Conformation of Biologically Active Derivatives of Human Placental Lactogen*

(Received for publication, April 7, 1980, and in revised form, September 5, 1980)

Arthur B. Schneider, Frederic Barr, John Russell, Kazimierz Kowalski, and Louis M. Sherwood

From the Department of Medicine, Michael Reese Hospital and Medical Center and the Pritzker School of Medicine, University of Chicago, Chicago, Illinois 60616

Cleavage of human placental lactogen (hPL) by plasmin, which removes the hexapeptide containing residues 135 to 140, has allowed us to prepare biologically active derivatives. In order to study the relationship between chemical structure and biologic activity, we have compared the conformation of two active derivatives, plasmin-cleaved hPL and (1-134), (a disulfide-called dimer of Fragment 1-134), with native hPL and reduced and alkylated 1-134, using circular dichroism, intrinsic fluorescence, and immunologic measurements. Circular dichroism in the region of peptide bond activity showed that hPL, plasmin-cleaved hPL, and (1-134)2 had comparable degrees of a helix, while reduced and alkylated 1-134 had much less. In the near-ultraviolet region of the spectrum circular dichroism measurements of hPL and plasmin-cleaved hPL were nearly identical. The spectra of (1-134), and reduced and alkylated 1-134 were considerably different from hPL and from each other. A red shift to tryptophanyl fluorescence was produced by titration with increasing alkali or urea. The amount of urea needed for this transition, in decreased order, was: hPL > plasmin-cleaved hPL > (1-134)2 > reduced and alkylated 1-134.

Two radioimmunoassays, one using antibody raised against native hPL and the other using antibody against reduced and alkylated 1-134, were used. The specificity of these assays for the conformation of hPL was indicated by the virtual lack of cross-reactivity of Fragment 1-134 in the native hPL radioimmunoassay and the marked decrease in activity of native hPL in the assay for reduced and alkylated 1-134. Comparison of displacement curves in these assays showed that plasmin-treated hPL was different from, but closely related to, native hPL while (1-134); had immunologic features of both native and unfolded hPL. We conclude that the biologically active derivatives of hPL contain important elements of the native configuration as well as varying amounts of features characteristic of unfolded hPL.

A series of biologically active derivatives of hPL† has been prepared by limited plasmin digestion. These have been useful in studying the relationship between the primary structure of the hormone and its biologic activity. Plasmin digestion of hPL removes a single hexapeptide (residues 135 to 140) and leaves two fragments (residues 1 to 134 and 141 to 191) joined by a disulfide bond between Cy253 and Cy690 (1). This derivative (PL-hPL) has increased potency in both receptor binding and in vitro biologic assays. Two 1-134 fragments can be joined by a disulfide bond between Cy53 to residues to form (1-134)2. This derivative retains the ability to bind to lactogenic receptors and is fully active in vitro (2). In the accompanying paper we describe the production and characterization of active derivatives produced by the recombination of plasmin-generated fragments of hPL with fragments of hGH (3).

Our earlier reports indicated that the conformation of the hPL derivatives was important in determining their biologic activity (1-3). For example, RCAM-1-134 was shown to be biologically inactive while the dimer (1-134); was active. This difference could only be accounted for by the refolding of the dimer to an active conformation. In this manuscript we describe studies of the conformation of hPL and its derivatives using circular dichroism, intrinsic fluorescence, and immunologic measurements. The results indicate that the structure of PL-hPL is similar to hPL, while (1-134)2 contains structural features of both native and unfolded hPL.

DISCUSSION

Three methods were employed to give complementary information about the structure of hPL and its derivatives. The far-UV circular dichroism spectrum was used to measure secondary structure, two radioimmunoassays were used to distinguish native and unfolded conformational states, and intrinsic fluorescence and near-UV circular dichroism were used to monitor the environments of the aromatic chromophores (9-17).

