INTRODUCTION

Xylanases are biotechnologically relevant enzymes for enzymatic lignocellulose degradation. The current knowledge regarding structural dynamics and the kinetics of structural fluctuations of these proteins during their catalytic cycle is obscure. We have applied advanced fluorescence spectroscopy techniques to study the biophysical and dynamical properties of the GH11 endo-1,4-beta-xylanase from *Bacillus subtilis* (XynA).

OBJECTIVES

To obtain insights on the global and local conformational changes of a XynA during its catalytic cycle through the use of fluorescence spectroscopy techniques. These techniques have been correlated with biophysical and dynamical properties of this enzyme with a view for biotechnological applications.

MATERIALS AND METHODS

Heterologous XynA was modified at N-terminus with the fluorescent dye AF488, and the fluorescence autocorrelation curve was measured over increasing arabinoxylan concentrations by fluorescence correlation spectroscopy (FCS). These results were correlated with fluorescence emission spectra and anisotropy measurements over increasing arabinoxylan concentrations at both 20°C and 55°C using an acrylodan derivative of the XynA labeled specifically on the thumb domain of the enzyme.

RESULTS

FCS measurements of the AF488-XynA showed increased diffusion times and altered autocorrelation curve profiles that could be fitted with a T+3D+2D function model, suggesting the formation of xylanase-arabinoxylan complexes and the appearance of a two-dimensional motion of the protein. Changes in the emission parameters of the XynA acrylodan derivative with increasing arabinoxylan concentration and temperature were detected.

CONCLUSIONS

Estimation of the $K_D$ of the XynA/substrate interaction may be possible by using FCS. Evidence for the control of substrate access to the enzyme catalytic site through dynamics of the xylanase thumb domain was obtained, and this thumb motion appears to be influenced by temperature.