Cloning and Sequence Determination of Human Placental Aldose Reductase Gene*

Stephen Chung† and Joseph LaMendola
From the Department of Biological Chemistry, University of Illinois, Chicago, Illinois 60612

The human aldose reductase gene has been cloned by screening a human placental cDNA library with antibodies against bovine lens aldose reductase. The nucleotide sequence of the entire coding region has been determined. The deduced amino acid sequence indicates that the human enzyme is 84% identical to the bovine lens aldose reductase and 85% identical to the rat lens aldose reductase. It is also very similar to the human aldehyde reductase, the bovine prostaglandin F synthase, and to the European common frog rho-crystallin. The deduced amino acid sequence also indicates that maturation of aldose reductase involves removal of the N-terminal methionine.

Aldose reductase is an enzyme that converts sugars into their polyols. The major substrate in the cell is glucose, which is reduced to sorbitol. The enzyme was first identified in seminal vesicles and placental tissues (Hers, 1960) and later found in a number of other tissues including lens, kidney, liver, and brain (Gabbay, 1975). Since sorbitol does not readily diffuse out of the cell, and its conversion to fructose is slow, in some tissues it constitutes a significant fraction of the cellular osmolite. Thus, aldose reductase has been implicated to be involved in the regulation of cellular osmotic pressure in these tissues. In a rabbit kidney cell line that is able to grow in high salt medium, adaptation to high salt environment results in high level of aldose reductase and high concentration of sorbitol (Bagnasco et al., 1987). In diabetic patients where the blood sugar level is high, this enzyme has been implicated to be involved in some of the clinical complications such as cataract formation (Kinoshita, 1974), retinopathy (Buzney et al., 1977), neuropathy (Gabbay, 1973), and possibly kidney failure (Corder et al., 1979). Treatment with inhibitors of aldose reductase alleviates some of these symptoms (Kinoshita, 1974; Tomlinson et al., 1982; Judzewitsch et al., 1983). One hypothesis is that in the hyperglycemic patients, the aldose reductase activity increases cellular osmotic pressure, leading to swelling of the cells, and in some yet undefined fashion, causes some of these clinical complications. However, it was also observed that treatment with high doses of myoinositol also restores the nerve conduction velocity of diabetic animals without reducing the cellular osmotic pressure (Mayer and Tomlinson, 1983). Thus, it is essential to understand the function of this enzyme in the cell and its role in diabetic complications. Our approach is to clone the gene for this enzyme and to observe the effect when the expression of the gene is modulated by introducing modified cloned genes into the cell. The cloned gene will also aid in elucidating the structure of aldose reductase for designing specific inhibitors of this enzyme for clinical applications. In this report, we describe the cloning and sequence determination of this gene and discuss its homology with a family of related proteins.

MATERIALS AND METHODS

Cloning of Aldose Reductase Gene—The human placental cDNA library constructed in Agt11 vector was purchased from Clonetech (Palo Alto, CA). The library was screened with anti-bovine lens aldose reductase antibodies (gift from Dr. C. Doughty, University of Illinois at Chicago) according to the protocol suggested by Clonetech. Approximately 10,000 plaques were plated on each 90-mm diameter plate and incubated at 42°C for 4 h. A dry nitrocellulose filter saturated previously with 10 mM isopropyl-1-thio-β-D-galactopyranoside was placed on top of the agar, and the plates were incubated for another 4 h at 37°C. The filters were then rinsed with TBST (50 mM Tris, pH 7.9, 150 mM NaCl, 0.05% Tween-20), and incubated with coating solution (10 mg/ml turkey egg albumin in TBST) at room temperature for 30 min. The filters were then rinsed with TBST and incubated with rabbit anti-bovine aldose reductase antibodies (500-fold dilution of the serum in TBST, 5 ml/filter) at room temperature for 1 h. The filters were washed with TBST to remove the bound primary antibodies and incubated with anti-rabbit IgG antibodies conjugated to alkaline phosphatase (Promega, Madison, WI). Color development of the filters was performed as suggested by the manufacturer. Positive clones give a dark purple color. The clones were purified and rescreened with antibodies as described above.

