Hall, K., Brandt, J., Enberg, G., and Fryklund, L. (1979) J. Clin. Endocrinol. Metab. 48, 271-278.
11. Furlanetto, R.W. (1980) J. Clin. Endocrinol. Metab. 51, 12-19.
12. White, R.M., Nissley, S.P., Moses, A.C., Rechler, M.M., and Johnsonbaugh, R.E. (1981) J. Clin. Endocrinol. Metab. 53, 49-57.
13. Daughaday, W.H., Ward, A.P., Goldberg, A.C., Trivedi, B., and Kapadia, M. (1982) J. Clin. Endocrinol. Metab. 55, 916-921.
14. Schalch, D.S., Heinrick, U.E., Draznin, B., Johnson, C.J., and Miller, L.L. (1979) Endocrinology 104, 1143-1151.
15. Clemmons, D.R., Underwood, L.E., and Van Wyk, J.J. (1981) J. Clin. Invest. 67, 10-19.
16. Binoux, M., Lassarre, C., and Hardouin, N. (1982) Acta Endocrinol. (Copenh.) 99, 422-430.
17. Chochinov, R.H., Ketupanya, A., Mariz, I.K., Underwood, L.E., and Daughaday, W.H. (1976) J. Clin. Endocrinol. Metab. 42, 983-986.
18. Chochinov, R.H., Mariz, I.K., Hajek, A.S., and Daughaday, W.H. (1977) J. Clin. Endocrinol. Metab. 44, 902-908.
19. Bala, R.M., Wright, C., Bardal, A., and Smith, G.R. (1978) J. Clin. Endocrinol. Metab. 46, 649-652.
20. Drop, S.L.S., Valiquette, G., Guyda, H.J., Corvol, M.T., and Posner, B.I. (1979) Acta Endocrinol. (Copenh.) 90, 505-518.
21. Póvoa, G., Enberg, G., Jörnvall, H., and Hall, K. (1984) Eur. J. Biochem. 144, 199-204.
22. Hall, K., Takano, K., and Fryklund, L. (1974) J. Clin. Endocrinol. Metab. 39, 973-976.
23. Takano, K., Hall, K., Ritzén, M., Iselius, L., and Sievertsson, H. (1976) Acta Endocrinol. (Copenh.) 82, 449-459.

24. Enberg, G., and Hall, K. (1984) Acta Endocrinol. (Copenh.) 107, 164-168.
25. Jörnvall, H., and Philipson, L. (1980) Eur. J. Biochem. 104, 231-247.
26. Zimmermann, C.L., Appella, E., and Pisano, J.J. (1977) Anal. Biochem. 77, 569-573.
27. Zumstein, P.P., Läubli, U., and Humbel, R.E. (1983) Insulin like growth factors. Proc. Symp. Somatomedins. Abstract, p. 33.
28. Chernausek, S.D., Chatelain, P.G., Svoboda, M.G., Underwood, L.G., and Van Wyk, J.J. (1985). Biochem. Biophys. Res. Commun. 126, 282-288.
29. Hall, K. (1972) Acta Endocrinol. (Copenh.) Suppl. 163.
30. Posner, B.I., Guyda, H.J., Corvol, M.F., Rappaport, R., Harley, C., and Goldstein, S. (1978) J. Clin Eneocrinol. Metab. 47, 1240-1251.
31. Bala, R.M., and Bhaumick, B. (1979) Can. J. Biochem. 57, 1289-1298.
32. Svoboda, M.G., Van Wyk, J.J., Klapper, D.J., Fellows, R.E., Grissom, F.E., and Schleuter, R.J. (1980) Biochemistry 19, 790-797.
33. Kuffer, A.D., and Herington, A.C. (1984) Biochem. J. 223, 97-103.