

# Small Angle X-ray Scattering of Dimeric Yeast Hexokinase in Solution\*

(Received for publication, October 19, 1978)

Robert C. McDonald, Donald M. Engelman, and Thomas A. Steitz

From the Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06520

Small angle x-ray scattering measurements on dimeric yeast hexokinase B at pH 5.5 in acetate buffer yield a radius of gyration of  $31.28 \pm 0.23 \text{ \AA}$ . This measured value is comparable to the radius of gyration of  $31.5 \text{ \AA}$  calculated from the refined coordinates of the dimer in the BII crystal form. The hexokinase dimer found in the BI crystal form has a radius of gyration of  $42 \text{ \AA}$  calculated from the atomic coordinates. Thus, the measured radius of gyration is consistent with the BII dimer being the predominant species in solution and rules out the existence of the BI dimer as a major species under these conditions.

In solution, yeast hexokinase B is an  $M_r = 104,000$  dimer of identical subunits (1) which tends to dissociate into monomers as the ionic strength, temperature, or pH is raised (1-3). Both the monomer and dimer forms are enzymatically active (4). Two crystal forms of hexokinase B (BI and BII) have been obtained which contain a dimer in the asymmetric unit (4-7). The crystal structures that have been determined show that the subunit orientations in these two dimers (4, 5) are substantially different (Fig. 1). The BI dimer (6, 7) is considerably more elongated than the BII dimer (6, 7). In order to establish which of these two dimer structures is the predominant species in solution, we have used small angle x-ray scattering to measure the radius of gyration. Comparison of this measured radius of gyration with those calculated from the two crystal structures shows that the BI dimer cannot be the predominant dimer species in solution under the conditions of these experiments, while the BII dimer could well be.

## MATERIALS AND METHODS

Yeast hexokinase B was purchased from Worthington Biochemical Corp. (Catalogue No. HKP-2, Lot 56K393) as an ammonium sulfate precipitate and was used without further purification. The precipitate was centrifuged, dissolved in doubly distilled water to a concentration of  $15 \text{ mg/ml}$ , and dialyzed against  $50 \text{ mM}$  sodium acetate, pH 5.5,  $10^{-5} \text{ M}$  phenylmethanesulfonyl fluoride and  $0.1 \text{ mM}$  dithiothreitol. Measurements were also made on the same enzyme dialyzed against  $50 \text{ mM}$  Hepes<sup>1</sup> at pH 6.5. The protein concentrations were obtained from absorbance measurements based on an  $E_{280}^{1\%}$  of 9.47 (8). Polyacrylamide disc gel electrophoresis of the Worthington hexokinase under nonreducing conditions demonstrated that the enzyme had not been proteolytically degraded and was more than 95% pure (9).

The small angle scattering measurements were made using a Tennelec PSD100 position-sensitive detector as described previously.<sup>2</sup>

\* This research was supported in part by Grant GM-18268 and Postdoctoral Fellowship 5-F32-GM-05533 from the United States Public Health Service. 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.

<sup>1</sup> The abbreviation used is: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid.

<sup>2</sup> R. C. McDonald, T. A. Steitz, and D. M. Engelman (1979) *Biochemistry*, **18**, 338-342.

The coordinates for the BI and BII dimer molecules were generated using the refined coordinates of the hexokinase monomer, BIII (10) and the appropriate matrices and vectors relating the monomer and dimer subunits (Table I). The matrices and vectors relating the monomer to the BII dimer subunits were determined and described previously (7). For the BI dimer, which has only been solved at low resolution (5), the appropriate matrices and vectors were determined (7) by using the coordinate heavy atom sites which are in common between the monomer and each of the dimer subunits and are given in Table I.

Using the atomic coordinates of these two dimer molecules, radii of gyration were calculated using the relationship

$$Rg = \left[ \frac{\sum Z_i R_i^2}{\sum Z_i} \right]^{1/2}$$

where  $Rg$  is the radius of gyration,  $Z_i$  is the atomic number for atom  $i$  and  $R_i$  is its distance from the centroid of the electron distribution in the molecule.

## RESULTS

Guinier plots (12) obtained from four concentrations of dimeric hexokinase in acetate buffer are shown in Fig. 2. A plot of  $I(0)$ , the intercept of the Guinier slope at zero angle, as a function of concentration is linear for the dimer at pH 5.5, indicating that no further aggregation of the dimer or dissociation into monomers occurs in these experiments.

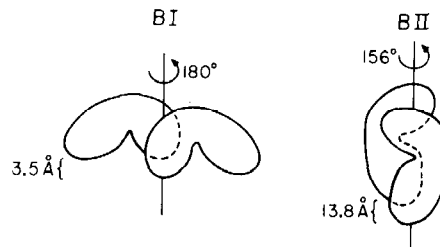


FIG. 1. Schematic drawings of the rather elongated BI dimer (4, 5) on the left and the BII dimer (6, 7) on the right. The two subunits of the BI dimer are related by a  $180^\circ$  rotation and a translation of  $3.5 \text{ \AA}$  along the rotation axis, while the subunits of the BII dimer are related by a rotation of  $156^\circ$  and a translation of  $13.8 \text{ \AA}$  along the rotation axis.

