Crystallization and preliminary X-ray analysis of human brain hexokinase

Alexander E. Aleshin, Chenbo Zeng, Herbert J. Fromm, Richard B. Honzatko*

Department of Biochemistry and Biophysics, 1210 Molecular Biology Bldg., Iowa State University, Ames, IA 50011, USA

Received 1 April 1996

Abstract Human brain hexokinase type I, expressed in *Escherichia coli*, has been crystallized from polyethylene glycol 8000 in the presence of inorganic phosphate. The crystals are hexagonal needles of diameter 0.25 mm, diffracting to a resolution of 3.5 Å on a rotating-anode/area-detector system. The crystals belong to the space group $P3_121/P3_221$ with cell dimensions $a = b = 171.5$ Å and $c = 99.4$ Å. The specific volume of the crystal is 4.2 Å^3/Da, suggesting an asymmetric unit with a single 100 kDa molecule and a solvent content of 71% by volume or two molecules of hexokinase with a solvent content of 41%. The complex of hexokinase with glucose crystallizes under similar conditions, giving crystals of the same morphology.

Key words: Brain hexokinase; Hexokinase type I; X-ray crystallography; Crystallization

Hexokinase I (ATP: D-hexose 6-phosphotransferase, EC 2.7.1.1) is one of four mammalian hexokinase isozymes. It is the pacemaker of glycolysis in brain and red blood cells [1,2]. In fact, the human brain uses approximately 50% of the total chemical energy expended by the human body, a demand met almost entirely by the activity of hexokinase type I in the brain [2].

Type I and II isozymes have a number of features which distinguish them from other mammalian hexokinases and from eukaryotic glucokinases, such as yeast hexokinase. Hexokinases type I and type II bind to the outer membrane of mitochondria [3] and are inhibited strongly by their reaction product glucose-6-phosphate [1,4–6]. Hexokinase type I differs from hexokinase type II in that the product inhibition can be reversed by physiological levels of inorganic phosphate [1,4,5]. These properties of hexokinase type I may make it a critical regulator of glycolysis in brain tissue and erythrocytes [1,2,4,7].

Human brain hexokinase is a 100 kDa enzyme, consisting of two highly homologous (50% identity) sequence domains. Each sequence domain in turn is homologous (30% identity) to yeast hexokinase and human glucokinase (hexokinase type IV). Presumably, human brain hexokinase evolved from a 50 kDa yeast-like enzyme by gene duplication and fusion [8,9]. Hexokinase type I, lacking the N-terminal sequence domain (mini-hexokinase), has the same kinetic properties toward glucose, ATP, and glucose-6-phosphate as intact brain hexokinase type I [10]. Mini-hexokinase, however, exhibits inhibition by glucose-6-phosphate, which cannot be reversed by phosphate [11,12]. The N-terminal sequence domain is inactive, generally being regarded as a modulator of activity [10]. Glucose-6-phosphate binds near or at the ATP site on the C-terminal sequence domain [1,12], rather than, as originally suggested [2,13], to an allosteric site on the N-terminal sequence domain. On the basis of kinetics [1], the binding site for glucose-6-phosphate is different from that of glucose.

The structure of yeast hexokinase is known [14–16], but the Protein Data Bank [17] has only two relevant entries with sequences deduced from 2.1 Å and 3.5 Å electron density maps. The binding of glucose to the active site causes a 12° rotation of one of the structural domains of the yeast enzyme, transforming it into an active form [18]. Though the structure of yeast hexokinase has been used for modeling the glucose and ATP binding sites of brain hexokinase ([19]; Zeng, C., Aleshin, A.E., Harrison, R.W., Chen, G. and Fromm, H.J., personal communication), the yeast enzyme is of little help in understanding the regulatory properties of hexokinase type I. Furthermore, yeast hexokinase and hexokinase type I exhibit significant differences in their interaction with glucose-6-phosphate [1].

Reported here are the crystallization conditions and preliminary X-ray diffraction investigation of human brain hexokinase type I. The cloned enzyme was produced in *Escherichia coli* strain BL21 (DE3) and purified as described earlier [12,20]. The initial conditions for crystallization were discovered by the method of hanging drops, using a sparse screening matrix [21]. Crystals grew at room temperature under the following conditions: 5 μl of the protein solution of concentration 20 mg/ml in 20–40 mM sodium phosphate buffer (pH 6.5) was mixed with the same amount of a precipitant solution containing 3–5% (w/v) polyethylene glycol 8000 (Sigma) and 0.1 M sodium cacodylate buffer at pH 6.5. The drops were equilibrated against 0.7 ml of the precipitant solution. Long hexagonal needles of diameter 0.1–0.3 mm appeared within 1 week. Crystals of similar morphology also appeared under the same conditions, but with 10 mM glucose present in the protein solution. Taking into account that the constants of dissociation for phosphate and glucose are 22 μM [22] and 60 μM [12], respectively, we suggest that the first crystal form contains bound phosphate and the second one both ligands. The addition of glucose did not change the morphology of crystals, suggesting that hexokinase type I in both complexes may have similar conformations (Fig. 1).

We collected data from crystals of brain hexokinase type I, complexed with phosphate, on a Siemens area detector mounted on an X-ray generator with a Cu rotating anode. We observed diffraction to a resolution of 3.5 Å. Due to problems of decay, however, we limited data collection to 3.8 Å resolution. The data set contains 33375 observations, 15449 of which are unique reflections (91% of data expected to 3.8 Å). Data reduction with XENGEN [23] gave R_{mrg} equal to 0.07.

