TEMPERATURE DEPENDENT TRANSITION OF LYSOZYME-β-ARYL DI-N-ACETYLCHITOBIOSIDE COMPLEX

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Summary

At ambient temperature (32°C), the acetamido methyl protons (A) proximal to the aryl aglycone of β-p-methoxyphenyl di-N-acetyl chitobioside undergo an upfield shift and line broadening while the acetamido methyl protons (B) distal from the aryl aglycone do not exhibit isotropic shift. However, with an elevation of temperatures, protons (B) gradually shift upfield while proton (A) shift back to the original position. The significance of this transition is discussed in terms of the modes of interaction between lysozyme and β-aryl di-N-acetylchitobiose.

The application of nuclear magnetic resonance (NMR) spectroscopy to obtain information concerning kinetics and geometry for the interactions of lysozyme and N-acetylchitooligoses is well documented (Raftery et. al., 1969; Butchard et. al., 1972; Wien et. al., 1972). In the presence of lysozyme, acetamido methyl protons proximal to the aryl aglycone of β-aryl di-N-acetylchitobiosides (Otson et. al., 1973) and those at the reducing terminus of di-N-acetylchitobiose (Dahlquist and Raftery, 1969) undergo upfield shift which is attributed to the interaction of the pyranose ring with subsite C of the enzyme. This communication reports the nuclear magnetic resonance evidence for the temperature dependent transition of lysozyme -β-ary di-N-acetylchitobioside interaction.

Materials and Methods

Six times recrystallized lysozyme from Hen's egg white was purchased from Miles Laboratories, Inc. β-p-Methoxyphenyl di-N-acetylchitobioside was synthesized as described (Tsai et. al., 1969). Nuclear magnetic resonance spectra were taken with a Varian XL-100 NMR spectrometer.

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β-p-Methoxyphenyl di-N-acetylchitobioside (10mg.) was dissolved in 0.4 ml of D_2O containing 0.3% of sodium 2,2-dimethyl-2-silapentane-5-sulfonate as the internal standard. The pD was adjusted to 7.0 with NaoD. Spectra were taken at different temperatures at a sweep width of 50 Hz immediately after the additions of 20 mg of lysozyme. Rates of lysozyme catalysis were followed by analyzing chloroform soluble products spectrophotometrically (Tsai et. al., 1969).

Results and Discussion

The active site of lysozyme lies in a cleft which can accommodate six pyranose rings designated as subsites A to F (Blake et. al., 1967). The binding of N-acetylchitooligoses between subsite D and E yields productive complexes (Blake et. al., 1967; Rupley and Gates, 1967). Because of the thermodynamically favourable contribution of the subsite C to the binding (Rupley et. al., 1967; Chipman et. al., 1967), di-N-acetylchitobiose and β-aryl di-N-acetylchitobiosides interact preferentially with subsite C to give the nonproductive complexes which can be readily detected by ultraviolet (Rupley et. al., 1967), fluorescence (Lehrer and Fasman, 1967), circular dichroic (Miwa and Nishima, 1972) and nuclear magnetic resonance (Raftery, et. al., 1969; Butchard et. al., 1972; Wien et. al. 1972) spectroscopic methods.

There are two useful parameters which provide information concerning the mode of diamagnetic interactions between small molecules and proteins by NMR spectroscopy. The line broadening measures the restriction of small molecules in its association with macromolecules and the change of the chemical shift arises from the change in chemical environment of the group involved when it interacts with proteins. The nuclear magnetic resonance spectrum of β-p-methoxyphenyl di-N-acetylchitobioside at 100 MHz exhibits two singlets at δ2.04 and 2.08 ppm corresponding to two acetamido methyl groups proximal (A) to and distal (B) from the aryl aglycone (Otson et. al., 1973) Fig. 1 shows that, upon the addition of lysozyme at 32°C,

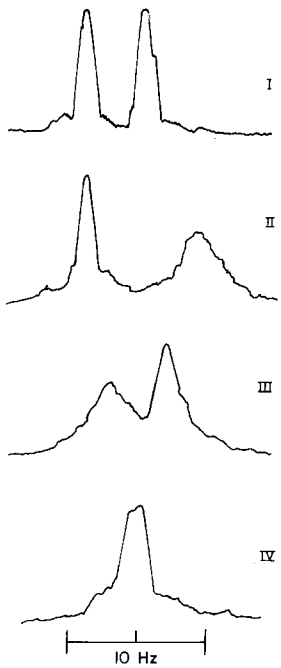

Fig. 1 NMR spectra of acetamido methyl protons of β-p-methoxyphenyl di-N-acetylchitobioside in the absence (I) and the presence of lysozyme at 32° (II), 50° (III) and 65°C (IV).

the signal for the acetamido methyl protons (A) shifts upfield by 4 Hz with a concurrent line broadening while that of the acetamido methyl protons (B) undergoes only slight line broadening without noticeable isotropic shift.

With the elevation of temperatures, the chemical shift of the acetamido methyl protons (A) shifts downfield whereas that of acetamido methyl protons (B) shifts upfield until the two resonance peaks overlap at δ2.04 ppm at 65°C. This is accompanied by a decreasing line width for the acetamido methyl protons (A) and the line broadening of the acetamido methyl protons (B). Lysozyme is known to be stable within the temperature range studied (Hayashi et. al., 1968; McDonald et. al., 1971), therefore, these observations are best explained by the temperature dependent transition of the lysozyme - chitobioside complex.

At 32°C, β-p-methoxyphenyl di-N-acetychitobioside interacts with

lysozyme by placing the pyranose ring proximal to the aryl aglycone at subsite C as indicated by an upfield shift (4 Hz) and line broadening of the acetamido methyl protons (A). This interaction yields a nonproductive complex. Whereas, at an elevated temperature (65°C), the acetamido methyl protons (B) undergoes the upfield shift (4 Hz) and line broadening implying the interaction of the pyranose ring distal from the aryl aglycone with subsite C.

Fig. 2 shows that the rate of lysozyme catalyzed hydrolysis of β-p-methoxyphenyl di-N-acetylchitobioside at 35°C displays an induction period which disappears at an elevated temperature (55°C). This is in

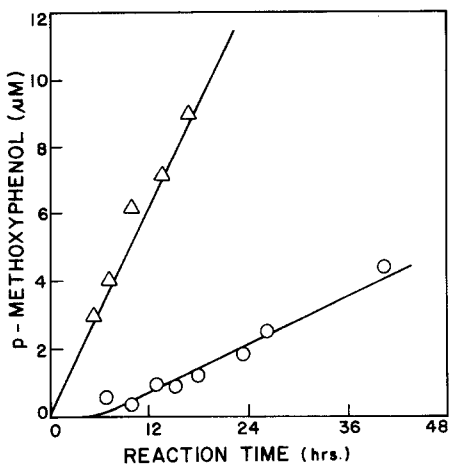

Fig. 2 Temperature dependent hydrolysis of β-p-methoxyphenyl di-N-acetylchitobioside catalyzed by lysozyme at 35° (O) and 55°C (Δ).

agreement with the preferential productive interaction of β-p-methoxyphenyl di-N-acetylchitobioside with lysozyme at high temperatures from NMR studies. Although the mechanism for the transition is unknown, it is interesting to note that the thermodynamically unfavourable productive interaction between β-aryl di-N-acetylchitobioside and lysozyme can be overcome by an elevated temperature.

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