X-RAY CRYSTALLOGRAPHIC STUDY OF BINDING OF COBALT ION TO HEN EGG-WHITE LYSOZYME

Ada YONATH*, Anita SMILANSKY* and Nathan SHARON**
Departments of Structural Chemistry and Biophysics**, The Weizmann Institute of Science, Rehovoth, Israel*

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1. Introduction

In a recent study [1] we have shown that Cu^{++}, an inhibitor of hen egg-white lysozyme, binds to the enzyme close to the carboxyl side chain of Asp 52. We have now studied by X-ray crystallography the binding to lysozyme crystals of Co^{++}, another divalent ion inhibitor of the enzyme [2], and found it attached to the active site at almost the same location as Cu^{++}.

2. Materials and methods

The present studies were carried out essentially as described by Moult et al. [3]. Tetragonal crystals of hen egg-white lysozyme [4] were grown at pH 4.7 in 0.02 M acetate buffer, containing 5% NaCl (w/v). Individual crystals (space group $P4_32_12$, a=b=79.1 Å, c= 37.9 Å) about 0.6 mm in the longest dimension, were transferred to 1.0 mm diameter quartz capillaries containing 0.1 ml mother liquor. About 2 mg of solid $CoSO_4$ was added to the capillary to obtain an approximate molar ratio of 1:100 lysozyme to $CoSO_4$. Soaking was done at room temperature for 3–4 days. The excess of mother liquor was removed from the capillary with a syringe and filter paper. Precession photographs (μ= 18°, minimum spacing=2.5 Å) of the hko, okl and hhl centrosymmetric zones, were taken with two films in a pack and exposures of 40–45 hr, using a copper X-ray tube running at 40 kV and 28 mA and a nickel filter. The maximum exposure time for a crystal was 55 hr. The crystals were isomorphous with those of native lysozyme and showed only small changes in intensities. A microdensitometer (Optronics photoscan P-1000) was used for complete recording of the diffraction patterns, and the integrated intensities of the reflections were obtained by processing on an IBM 370/165 computer, after corrections for background, and for Lorenz and polarization factors. Intensities of equivalent reflections within a pack were scaled together, and compared to the corresponding data for the native crystals. Difference Fourier projections were calculated using the amplitudes of native lysozyme (D. C. Phillips, personal communication) and of Co^{++}–lysozyme, with phases of the native enzyme, and weights based on the native figures of merit.

3. Results and discussion

The three difference electron density maps show the presence of one major peak. The fractional coordinates for this peak (A in fig.1a and 1b) are

$x = 0.10$ $y = 0.30$ $z = 0.58$

The closest distances from the coordinates of this peak to atoms of the lysozyme molecule were found to be 3.5 Å to $O^{\delta 1}$ and 1.8 Å to $O^{\delta 2}$ of Asp 52, and about 5 Å from the carboxyl end of glutamic acid 35. We believe that the insertion of the Co^{++} ion probably causes some slight distortion of Asp 52 side chain, as occurs with $CuSO_4$ [1]. This movement might not be big enough to be observed in an electron density projection map and it might also be hidden within approximately 4.5 Å diameter of the Co^{++} peak.

Comparison of the binding of Co^{++} and Cu^{++} to lysozyme shows that they both occur in the active site at almost identical locations, only 0.8 Å apart. How-

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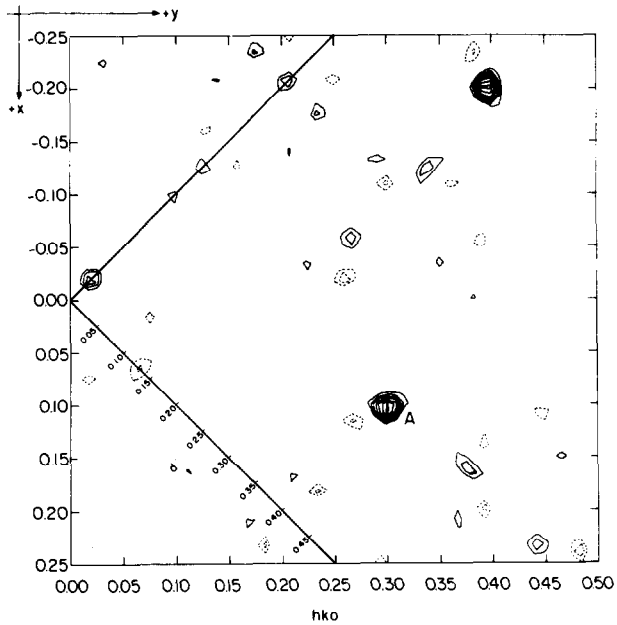

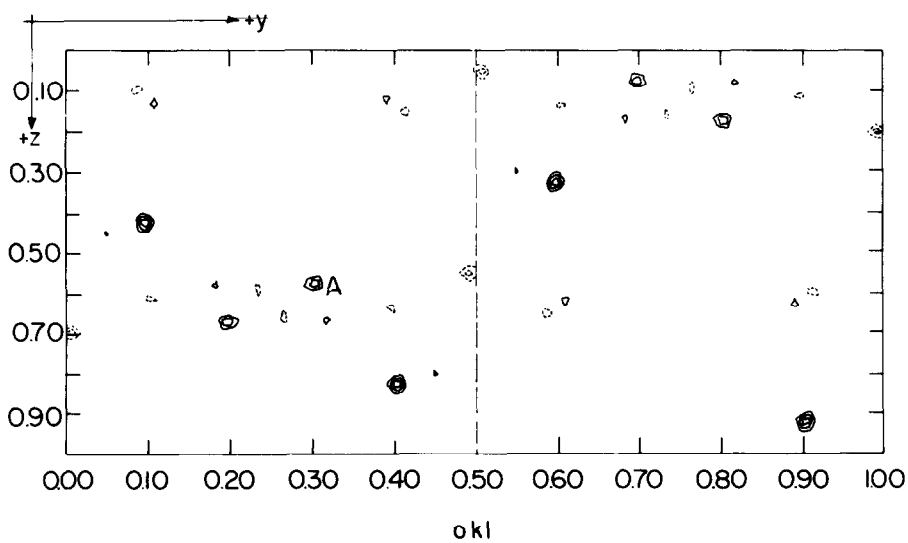

Fig.1. Fourier projections along c (1a) and a (1b) of the difference in electron density of native tetragonal lysozyme and the cobalt derivative. Only one of the equivalent symmetry related peaks is labelled (A) and its coordinates are given in the text.

ever, Cu^{++} also binds in a second location near the salt bridge of Lys 13–Leu 129 whereas Co^{++} does not bind at that location at all. This difference in binding between the two ions may be explained by the higher acidity of the Cu^{++} ion. [5]

As the electron density maps were very clear and did not contain any appreciable holes or extra peaks, we conclude that there are no marked conformational changes in the protein itself upon binding of Co^{++}.

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