HIGH TEMPERATURE CRYSTALLIZATION OF LYSOZYME: AN EXAMPLE OF PHASE TRANSITION*

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1. Introduction

The crystallization of proteins is unfortunately very often almost empirical. If we could master external factors which are the easiest to handle, then perhaps more general methods could be established. From this point of view this report is an attempt to show the importance of constant temperatures (until 55° in this Note) for crystallization of enzymes. Experiments achieved in this sense with hen egg-white lysozyme (EC 3.2.1.17) allowed besides to characterize a phase transition.

The great variety of crystalline forms obtained from a given enzyme is a well known phenomenon. It depends mainly on *internal factors:* a) different representatives of an enzyme family crystallize in quite different systems: hen [1], duck [2] and human [3] lysozyme crystals for example, are all different; b) when only one representative is considered (hen lysozyme) several crystalline forms have been described depending first on the ion bound to the enzyme: Cl^-, Br^-, NO_3^-, SO_4^{2-} [4] etc.; c) furthermore if we consider one form of hen lysozyme for example hydrochloride, we can obtain various types of crystals. Their occurrence depends on factors such as the protein concentration, the buffer, the pH, the precipitating salt, the presence of small amounts of heavy atoms, ions, dyes and traces of other materials [5, 6].

* 84th Communication on lysozymes.

In the case of inorganic substances one particular structure can only exist within a definite range of temperature and if it lies outside this range there may be a rapid reorganization of the building units to form a different arrangement. An extreme example is NH_4NO_3 which exists in five different crystalline forms each of which changes to another at a definite temperature.

From this point of view hen lysozyme has a behaviour which resembles an inorganic substance: there exists a polymorphism depending on one *external physical parameter:* the temperature leading to two forms A and B, the former seeming more metastable.

Fig. 1. Crystals of hen egg-white lysozyme obtained at pH 4.7 and 37° (orthorhombic form, called B form in this note). Magnification × 5.

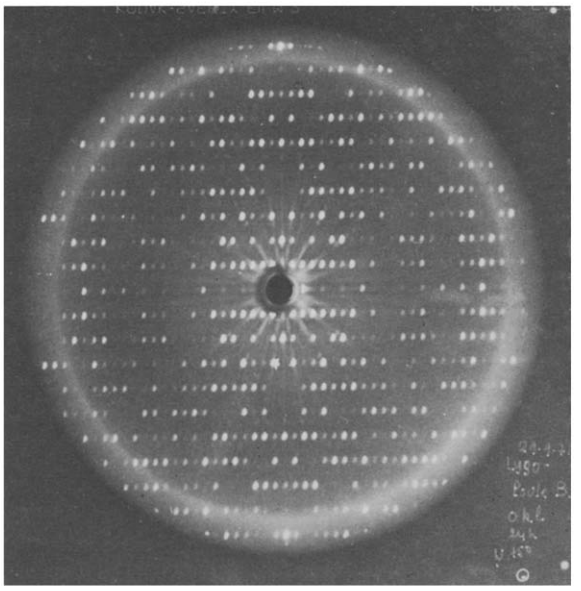

Fig. 2. X-ray diffraction pattern of the orthorhombic crystals of hen egg-white lysozyme obtained at pH 4.7 and 37° (B form in this note, see fig. 1).

Fig. 3. Transition of tetragonal crystals (A form) of hen egg-white lysozyme into orthorhombic crystals (B form) at 25°. Magnification × 5.

2. Materials and methods

Three commercial samples of hen egg-white lysozyme were used throughout this study (lysozyme 1 × crystallized, spray dried, LYSD 641 from Worthington; lysozyme 6 × crystallized, lot 7107, from Miles; lysozyme 6 × crystallized, lot 902, from Seikagaku); the quantitative amino acid composition of the enzyme was assayed by usual methods. According to the procedures of Alderton and Fevold [7], Palmer et al. [8] and Blake et al. [1], all our crystallization experiments were achieved as follows: to 50 mg lysozyme, dissolved in 0.5 ml water, were added 0.0625 ml 0.2 M acetate buffer, pH 4.7 and 0.1875 ml water; after centrifugation, 0.75 ml of a 10% NaCl solution was added to the clear supernatant followed by a drop of toluene. The crystals were analyzed by the precession camera technique and their water content measured by a thermogravimetric method.

3. Results

3.1. *Crystallization*

In a first series of experiments, the crystallization of hen lysozyme was achieved at 18° and 37°. At 18° the classical tetragonal form [1, 8] called A in this note, was obtained ($P4_32_12$, $a = 79.1$, $c = 37.9$, $\rho = 1.25$, content of water: 33.5%, M.W. = 14,700). At 37°, an orthorhombic form, called B in this note, appeared ($P2_12_12_1$, $a = 56.3$, $b = 73.8$, $c = 30.4$; $\rho = 1.24$, content of water: 36%, M.W. = 15,000) (figs. 1 and 2). It was different from the crystalline form described by Palmer et al. [8]. The question thus arose what happened between 18° and 37°, as well as at higher temperatures.

3.2. *Transition point*

The transition point was around 25°. At this temperature, both forms were present (fig. 3). If pregrown A crystals were put at 25°, they dissolved from the walls of the tubes and a slow transformation of the A into the B form was observed. The B crystals obtained at 25° were of a rare optical quality.

From 37° to 55°, only the B form was observed.

These crystals, also of an excellent optical quality, appeared rather quickly and reached several millimeters. Above 55°, lysozyme precipitated, but already at 45° a precipitation was observed as a function of time after the appearance of the crystals. To preserve these latter, it was necessary to remove them in time; at 37° they seemed stable.

The reversibility of the phenomenon was more difficult to verify and is actually under investigation.

4. Conclusion

This note points out for the first time at our knowledge that high temperatures (until 55°) are compatible with crystallization of an enzyme, hen lysozyme; this observation was already verified in the case of other lysozymes. The crystals grown under these conditions were frequently rather large and of a rare optical quality, probably suitable for studies by other physical methods such as neutron diffraction. Of course, other external physical parameters need to be more intensively studied.

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