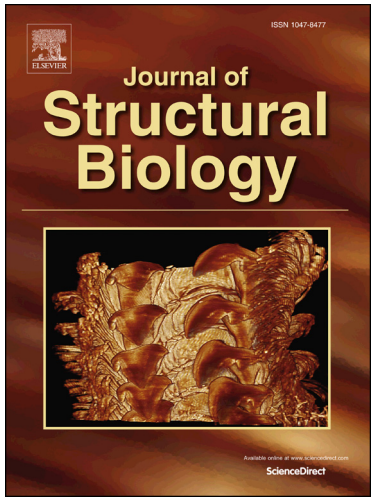

X-ray Diffraction Measurement of Cosolvent Accessible Volume in Rhombohedral Insulin Crystals

Alexei S. Soares, Donald L.D. Caspar

PII: S1047-8477(17)30135-1
DOI: http://dx.doi.org/10.1016/j.jsb.2017.08.004
Reference: YJSBI 7090

To appear in: *Journal of Structural Biology*

Received Date: 1 May 2017
Revised Date: 11 August 2017
Accepted Date: 20 August 2017

Please cite this article as: Soares, A.S., Caspar, D.L.D., X-ray Diffraction Measurement of Cosolvent Accessible Volume in Rhombohedral Insulin Crystals, *Journal of Structural Biology* (2017), doi: http://dx.doi.org/10.1016/j.jsb.2017.08.004

X-ray Diffraction Measurement of Cosolvent Accessible Volume in Rhombohedral Insulin Crystals

Alexei S. Soares[1] and Donald L.D. Caspar[2†]

[1]Photon Sciences Directorate, Brookhaven National Laboratory, Upton, NY, 11973-5000, USA
[2]Institute of Molecular Biophysics, Florida State University, Tallahassee, FL 32306, USA

†Correspondence: dcaspar@fsu.edu

Keywords: solvent; co-solvent; insulin; crystallography; refinement; trehalose;

Abstract

X-ray crystallographic measurement of the number of solvent electrons in the unit cell of a protein crystal equilibrated with aqueous solutions of different densities provides information about preferential hydration in the crystalline state. Room temperature and cryo-cooled rhombohedral insulin crystals were equilibrated with 1.2M trehalose to study the effect of lowered water activity. The native and trehalose soaked crystals were isomorphous and had similar structures. Including all the low resolution data, the amplitudes of the structure factors were put on an absolute scale (in units of electrons per asymmetric unit) by constraining the integrated number of electrons inside the envelope of the calculated protein density map to equal the number deduced from the atomic model. This procedure defines the value of F(000), the amplitude at the origin of the Fourier transform, which is equal to the total number of electrons in the asymmetric unit (i.e. protein plus solvent). Comparison of the F(000) values for three isomorphous pairs of room temperature insulin crystals, three with trehalose and three without trehalose, indicates that 75 +/- 12 electrons per asymmetric unit were added to the crystal solvent when soaked in 1.2M trehalose. If all the water in the crystal were available as solvent for the trehalose, 304 electrons would have been added. Thus, the co-solvent accessible volume is one quarter of the total water in the crystal. Determination of the total number of electrons in a protein crystal is an essential first step for mapping the average density distribution of the disordered solvent.

1. Introduction