The crystals of hexokinase type I belong to space group $P3_221/P3_121$. The unit cell dimensions are $a = b = 171.5$ Å

*Corresponding author.

PII S0014-5793(96)00688-6

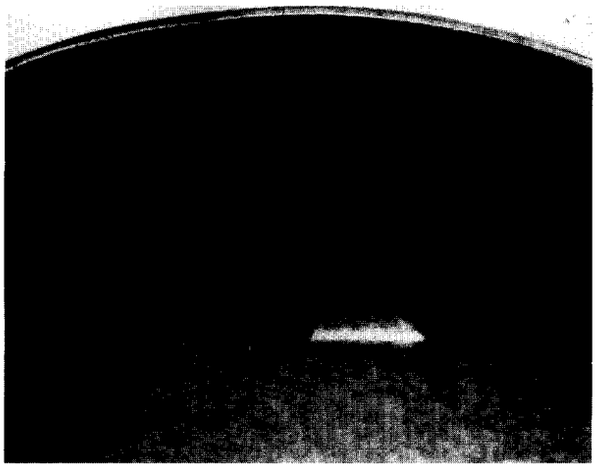

Fig. 1. Photograph of human brain hexokinase crystal. The diameter of the crystal is 0.25 mm.

and $c = 99.4$ Å. If one protein molecule lies in the asymmetric unit, the V_m is 4.2 Å^3/Da and the fractional volume occupied by the solvent is 71%. These values are almost two-fold greater than the average value observed in other protein crystals [24], which raises the possibility of two molecules of hexokinase type I in the asymmetric unit with a solvent content of 41%. Protein crystals with trigonal cells, however, generally have a high content of solvent [24]. The susceptibility of the crystal to damage by X-rays and its modest diffraction limit are properties consistent with a loosely packed asymmetric unit, containing a single enzyme molecule and a high solvent content.

Acknowledgements: This work was supported by Grant 95-37500-1926 from the United States Department of Agriculture and Grant NS 10546 from the National Institutes of Health.

References

[1] Purich, D.L., Fromm, H.J. and Rudolph, F.B. (1973) Adv. Enzymol. Relat. Areas Mol. Biol. 35, 249–326.
[2] Wilson, J.E. (1985) in Regulation of Carbohydrate Metabolism (Beitner, R., Ed.), Vol. 1, pp. 45–85, CRC Press, Boca Raton, FL.
[3] Gots, R.E., Gorin, F.A. and Bessman, S.P. (1972) Biochem. Biophys. Res. Commun. 49, 1249–1255.
[4] Lowry, O.H. and Passoneau, J.V. (1964) J. Biol. Chem. 239, 31–42.
[5] Fromm, H.J. (1981) in The Regulation of Carbohydrate Formation and Utilization in Mammals (C.M. Veneziale, Ed.), pp. 45–68, University Park Press, Baltimore, MD.
[6] Ureta, T. (1975) in Isozymes III (C.L. Markert, Ed.), pp. 575–601, Academic Press, New York.
[7] Rapoport, S. (1968) Essays Biochem. 4, 69–103.
[8] Easterrby, J.S. and O'Brien, M.J. (1973) Eur. J. Biochem. 38, 201–211.
[9] Colowick, S.P. (1973) in The Enzymes (Boyer, P.D., Ed.) 3rd edn., Vol. 9, pp. 1–48, Academic Press, New York.
[10] White, T.K. and Wilson, J.E. (1989) Arch. Biochem. Biophys. 274, 375–393.
[11] Magnani, M., Bianchi, M., Casabianca, A., Stocchi, V., Daniele, A., Altruda, F., Ferrone, M. and Silengo, L. (1992) Biochem. J. 285, 193–199.
[12] Zeng, Ch. and Fromm, H.J. (1995) J. Biol. Chem. 270, 10509–10513.
[13] Crane, R.K. and Sols, A. (1954) J. Biol. Chem. 268, 597–606.
[14] Anderson, C.M., Stenkamp, R.E., McDonald, R.C. and Steitz, T.A. (1978) J. Mol. Biol. 123, 207–219.
[15] Bennett, W.S., Junior, T.A. and Steitz, T.A. (1980) J. Mol. Biol. 140, 183–210.
[16] Harrison, R. (1985) Crystallographic Refinement of Two Isozymes of Yeast Hexokinase and Relationship of Structure to Function, Ph.D. Thesis, Yale University, New Haven, CT.
[17] Bernstein, F.C., Koetzle, T.F., Williams, G.J.B., Meyer, E.F., Brice, M.D., Rodgers, J.R., Kennard, O., Shimanouchi, T. and Tasumi, T. (1977) J. Mol. Biol. 112, 535–542.
[18] Bennett, W.S., Junior, T.A. and Steitz, T.A. (1980) J. Mol. Biol. 140, 211–230.
[19] Andreone, T.L., Printz, R.L., Pilkis, S.J., Magnuson, M.A. and Granner, D.K. (1989) J. Biol. Chem. 264, 363–369.
[20] Liu, F., Dong, Q., Myers, A.M. and Fromm, H.J. (1991) Biochem. Biophys. Res. Commun. 177, 305–311.
[21] Jancarik, J. and Kim, S.H. (1991) J. Appl. Crystallogr. 24, 409–411.
[22] Ellison, W.R., Lueck, J.D. and Fromm, H.J. (1975) J. Biol. Chem. 250, 1864–1871.
[23] Howard, A., Gililand, G., Finzel, B., Poulos, T., Ohlendorf, D. and Salemme, F. (1987) J. Appl. Crystallogr. 20, 383–387.
[24] Matthews, B.W. (1986) J. Mol. Biol. 33, 491–493.