Purification of DNA from Agt11 Clones—The plaque-purified clones were grown in liquid culture, and the phage particles were precipitated from the lysate by polyethylene glycol 6000. DNA was extracted from phage particles by equal volumes of phenol and chloroform three times.

DNA Hybridization—The hybridization solution containing 50% formamide, 4 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate), 4 × Denhardt (1 × Denhardt = 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, 0.02% Ficoll), 2.5 mM EDTA, and 1 μg/ml sonicated denatured calf thymus DNA. Hybridization was performed at 42°C for 48 h, and the filters were washed with 0.1 × SSC and 0.1% sodium dodecyl sulfate at 53°C.

DNA Sequence Determination—The nucleotide sequence was determined by the dideoxynucleotide chain termination method of Sanger et al. (1977). Unidirectional deletion of the cDNA in the Bluescript vector was constructed according to the procedure recommended by Stratagene of California. Both strands were sequenced with the exception of about 30 bases at the 3′-untranslated region where only one strand was sequenced (see Fig. 1).

RESULTS AND DISCUSSION

Identification of Aldose Reductase Clones—Approximately 500,000 clones of the human placental cDNA library were screened by antibodies against the bovine lens aldose reduc-
Human Placental Aldose Reductase cDNA Sequence

The nucleotide sequence of approximately 300 base pairs from the EcoRI restriction site of the vector was determined. The cDNA released from the vector by EcoRI digestion was inserted into the EcoRI site of the plasmid vector. The sequence from both ends of the insert can be determined in this vector.

The nucleotide sequence of approximately 300 bases from each end of the cDNA insert was determined, and the deduced amino acid sequence was compared with the bovine aldose reductase sequence that was determined by peptide sequencing and with the partial rat aldose reductase sequence. The nucleotide sequence of the 1.4-kilobase cDNA insert contains the entire coding region of aldose reductase. The ATG codon at position 37 encodes the first amino acid methionine, and the codon TGA at position 985 signifies translation termination. There is no other open reading frame longer than 67 nucleotides, indicating that the protein beginning at the ATG codon at position 37 is the protein product of this gene. Furthermore, this translation reading frame is the same as that of β-galactosidase of the vector. This is in agreement with the fact that the clone was identified by antibodies recognition of the β-galactosidase-aldose reductase fusion protein.

We think that clone B1 is a cDNA clone of the human aldose reductase gene because of its high degree of homology with the bovine and rat aldose reductases. As shown in Fig. 3 and Table I, 85.4% of the amino acids in B1 protein are identical to those of bovine aldose reductase. Similarly, B1 shares 84.8% identity with the portion of the rat aldose reductase that has been sequenced. In the region of the B1 protein where the corresponding rat aldose reductase sequence is known, there are 43 amino acids that are not identical to the rat enzyme. Twenty of these 43 amino acids are identical to the corresponding amino acids in both bovine and rat aldose reductase. Therefore, there are only 23 amino acids on B1 protein that are not identical to either the rat or bovine enzyme. Among these 23 amino acids, 12 of them can be considered as having similar functional groups as their corresponding amino acids in either the rat or bovine aldose reductase, leaving only 11 among the 284 amino acids in this region that are different from the two known aldose reductases. This indicates that B1 protein is structurally and functionally very similar to the rat and bovine aldose reductase.

1 C. Doughty, J. Biol. Chem., submitted for publication.
Human Placental Aldose Reductase cDNA Sequence

Acknowledgment — We thank Drs. Clyde Doughty and Sylvia Shade for providing us with anti-bovine aldose reductase antisera, the unpublished sequence of the enzyme, and for helpful discussions.