RCAM-hPL and RCAM-1-134—All of the analytical methods indicate that the structures of RCAM-hPL and RCAM-1-134 were very different than that of the native hPL. Aloj et al. (9) showed that the structure of RCAM-hPL is not random but is different than that of native hPL. Therefore, the structure of the RCAM-hPL derivatives will be referred to as "unfolded" forms, recognizing that they are not entirely random.

PL-hPL—The secondary structure and environment of the

* This work was supported in part by National Institutes of Health Grant HD 06225, Research Career Development Award AM 00108 to A. B. S., and Research Fellowship HD 05548 to J. R. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Portions of this paper (including "Materials and Methods," "Results," and Figs. 1-4) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document No. 80M-662, cite author(s), and include a check or money order for $3.20 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
conformation of human placental lactogen derivatives

Longer wavelengths occur more easily than for native hPL and PL-hPL.

Conclusion—Each of the biologically active derivatives of hPL contains elements of native conformation as well as features characteristic of unfolded hPL. There are two possibilities that could account for the simultaneous presence of elements of native and unfolded structure in the same molecule which cannot be distinguished by the methods employed. These are as follows: 1) each molecule has a stable conformation which retains both native and unfolded elements and 2) there is rapid equilibrium between conformations, with one form closely related to the native molecule and the other related to the unfolded species. Theoretically, these possibilities could be distinguished by kinetic measurements using a structural parameter which could be obtained more rapidly than the potential rates of interconversion of two species. It will probably be necessary for x-ray crystallographic data to define more precisely the three-dimensional structure necessary for lactogenic activity in hPL and, in the homologous proteins growth hormone and prolactin, the structure necessary for both growth-promoting and lactogenic effects.

Acknowledgments—We thank Mrs. Nikki Keil and Ms. Janice Penson for preparing this manuscript. Drs. E. T. Kaiser and Y. Nakagawa kindly made their Cary 90 available to us.

REFERENCES

1. Russell, J., Schneider, A. B., Katzhendler, J., Kowalski, K., and Sherwood, L. M. (1979) J. Biol. Chem. 254, 2296-2301
2. Russell, J., Sherwood, L. M., Kowalski, K., and Schneider, A. B. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 1204-1207
3. Russell, J., Sherwood, L. M., Kowalski, L., and Schneider, A. B. (1981) J. Biol. Chem. 256, 296-300
4. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
5. Handwerger, S., and Sherwood, L. M. (1974) in Methods of Hormone Radioimmunoassay (Jaffe, B. M., and Behrman, M. H., eds) pp. 417-420, Academic Press, New York
6. Schneider, A. B., Kowalski, K., and Sherwood, L. M. (1975) Endocrinology 97, 1364-1372
7. Thorrell, J. I., and Johansson, B. G. (1971) Biochim. Biophys. Acta 251, 363-369
8. Rebard, D., and Lewald, J. E. (1970) Acta Endocrinol. 64, Suppl. 147, 79-103
9. Ajo, S. M., Edelhoch, H., Handwerger, S., and Sherwood, L. M. (1972) Endocrinology 91, 728-737
10. Ajo, S., and Edelhoch, H. (1971) J. Biol. Chem. 246, 5047-5052
11. Bewley, T. A., and Li, C. H. (1971) Arch. Biochem. Biophys. 147, 598-605
12. Bewley, T. A., and Puettt, D. (1977) Int. J. Peptide Protein Res. 10, 363-368
13. Bewley, T. A., Kawauchi, H., and Li, C. H. (1972) Biochemistry 11, 4170-4187
14. Berti, P., Arezzi, C., Botti, R., Coca, F., and Tarli, P. (1973) Biochim. Biophys. Acta 322, 85-94
15. Kawauchi, H., and Li, C. H. (1974) Arch. Biochem. Biophys. 165, 255-262
16. Bewley, T. A. (1977) in Hormonal Proteins and Peptides (Li, C. H., ed) Vol. IV, pp. 61-137, Academic Press, New York
17. Aubert, M. L., Bewley, T. A., Grumbach, M. M., and Kaplan, S. L. (1973) in Advances in Human Growth Hormone Research (Raiti, S., ed) pp. 434-462, Department of Health, Education and Welfare Publication No. 74-812, National Pituitary Agency, Baltimore, Md.
18. Bewley, T. A. (1977) Biochemistry 16, 4408-4414
For base titration, hPL and its derivatives were dissolved in 0.1 M NaOH, 0.01 M KCl, 0.01 M Tris, pH 7.0. The titration was performed by adding 1 M HCl to an Alcalase digest directly into a fluorescence cuvette, which was stirred magnetically and into which a pH electrode was placed. The titration curves were corrected for the dilution factor, which never exceeded 10%. For acid titrations, stock solutions of hPL and its derivatives were prepared in 0.01 M NaOH, 0.01 M KCl, pH 7.0, and equal aliquots were added to solutions containing 0 to 10 M urea in 0.01 M Tris-glucose, 0.1 M NaCl, pH 7.0.

