CONTRIBUTION TO THE STUDY IN SOLUTION AND SOLID STATE OF THE RABBIT ALDOLASE TEMPERATURE DEPENDENCE

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1. Introduction

Attempts to crystallize proteins and enzymes have been carried out mostly at room or lower temperatures. However, in many animals, chemical reactions usually take place around 37°C. Previously we have demonstrated that hen lysozyme (EC 3.2.1.17) can crystallize at higher temperatures than was generally thought [1]. These high temperature crystals are orthorhombic while the classical crystals are tetragonal. The possibility was considered that lysozyme undergoes a conformational transition at ~25°C which could account for the existence of the two crystalline forms: such a temperature-dependent structural change was demonstrated in solution by different techniques [2,3]. Evidence of the existence of nondenaturational structural transition in proteins studied in solution was also provided by finding of breaks in the Arrhenius plots for various reactions as noted for lysozyme [4], for ribonuclease [5] and a few other proteins, quoted [6]. Our attention was by accident focused on [7] describing two forms of aldolase (EC 4.1.2.13) as a function of dilution and temperature: however the experiments in [7] were at $-10 < T < 23$–25°C and no radiocrystallographic study was done. Here we report a study of the Arrhenius plot obtained for rabbit muscle aldolase and some X-ray data concerning the two kinds of crystals obtained at low and higher ($\leqslant$40°C) temperature.

2. Materials and methods

Aldolase from rabbit muscle was obtained from Sigma as a crystalline suspension in a 3.2 M ammonium sulfate solution of pH 6 (10 mg protein/ml). All other reagents were purchased from Merck or Prolabo.

Our crystallization experiments were done at pH 4–8 and 4–50°C as follows: to the aldolase solution (0.3 ml) was added 0.3 ml water. The pH was adjusted with dilute NaOH or HCl before addition of ~0.09 ml of a buffered saturated ammonium sulfate solution until appearance of a slight turbidity.

The kinetic determinations were achieved at pH 7.4 and increasing temperatures as in [8]. The crystals were analyzed by the precession camera technique.

3. Results and discussion

3.1. *Arrhenius plot*

The variations of log V were plotted versus $1/T$ (fig.1): a sharp break in the Arrhenius plot was noted at 18°C.

In the case of hen lysozyme the break in the Arrhenius plots occurs at 25°C: we reported a series of results indicating that their non-linearity may be related to a conformational change [4]. Simultaneously low ($T < 25$°C) and high ($T > 25$–60°C) temperature crystals were obtained: they were different and a phase transition was observed at ~25°C [1]. Studies with ribonuclease [5] suggested a conformational change at ~32°C based on a non-linear Arrhenius plot in this temperature range, but no crystallographic results have been reported at $T > 32$°C. In the case of aldolase we completed the observations made in solution which were in favour of a conformational change at ~18°C by experiments done in solid state.

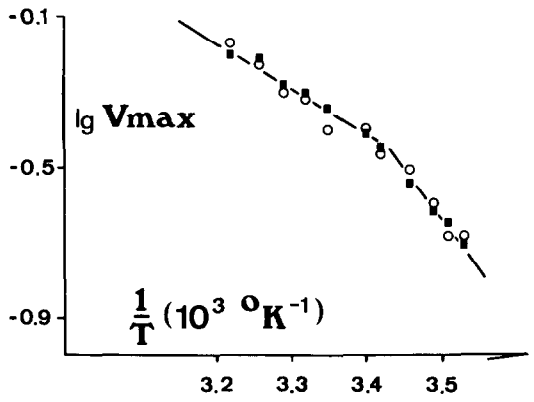

Fig.1. Arrhenius plot of rabbit muscle aldolase established at pH 7.4.

3.2. *Crystallographic studies*

Many crystallographic investigations have been devoted to aldolase since the observations in [7]. The crystalline properties of two kinds of crystals were described [9]: (i) hexagonal bipyramidal crystals, small sized and extremely susceptible to radiation damage; (ii) monoclinic $P2_1$, of which native and *p*-chloromercuribenzoate (PCMB)-derivative crystals were obtained. Unfortunately they were not isomorphous. Nevertheless it was shown that aldolase must have a tetrameric structure [9]. In [10] a new form (iii) was found to be also hexagonal; comparing the crystalline forms previously studied it was concluded [10] that the molecule had 222 symmetry to at least 4 Å resolution. A ivth form has been studied [11]. It was found to be hexagonal again and having a doubling of *C* axis dimension when compared to that in [10]: here again the cell dimensions together with an extreme sensitivity to radiation exposure made the structure analysis impracticable.

