ON THE BINDING OF *N*-ACETYLGLUCOSAMINE AND ITS SHORT POLYMERS TO HEN LYSOZYME AT PHYSIOLOGICAL TEMPERATURE (40°C)*

P. JOLLÈS, J. SAINT-BLANCARD, M. ALLARY, J.-P. PÉRIN and P. COZZONE

Laboratory of Proteins, University of Paris V, 45, rue des Saints-Pères, F-75270 Paris Cedex 06, France and Centre de Transfusions Sanguine des Armées, J. Julliard F-92140, Clamart, France

Received 7 May 1975

1. Introduction

We have demonstrated that hen lysozyme (EC 3.2.1.17) crystals can be obtained between 25 and 60°C [1]. These high temperature crystals which we called form B are orthorhombic while the classical crystals obtained between −36 and 25°C are tetragonal (form A) [1,2]. A crystals can convert into B crystals under the sole influence of temperature regardless of pH, ionic strength, protein concentration or nature of the precipitating salt; the only requirement for this transition to occur is the presence of the liquid phase [2]. It was thus reasonable to consider the possibility that the lysozyme molecule undergoes a conformational transition around 25°C which would account for the existence of the two crystalline forms.

Only the determination of the structure of the high temperature form can answer this question and a X-ray crystallographic study is under progress. Meanwhile by ^{13}C NMR spectroscopy a specific temperature-dependent conformational transition of lysozyme occurring between 20 and 30°C in solution could be detected [3]. This localized conformational change was noted in the enzyme molecule in the vicinity of subsites D and E of the active site, more particularly on one edge of the cleft of the molecule which plays an important role when the behaviour of lysozyme towards substrates and inhibitors is considered.

Detailed studies concerning the influence of pH and ionic strength on the apparent affinity constant [4] of lysozyme for the bacterial substrate as well as on the inhibition of the enzyme by GlcNAc** and its short polymers have been performed [5,6], but only at 20°C. In the light of our observations concerning a temperature-dependent conformational transition in lysozyme, we decided to determine the apparent affinity constant and the inhibition constants at higher temperatures.

Hen egg-white lysozyme was a six times crystallized sample from Miles. GlcNAc was obtained from Koch-Light; chitobiose, chitotriose and chitotetraose were isolated from acid hydrolysates of chitin [7].

The apparent affinity constant for the bacterial substrate Ka_{app} and the inhibition constants K_i in the presence of GlcNAc and its short polymers were determined according to procedures previously described by our group (Locquet et al. [4] and Saint-Blancard et al. [6]). The effect of binding small oli-

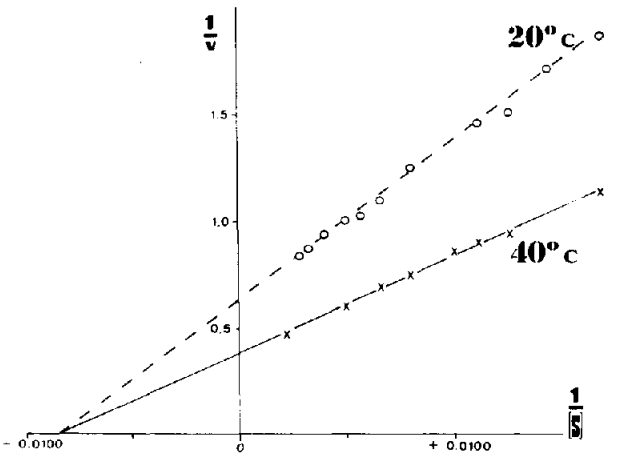

Fig.1. Variation of $1/V$ vs. $1/[S]$ for hen lysozyme at 20 and 40°C determined according to Locquet et al. [4].

* 100th communication on lysozymes.

** *Abbreviation*: GlcNAc, *N*-acetylglucosamine.

gomers of GlcNAc on the spectrophotometric titrations and fluorescence emission of lysozyme was studied according to Dahlquist et al. [8] and Mulvey et al [9].

Fig.1 indicates the determination of the apparent affinity constant of lysozyme at 20 and 40°C: its value, 115 ± 10 mg/l, was the same at both temperatures. The inhibition constants K_i were determined at different temperatures between 20° and 40°C by three different procedures. Various experimental conditions were employed: thus the assays were achieved at three different values of pH (5.5; 6.2, 7.5) and of ionic strength (0.100; 0.107 and 0.181) in the presence of

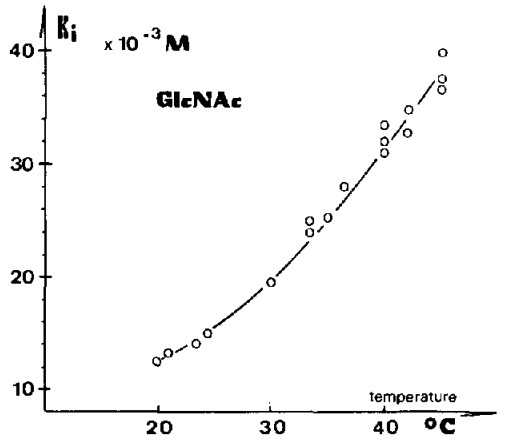

Fig.2. Inhibition constant K_i of hen lysozyme in the presence of GlcNAc determined at different temperatures by the kinetical method [4,6].