TABLE I

Matrices and vectors relating coordinates of BI and BIII subunits

Trans- formed coordinate <sup>a</sup>	A <sup>b</sup>	Initial coord- inate <sup>a</sup>	T <sup>b</sup>
$x'$	$\begin{bmatrix} -0.8745 & 0.4850 & 0.0031 \\ 0.0144 & 0.01953 & 0.9997 \\ 0.4878 & 0.8743 & 0.0240 \end{bmatrix}$	$x$	$\begin{bmatrix} 131.92 \\ -77.60 \\ -2.69 \end{bmatrix}$
$y'$		$y$	
$z'_{\text{BIII}}$		$z_{\text{BI sub 1}}$	
$x'$	$\begin{bmatrix} 0.8771 & -0.4784 & 0.0285 \\ 0.0042 & -0.0518 & -0.9987 \\ 0.4802 & 0.8761 & -0.0434 \end{bmatrix}$	$x$	$\begin{bmatrix} 23.44 \\ -12.60 \\ 26.50 \end{bmatrix}$
$y'$		$y$	
$z'_{\text{BIII}}$		$z_{\text{BI sub 2}}$	

<sup>a</sup>  $x, y, z$  are coordinates in Angstroms.

<sup>b</sup> A is the matrix and T is the vector relating initial coordinates in the space of the first subunit to the transformed coordinates in the space of the second subunit.

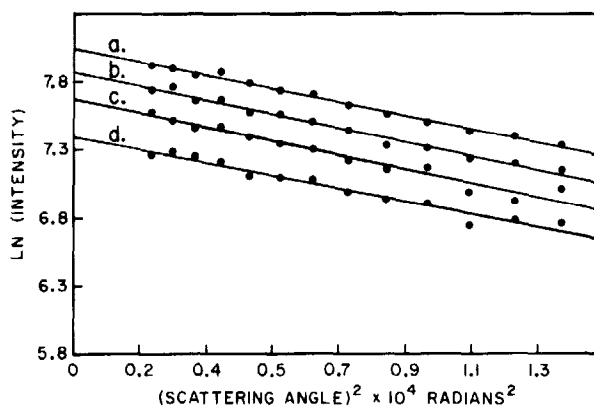


FIG. 2. Examples of data used in the determination of radii of gyration of the hexokinase dimer at a series of concentrations. In each case, the data are represented on a Guinier plot, and the line which is shown is the variance-weighted least squares fit to the experimental data points. From top to bottom, the protein concentrations used were (a) 12, (b) 10, (c) 8, and (d) 6 mg/ml.

Since no statistically significant variation of the radius of gyration was found for protein concentrations between 6.0 and 12.0 mg/ml, the variance-weighted average of the four radii of gyration measurements was computed. A value of  $31.28 \pm 0.23$  Å was obtained. The radius of gyration was also measured at a protein concentration of 12 mg/ml in 50 mM Hepes buffer, pH 6.5. Again, the radius of gyration was found to be  $30.0 \pm 0.8$  Å. These measured radii of gyration of dimeric yeast hexokinase can be compared with the values calculated from the atomic coordinates of the BI dimer, 40.2 Å, and the BII dimer, 31.3 Å. Clearly, the measured radius of gyration is nearest to that of the BII dimer.

#### CONCLUSION

The existence of two different crystalline dimer structures (4-7) raises the important question of which of these dimer forms exists in solution. This point is of particular interest because both of these dimer structures show an unsymmetrical or heterologous association of subunits which, in the case

of the dimer in the BII crystal form, results in a nonsymmetric binding of substrate ligands (11).

The radius of gyration of dimeric yeast hexokinase measured in acetate buffer at pH 5.5 or in Hepes buffer at pH 6.5 is 31.3 Å. Since the radius of gyration calculated from the atomic coordinates of the BI dimer is 42 Å, it is clear that this elongated dimer is not the predominant dimer in solution under the conditions of these measurements. The radius of gyration calculated from the coordinates of the BII dimer is 31.5 Å, very nearly the same as the measured radius of gyration. We would, of course, like to be able to say that the BII dimer is the predominant dimer species in solution. However, with these solution x-ray scattering data alone, we cannot rule out the possibility that the major dimer species is yet a third dimer structure, perhaps even showing a symmetric association of subunits, which also has a radius of gyration of 31 Å. Nevertheless, the existence in solution of the BII dimer with its asymmetrically associated subunits is consistent with the present radius of gyration measurements.

#### REFERENCES

1. Lazarus, N. R., Derechin, M., and Barnard, E. A. (1968) *Biochemistry* **7**, 2390-2400
2. Ramel, A., Barnard, E. A., and Schachman, H. K. (1963) *Angew. Chem. Int. Ed. Engl.* **2**, 745-747
3. Schulze, I. T., and Colowick, S. P. (1969) *J. Biol. Chem.* **244**, 2306-2316
4. Steitz, T. A. (1971) *J. Mol. Biol.* **61**, 695-700
5. Steitz, T. A., Fletterick, R. J., and Hwang, K. J. (1973) *J. Mol. Biol.* **78**, 551-561
6. Anderson, W. F., Fletterick, R. J., and Steitz, T. A. (1974) *J. Mol. Biol.* **86**, 261-269
7. Steitz, T. A., Fletterick, R. J., Anderson, W. F., and Anderson, C. M. (1976) *J. Mol. Biol.* **104**, 197-222
8. Colowick, S. P. (1973) in *The Enzymes* (Boyer, P. D., ed) 3rd Ed, Vol. 9, pp. 1-48, Academic Press, New York
9. Schmidt, J. J., and Colowick, S. P. (1973) *Arch. Biochem. Biophys.* **158**, 458-470
10. Anderson, C. M., Stenkamp, R. E., and Steitz, T. A. (1978) *J. Mol. Biol.* **123**, 15-33
11. Anderson, W. F., and Steitz, T. A. (1975) *J. Mol. Biol.* **92**, 279-287
12. Guinier, A. (1939) *Ann. Phys. (Lepzig)* **12**, 161-237