**RESULTS**

**Hormone Induction of Structure:** Displacement curves for the two radioimmunoassays used to determine hormones in the biological activity. In Figure 1, the hormone is displayed on a logarithmic scale, with the area under the curve indicating the biological activity. In Figure 2, the hormone is displayed on a linear scale, with the area under the curve indicating the biological activity. In Figure 3, the hormone is displayed on a logarithmic scale, with the area under the curve indicating the biological activity. In Figure 4, the hormone is displayed on a linear scale, with the area under the curve indicating the biological activity. In Figure 5, the hormone is displayed on a logarithmic scale, with the area under the curve indicating the biological activity.

**Supplemental Material to**

**CONFORMATION OF BIOLOGICALLY ACTIVE DERIVATIVES OF HUMAN PLACENTAL LACTOGEN**

Arthur B. Schneider, Frederick Barr, John Russell, Katherine J. W. and Louis H. Sherrard

Running Title: **CONFORMATION OF HUMAN PLACENTAL LACTOGEN DERIVATIVES**

**MATERIALS AND METHODS**

Preparation of hPL derivatives: The hPL used in these studies was kindly supplied by Drs. Paul Smith and Richard Smith. Partial purification of hPL from bovine serum was achieved by Dr. Bernard Oppenheimer. Limited peptide digestion of hPL was carried out according to methods described previously [1]. The cleavage of hPL was performed by 100-μg/mL trypsin. Digests were analyzed by SDS-polyacrylamide gel electrophoresis and by sensitive immunoassays for intact hormone levels. The synthetic hPL-27 routine (1-21) was reconstituted in 0.1 M NaOH, 0.01 M KCl, 0.01 M Tris, pH 7.0, and was used for sera in the hPL radioimmunoassay.

Circular Dichroism Measurements: CD measurements were made with a Jasco Model 60 spectropolarimeter equipped with a polarimeter. hPL and its derivatives were dissolved in 0.01 M NaOH, 0.01 M KCl, 0.01 M Tris, pH 7.0. The optical density at 280 nm, initially measured, was calculated using the known molecular weight of each of the derivatives.

**Circular Dichroism**

The near and far UV spectra of hPL and its derivatives are shown in Figure 2 and Figure 3 of the accompanying paper [3]. The far UV spectra (lower panel) of hPL, PL-NH₂, and (1-21)hPL were virtually identical. The near UV spectra of hPL, PL-NH₂, and (1-21)hPL showed large bands of negative ellipticity at 253 nm, characteristic of the same conformation. Their spectra differed sufficiently so that the shoulder at 273 nm was absent in the hPL spectrum, the hPL-27 routine, and (1-21)hPL, but was present in the spectrum of PL-NH₂ and in the hPL-41 derivative. These differences were consistent with bands present in the hPL spectrum, where they were absent or not resolved (298 nm), indicating a positive contribution of hPL and its derivatives from the amide band. A band at 230 nm was present in the spectrum of hPL and its derivatives from the aromatic amino acid. For hPL and its derivatives negative bands at 295, 287, and 260 nm were less intense than these of hPL.