Vol. 114, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 18, 1983 Pages 325-330

AMINO ACID SEQUENCE HOMOLOGY BETWEEN RAT PROSTATIC STEROID BINDING PROTEIN AND RABBIT UTEROGLOBIN

Michael E. Baker

Department of Medicine, M-023
University of California, San Diego
La Jolla, California 92093

Received May 31, 1983

Using a computer program designed to detect evolutionary relationships between proteins, I find that the polypeptide chain of rabbit uteroglobin has amino acid sequence homology with the C_1 and C_2 polypeptide chains of rat prostatic steroid binding protein. Using this finding I suggest several interesting approaches for studying the biology of these proteins.

Two secretory proteins, rat prostatic steroid binding protein (1-6) and rabbit uteroglobin (7-13), share the property of binding the steroid hormones that induce their synthesis. In this report, I present evidence that uteroglobin and prostatic steroid binding protein also share amino acid sequence homology and therefore I suggest that there was a common ancestor for these proteins. Also, I show how the homology between prostatic steroid binding protein and uteroglobin suggests experiments that may prove useful in understanding the biology of prostatic steroid binding protein and of uteroglobin.

Prostatic steroid binding protein is a tetramer consisting of two subunits; an A subunit containing polypeptides C_1 (M_r ~10,200) and C_3 (M_r ~8,600) and a B subunit containing C_2 (M_r ~10,500) and C_3 (3, 14-19). C_3 also contains an oligosaccharide chain (14). Androgens induce prostatic steroid binding protein synthesis and bind to prostatic steroid binding protein, its B subunit, and C_2 chain with low affinity (Kd ~1μM). Liao and co-workers (2, 16, 20, 21) have shown that prostatic steroid binding protein, its A subunit, and C_1 polypeptide inhibit binding of the classical 5α-dihydrotestosterone-receptor complex to prostatic cell nuclei. Since the binding of steroid hormone-receptor complexes to nuclear acceptor sites is thought to be an essential part of steroid hormone action (22) they have suggested that prostatic steroid binding protein functions as part of a feedback mechanism to regulate androgen action in rat prostate (21).

Uteroglobin consists of two identical polypeptides (M_r ~8,000) that are joined by two disulfide bridges (23-25). Progesterone induces uteroglobin synthesis in rabbit uterus and binds to uteroglobin with low affinity (Kd ~1μM).

Methods

I have used the Relate and Align programs developed at the National Biomedical Research Foundation (26-31) to study relationships among the amino acid sequences of uteroglobin and chains C_1, C_2, and C_3 of prostatic steroid binding protein.

0006-291X/83 $1.50

As described by Barker and Dayhoff (30), "the Relate program compares all possible segments of a given length (in this report 35 amino acids) from one sequence with all segments of the same length from a second sequence. A segment score is accumulated from the pair scores of the amino acids occupying corresponding positions within the two segments. The pair scores are specified using an empirically derived mutation data matrix (26-31). The mean of a number of highest scores is determined for the given sequences and for 100 comparisons of random permutations of the sequences. The segment comparison score is calculated as the difference between the mean of the real sequence and the average value determined from the 100 randomized sequences divided by the standard deviation (SD) of the values from the randomized sequences. The segment comparison score is thus expressed in SD units. It is assumed that a score > 5 SD units ($p < 2.8 \times 10^{-7}$) indicates evolutionary relatedness of two proteins and scores between 3 SD ($p < 10^{-3}$) and 5 SD ($p < 2.8 \times 10^{-7}$) support relationship if there are other indications such as similarity of function".

The Align program (28, 31) calculates the best alignment between any pair of sequences given a matrix of amino acid pair scores (26-29) and a penalty for breaking a sequence (gap). This score is compared with that obtained from 100 random permutations of the two sequences. The alignment score is the number of standard deviations by which the maximum score for the real sequences exceeds the average maximum score for the random

Results and Discussion

A comparison of the C_1 and C_2 polypeptide chains of prostatic steroid binding protein using the Relate analysis (Table 1) and the Align analysis (Table 2) gives scores greater than 15 SD units, clearly demonstrating that these two polypeptides are related. This result agrees with conclusions reached by Parker et al. (18), using the amino acid sequence and by Chen et al. (16), using immunological criteria. In agreement with those studies I find that the C_3 polypeptide is not related to C_1 or C_2 (Table 1).

Using the Relate analysis (Table 1) I find that a comparison of uteroglobin with C_1 and C_2 of prostatic steroid binding protein gives scores of 6 SD ($p<10^{-9}$) and 5.4 SD ($p<2.5 \times 10^{-8}$) units respectively. Additional evidence for an evolutionary relationship between these proteins comes from the Align analysis (Table 2) of uteroglobin with C_1 and C_2, which gives scores of 5.15 and 5.25 respectively ($p<10^{-7}$).