REFERENCES

Bagnasco, S. M., Uchida, S., Balaban, R. S., Kador, P. F., and Burg, M. B. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 1718–1720
Buzney, S. M., Frank, R. N., Varma, S. D., Tanishima, T., and Gabbay, K. H. (1977) Invest. Ophthalmol. & Vis. Sci. 16, 392–396
Carper, D., Nishimura, C., Shinohara, T., Dietzchold, B., Wartburg, J. P., Bullock, B., and Gabbay, K. H. (1988) FEBS Lett. 220, 202–205
Corder, C. N., Braughler, J., M. L., and Culp, P. A. (1979) Folia Histochem. Cytotom. 17, 137–146
Gabbay, K. H. (1975) Annu. Rev. Biochem. 44, 1718–1720
Hers, H. G. (1960) Biochim. Biophy. Acta 37, 120–126
Jude, J. G., Aspin, J. B., Polansky, K. S., Weinberg, C. R., Halter, J. B., Halar, E., Pfeifer, M. A., Vukadinovic, C., Bernstein, L., Schneider, A. P., and Gabbay, K. H. (1988) Biochem. Biophys. Res. Commun. 153, 1051–1059
Kagamiyama, H., Gabbay, K. H., and Rubin, T. (1988) Biochem. Biophys. Res. Commun. 16, 1051–1055
Mayer, J. H., Halar, E., Pfeifer, M. A., Vukadinovic, C., Bernstein, L., Schneider, M., Liang, K.-Y., Gabbay, K. H., and Porte, Jr., D. (1985) Diabetes 34, 433–438
Nishimura, C., Graham, C., Hohman, T. C., Nagata, M., Robinson, W. G., Jr., and Carper, D. (1988) Biochem. Biophys. Res. Commun. 153, 1051–1059
Sanger, F., Nicken, S., and Coulson, R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
Tomarev, S. I., Zinovieva, R. D., Dolgilevich, S. M., Luchin, S. V., Krayev, A. S., Skryabin, K. G., and Gause, G. G., Jr. (1984) FEBS Lett. 171, 297–302
Tomlinson, D. R., Holmes, P. R., and Mayer, J. H. (1982) Neurosci. Lett. 31, 169–173
Watanabe, K., Fujii, Y., Nakayama, K., Ohkubo, H., Kuramitsu, S., Kayagamiyama, H., Nagashima, S., and Hayashi, O. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 11–15
Wermuth, B., Omer, A., Forster, A., di Francesco, C., Wolf, M., van Wittersberg, J. P., Bullock, R., and Gabbay, K. H. (1987) in Enzymology and Molecular Biology of Carbohydr Metabolism (Weiner, H., and Flynn, T. C., eds) pp. 297–307, Alan R. Liss, Inc., New York

Fig. 3. Amino acid sequence comparison between aldose reductase and related proteins. RAR, rat lens aldose reductase (Carper et al., 1987); BAR, bovine lens aldose reductase (Schade, S. M., Uchida, S., Balaban, R. S., Kador, P. F., and Burg, M. B. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 1718–1720)

Homology with the Family of Related Proteins—As noted by other authors, aldose reductase shares substantial amount of homology with aldehyde reductase (Wermuth et al., 1987), prostaglandin F synthase (Watanabe et al., 1988), and the rhocystallin of the frog lens (Tomarev et al., 1984). The complete amino acid sequence of human aldose reductase is presented. The other amino acid sequences are presented. The other amino acid sequences are

and that it is most likely a human aldose reductase.

As discussed in the following section and in Fig. 3, aldose reductase also shares substantial amount of homology with aldehyde reductase (Wermuth et al., 1987; Carper et al., 1987), prostaglandin F synthase (Watanabe et al., 1988), and European common frog lens rhocystallin (Tomarev et al., 1984; Carper et al., 1987; Watanabe et al., 1988). However, we think that the B1 protein is unlikely to be the human counterpart of these proteins for the following reasons. First, the homology between B1 and these proteins are below 50% (see Table I). Second, the B1 protein sequence can be aligned perfectly (without deletions or insertions) with the bovine and rat aldose reductase but not with other proteins. Therefore, we conclude that B1 protein is the human aldose reduc-