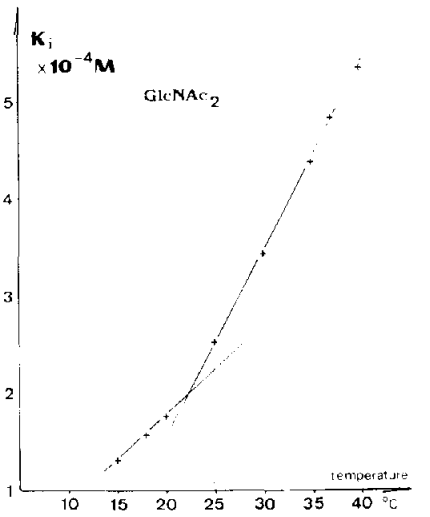

Fig.3. Inhibition constant K_i of hen lysozyme in the presence of chitobiose = $(GlcNAc)_2$ determined at different temperatures by fluorometric titrations according to Mulvey et al. [9].

GlcNAc (fig.2), chitobiose (fig.3), chitotriose and chitotetraose. These values of pH and ionic strength were previously used for most of the experiments performed at 20°C [6]. At 40°C (physiological temperature for birds), the inhibition constants K_i were 2–3 times higher than at 20°C (table 1 and fig.2 and 3). Moreover fig.2 and 3 show a change of slope of the line T vs. K_i at a temperature quite similar to that at which A crystals convert into the B form [1] and a confor-

Table 1

Inhibition constants K_i of lysozyme with GlcNAc and its short polymers determined at 20 and 40°C (physiological temperature) by various procedures: K, kinetical method [4,6]; S, spectrophotometric method [8], F, spectrofluorometric method [9]. K_i determined at other temperatures, see fig.2 and 3

Inhibitor	pH	I	method	K_i (M) 20°C	K_i (M) 40°C	K_i (40°C)/K_i (20°C)
GlcNAc	5.2	0.100	S	1.66×10^{-2}	2.47×10^{-2}	1.49
	6.2	0.107	K	1.30×10^{-2}	3.24×10^{-2}	2.49
	6.2	0.181	K	1.50×10^{-2}	4.00×10^{-2}	2.67
$(GlcNAc)_2$	7.5	0.100	F	1.76×10^{-4}	5.34×10^{-4}	3.03
$(GlcNAc)_3$	6.2	0.107	K	4.50×10^{-5}	13.00×10^{-5}	2.89
	7.5	0.100	F	1.63×10^{-5}	3.08×10^{-5}	1.89
$(GlcNAc)_4$	6.2	0.107	K	1.50×10^{-5}	3.60×10^{-5}	2.40
	7.5	0.100	F	1.35×10^{-5}	2.70×10^{-5}	2.00

mational transition occurs in solution [3].

The temperature-dependent conformational transition of lysozyme does not seem to have an effect on its behaviour towards the high molecular bacterial substrate; indeed it is well known that it is lysed more rapidly at 37°C or 40°C than at 20°C. However the conformational change completed at 40°C increases the values of the inhibition constants of the enzyme for the sugars indicating a diminution of the affinity of lysozyme for these compounds at higher temperatures. This observation might explain the difficulty to crystallize at 40°C an enzyme–inhibitor complex and have some importance at a biological viewpoint; many kinetical studies devoted to enzymes should be reexamined at more physiological conditions.

Acknowledgments

This research was supported in part by the I.N.S.E.R.M. (group U-116) and the C.N.R.S. (ER 102 and ATP 489906).

References

[1] Jollès, P. and Berthou, J. (1972) FEBS Lett. 23, 21–23.
[2] Berthou, J. and Jollès, P. (1974) Biochim. Biophys. Acta 336, 222–227.
[3] Cozzone, P., Opella, S. J., Jardetzky, O., Berthou, J. and Jollès, P. (1975) Proc. Natl. Acad. Sci. U.S.A. in the press.
[4] Locquet, J.-P., Saint-Blancard, J. and Jollès, P. (1968) Biochim. Biophys. Acta 167, 150–153.
[5] Saint-Blancard, J., Hénique, Y., Ducassé, D., Dianoux, A.-C. and Jollès, P. (1968) Bull. Soc. Chim. Biol. 50, 1783–1790.
[6] Saint-Blancard, J., Chuzel, P., Mathieu, Y., Perrot, J. and Jollès, P. (1970) Biochim. Biophys. Acta 220, 300–306.
[7] Rupley, J. A., Butler, L., Gerring, M., Hartdegen, F. J. and Pecoraro, R. (1967) Proc. Natl. Acad. Sci. U.S.A. 56, 1088–1092.
[8] Dahlquist, F. W., Jao, L. and Raftery, M. (1966) Proc. Natl. Acad. Sci. U.S.A. 56, 26–30.
[9] Mulvey, R. S., Gaultieri, R. J. and Beychok, S. (1973) Biochemistry, 12, 2683–2690.