This finding that rat prostatic steroid binding protein and rabbit uteroglobin are related stimulated me to examine reports from other laboratories about these proteins for other indications of relatedness.

First, I found that several investigators reported (1, 5, 6, 16, 32) that prostatic steroid binding protein binds progesterone and pregnenolone with higher affinity than testosterone or dihydrotestosterone. Thus, prostatic steroid binding protein and rabbit uteroglobin appear to share some steroid hormone binding specificities. However, prostatic steroid binding protein synthesis is stimulated by testosterone (4, 33, 34). This synthesis is

Table 1. SEGMENT COMPARISON SCORES OF RABBIT UTEROGLOBIN AND THE C_1, C_2, and C_3 CHAINS OF RAT PROSTATIC STEROID BINDING PROTEIN.

	Rat Prostatic Steroid Binding Protein		
	C_1 chain	C_2 chain	C_3 chain
Rabbit Uteroglobin	6.1 SD	5.4 SD	2.4 SD
C_1 chain		26.0 SD	0.8 SD
C_2 chain			-0.4 SD

These segment comparison scores were obtained using the Relate program (segment length 35 amino acids). Scores greater than 5 standard deviation units ($p < 2.8 \times 10^{-7}$) indicate evolutionary relatedness between two proteins.

Table 2. ALIGNMENT ANALYSIS OF RABBIT UTEROGLOBIN AND THE C_1 AND C_2 CHAINS OF RAT PROSTATIC STEROID BINDING PROTEIN.

	Rat Prostatic Steroid Binding Protein	
	C_1 chain	C_2 chain
Rabbit Uteroglobin	5.15 SD	5.25 SD
C_1 chain		15.7 SD

These scores were obtained using the Align program. Scores greater than 5 standard deviation units ($p < 2.8 \times 10^{-7}$) indicate evolutionary relatedness between two proteins.

inhibited by cyproterone, an antiandrogen (33, 34) and progesterone does not stimulate synthesis of prostatic steroid binding protein (34).

Second, several investigators reported (35-37) the presence of uteroglobin-like antigens in rabbit seminal fluid, seminal vesicle and vas deferens where prostatic steroid protein would be present. It may be that uteroglobin and prostatic steroid binding protein share antigenic determinants.

Implications and Applications

A. Rat prostatic steroid binding protein and rabbit uteroglobin, as their names imply, are thought to be specific for prostate and uterus. Immunological studies suggest that uteroglobin is present in oviduct where it is induced by estrogen and in lung where it is induced by glucocorticoids (36). Also, immunological studies suggest that prostatic steroid binding protein is present in small quantity in rat pituitary, cerebral cortex, pancreas, adrenal gland, and submaxillary gland (38). Confirmation of the presence of uteroglobin and prostatic steroid binding protein in those diverse tissues will await characterization of the proteins from those tissues. I suggest that uteroglobin and prostatic steroid binding protein are part of a family of related proteins under diverse endocrine control.

B. An interesting approach to studying the significance of the structure of prostatic steroid binding protein and uteroglobin for their biological activity is suggested by the alignment of uteroglobin with the C_1 and C_2 polypeptide chains of prostatic steroid binding protein presented in Figure 1. The alignment shows that the cysteines near the ends of uteroglobin and the C_1, C_2, (and C_3) chains of prostatic steroid binding protein are conserved. Uteroglobin does not have the cysteine present near the center of C_1, C_2, and C_3. The cysteine positions in these proteins suggests that oxidized hybrids between uteroglobin and C_1, C_2, and C_3 could be formed. These hybrids could prove useful in understanding the functioning of uteroglobin, prostatic steroid binding protein, and the structural requirements for progestin and androgen binding.

C. The report by Shyr and Liao (20) that rat prostatic steroid binding protein inhibits ^3H-estradiol-receptor binding to calf uterus nuclei suggests that prostatic steroid binding protein in seminal fluid may have a