Using temperature variations, we obtained between pH 4–7.5, at low temperatures and in the conditions described above, i.e., at $T < 18°C$ and particularly at

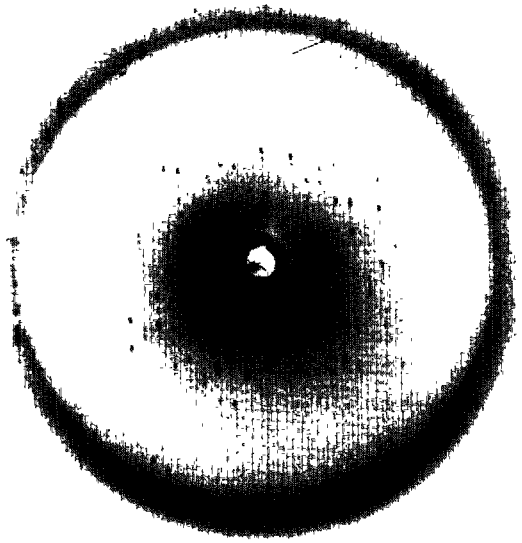

Fig.2. A 9° precession photograph of the hol zone of the monoclinic native aldolase crystals obtained at 40°C and pH 6.3.

4°C, small but well formed bipyramidal crystals. When analysed they were found to be hexagonal as in [9] and moreover very sensitive to X-ray action. At 18–50°C the crystals obtained between pH 4–8 were losange-shaped of several mm long and most of them twined. However a few of them were good crystals, especially at pH 6.3, and revealed to be monoclinic $P2_1$ (fig.2) with parameters very similar to those quoted in [9] (table 1). It is worth mentioning that our native crystals obtained at 40°C resemble more particularly their PCMB-crystals. It would be worthwhile to test the isomorphism and see if they are suitable for a crystallographic study. The crystal transformation in [7] has been difficult to exhibit. However in a few cases (pH 6.3) we observed the transformation of the bipyramidal crystals into the losange-shaped crystals while the reverse phenomenon did not occur up to now.

Table 1
Unit cell dimensions of monoclinic aldolase crystals

Ref.	Native aldolase		PCMB-aldolase	
[9]	a = 164.5 Å b = 57.3 Å c = 85.0 Å	β = 102°4	a = 163.7 Å b = 61.4 Å c = 81.6 Å	β = 103°7
This study (pH 6.3; 40°C)	a = 163 Å b = 61 Å c = 82 Å	β = 103°		

4. Conclusion

In the particular case of aldolase, there exists the possibility of obtaining good crystals up to 40°C. After the observation we have made with hen lysozyme crystals, this present study constitutes another example demonstrating the important role of relatively high temperatures in yielding good enzyme crystals. In addition two different types of crystals were obtained on both sides of the temperature (18°C) corresponding to the sharp break in the Arrhenius plot which might reflect a conformational change as in the other examples quoted above.

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References

[1] Jollès, P. and Berthou, J. (1972) FEBS Lett. 23, 21–23.
[2] Cozzonne, P., Opella, S. J., Jardetzky, O., Berthou, J. and Jollès, P. (1975) Proc. Natl. Acad. Sci. USA 72, 2095–2098.
[3] Saint-Blancard, J., Mazurier, J., Bournaud, M., Maurel, J.-P., Berthou, J. and Jollès, P. (1979) Mol. Biol. Rep. 5, 165–169.
[4] Saint-Blancard, J., Clochard, A., Cozzone, P., Berthou, J. and Jollès, P. (1977) Biochim. Biophys. Acta 491, 354–356.
[5] Matheson, R. R. and Scheraga, H. A. (1979) Biochemistry 12, 2446–2450.
[6] Privalov, P. L. (1979) Adv. Prot. Chem. 33, 189–192.
[7] Wolf, H. P. and Leuthardt, F. (1957) Helv. Chim. Acta 40, 237–246.
[8] Beisenherz, G., Boltze, H. J., Bücher, T., Czok, R., Garbade, K. H., Meyer-Arendt, E. and Pfleiderer, G. (1953) Z. Naturforsch. 8b, 555–557.
[9] Eagles, P. A. M., Johnson, L. N., Joynson, M. A., McMurray, C. H. and Gutfreund, H. (1969) J. Mol. Biol. 45, 533–544.
[10] Heidner, E. G., Weber, B. H. and Eisenberg, D. (1971) Science 171, 677–679.
[11] Sawyer, L. (1972) J. Mol. Biol. 71, 503–505.