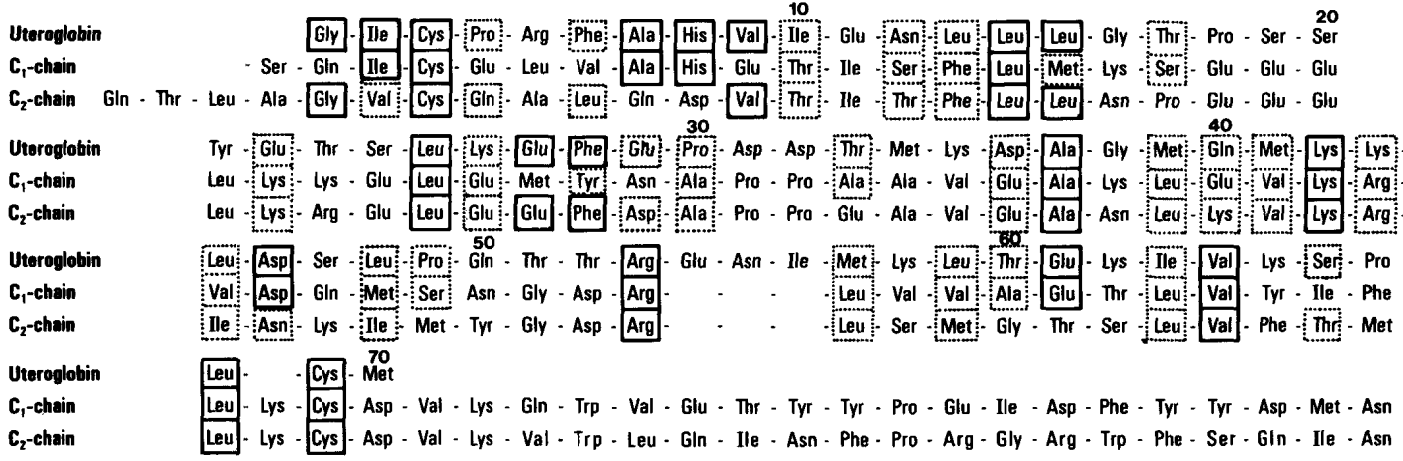

FIGURE 1. Alignment of rabbit uteroglobin and C_1 and C_2 polypeptides of rat prostatic steroid binding protein. Using the Align analysis, the best fit among these proteins is obtained with gap of 1 amino acid in uteroglobin (between Leu 68 and Cys 69) and 3 amino acids in C_1 and C_2 polypeptides of prostatic steroid binding protein, e.g. between Arg 54 and Leu 55 of C_1. The structure shows alignment of Cys 3 and Cys 69 of uteroglobin with cysteines on C_1 and C_2. Uteroglobin does not contain a third cysteine near the center that is present in the C_1, C_2, and C_3 polypeptides. Solid boxes show perfect homologies and the dotted boxes show readily accepted (26) semi-conservative amino acid homologies between uteroglobin and the C_1 and C_2 chains of prostatic steroid binding protein. The C_2 chain sequence is revised from that originally reported (18) (Malcolm Parker, personal communication).

function in the female reproductive system. This function could depend on the progesterone binding capability of prostatic steroid binding protein.

D. The homology between uteroglobin and prostatic steroid binding protein's C_1 chain suggests determining if uteroglobin inhibits nuclear binding of steroid hormone receptor complexes.

E. Recently, Mukherjee et al reported that either purified uteroglobin or crude prostatic fluid suppressed antigenicity of epididymal sperm (39) as well as early embryonic antigenicity (40). They proposed that uteroglobin suppresses sperm antigenicity in the female reproductive tract and the antigenicity of developing embryos during implantation. From their findings with prostatic seminal fluid and uteroglobin I suggest that prostatic steroid binding protein may have an immunosuppressive function.

F. Finally, estradiol mustard derivatives that bind to prostatic steroid binding proteins (32, 38), are also used in treating prostatic carcinoma (41). The homology between uteroglobin and prostatic steroid binding protein suggests that steroid mustards may prove useful in studying uteroglobin.

In summary, computer analysis of the amino acid sequences of rabbit uteroglobin and the C_1 and C_2 polypeptide chains of rat prostatic steroid binding protein indicated that these proteins have evolved from a common ancestor. This finding suggests several interesting approaches for studying the mechanism of action of these proteins.

Acknowledgements

I thank Dr. Winona Barker for her patient advice on the subtlties of using the Relate and Align programs. I thank Drs. W. Barker, M. Beato, R. Doolittle, D. Fanestil, J. Geller, S. Liao, A. Mukherjee, M. Parker and S. Yen for their constructive comments on an earlier version of this manuscript. This research was supported by NIH grant PHS R01 HD 14968.

REFERENCES

1. Karsznia, R., Wyss, R.H., Heinrichs, W.L., and Herrmann, W.L. (1969) Endocrinology 84, 1238-1246.
2. Fang, S. and Liao, S. (1971) J. Biol. Chem. 246, 16-24.
3. Ichii, S. (1975) Endocrinol. Jpn. 5, 433-437.
4. Heyns, W., Peeters, B. and Mous, J. (1977) Biochem. Biophys. Res. Commun. 7, 1492-1499.
5. Heyns, W. and DeMoor, P. (1977) Eur. J. Biochem. 78, 221-230.
6. Lea, O.A., Petrusz, P., and French, F.S. (1979) J. Biol. Chem. 254, 6196-6202.
7. Krishnan, R.S. and Daniel, J.C. (1967) Science 158, 490-492
8. Beier, H.M. (1968) Biochem. Biophys. Acta 160, 289-291.
9. Urzua, M.A., Stambaugh, R., Flickinger, G. and Mastroianni, L. (1970) Fertil. Ster. 21, 860-865.
10. Arthur, A.T. and Cowan, B.D. (1972) Fertil. Steril. 23, 85-92.
11. Arthur, A.T. and Daniel, J.C. (1972) Fertil. Steril. 23, 115-122.
12. Beato M. and Baier, R. (1975) Biochim. Biophys. Acta 392, 346-356.
13. Beato, M., Arneman, T., and Voss, H.J. (1977) J. Steroid Biochem. 8, 725-730.
14. Peeters, B., Rombauts, W., Mous, J. and Heyns, W. (1981) Eur. J. Biochem. 115, 115-121.
15. Peeters, B., Heyns, W., Mous, J. and Romhauts, W. (1982) Eur. J. Biochem. 123 55-62.
16. Chen, C., Schilling, K., Hiipakka, R.A., Huang, I. and Liao, S. (1982) J. Biol. Chem. 257, 116-121.
17. Liao, S., Chen, C. and Huang, I.-Y. (1982) J. Biol. Chem. 257, 122-125.
18. Parker, M., Needham, M. and White, R. (1982) Nature 298, 92-94.
19. Parker, M.G., White, R., Hurst, H., Needham, M. and Tilly, R. (1983) J. Biol. Chem. 259, 12-15.
20. Shyr, C. and Liao, S. (1978) Proc. Nat. Acad. Sci. 75, 5969-5973.
21. Chen, C., Hipakka, R.A. and Liao, S. (1979) J. Steroid Biochem. 11, 401-405.
22. Spelsberg, T.C. (1982) Biochemical Actions of Hormones, Vol. IX. pp. 141-204, Academic Press, New York,
23. Ponstingl, H., Nieto, A. and Beato, M. (1978) Biochemistry 17, 3908-3912.
24. Popp, R.A., Foresman, K.R., Wise, L.D. and Daniel, J.C. Jr. (1978) Proc. Nat. Acad. Sci. 75, 5516-5519.

25. Menne, C., Suske, G., Arnemann, J., Wenz, A.C., Cato, B., and Beato, M. (1982) Proc. Nat. Acad. Sci. USA 79, 4853-4857.
26. Dayhoff, M.O., Eck, R.V. and Park, C.V. (1972) Atlas of Protein Sequence and Structure. Vol. 5, pp. 89-99, National Biomedical Research Foundation, Washington, D.C.
27. Barker, W.C. and Dayhoff, M.O. Ibid, pp. 101-110.
28. Dayhoff, M.O. (1978) Atlas of Protein Sequence and Structure, Vol. 5, Suppl. 3. pp. 1-8. National Biomedical Research Foundation, Washington, D.C.
29. Schwartz, R.M. and Dayhoff, M.O. Ibid, pp. 353-358.
30. Barker, W.C. and Dayhoff, M.O. (1982) Proc. Nat. Acad. Sci. USA 79, 2836-2839.
31. Dayhoff, M.O., Barker, W.C. and Hunt, L.T. (1983) Meth. in Enzymol. 91, 524-545.
32. Forsgren, B., Gustafsson, J-A., Pousette, A., and Hogberg, B. (1979) Cancer Res. 39, 5155-5164.
33. Parker, M.G., Scrace, G.T. and Mainwaring, W.I.P. (1978) Biochem. J. 170, 115-121.
34. Heyns, W., VanDamme, B. and DeMoor, P. (1978) Endocrinology 103, 1090-1095.
35. Beier, H.M., Bohn, H., and Muller, W. (1975) Cell. Tiss. Res. 165, 1-11.
36. Feigelson, M., Noske, I.G., Goswami, A.K., and Kay, E. (1977) Annals N.Y. Acad. Sci. 286, 273-286.
37. Noske, I.G. and Golding, M. (1978) J. Reprod. Fert. 54, 193-196.
38. Forsgren, B., Bjork, P., Carlstrom, K., Gustafsson, J-A., Pousete, A. and Hogberg, B. (1979) Proc. Nat. Acad. Sci. USA 76, 3149-3153.
39. Mukherjee, D.C., Agrawal, A.K., Manjunath, R., and Mukherjee, A.B. (1983) Science 219, 989-991.
40. Mukherjee, A.B., Ulane, R.E. and Agrawal, A.K. (1982) Amer. J. Reprod. Immunol. 2, 135-141.
41. Jonsson, G., Hogberg, B. and Nilsson, T. (1977) Scan. J. Urol. Nephrol. 11, 231-238.