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Research paper

Analysis of the specificity and thermodynamics of the interaction between low affinity antibodies and carbohydrate antigens using fluorescence spectroscopy

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Abstract

The purpose of this work has been to examine whether fluorescence spectroscopy can be used to investigate weak or transient binding between monoclonal antibodies and carbohydrate antigens. In earlier studies we have demonstrated that the three monoclonal antibodies 39.4 (IgG2b), 39.5 (IgG2b) and 61.1(IgG3) bind weakly to the glycosidic α(1–4) bond present in e.g. maltose and panose. In this study these antibodies showed an enhancement in the fluorescence intensity of tryptophan upon binding in solution to these two carbohydrate antigens. Using a structural analog to maltose, cellobiose, no fluorescence intensity change was induced. Dissociation constants for these antibodies at different temperatures (5–40 °C) were obtained in the range of 0.003–0.2 mM and they were in accordance with earlier data from studies on affinity chromatography and surface plasmon resonance. Almost a doubling of the dissociation constants was observed for every 10 °C increase in temperature, giving an exothermal reaction with standard enthalpy change of −51 kJ/mol, for the association between antibody and carbohydrate antigen. It was seen that the extra glycosyl ring in panose increased the affinity more than eight times for the monoclonal antibody 39.5. A standard entropy increase of 21%, probably due to hydrophobic effects, is introduced by the extra glycosyl ring, while the enthalpy stays unaffected. This direct fluorescence approach to measure the binding and

Abbreviations: a.u., arbitrary unit; $[Ag]_{free}$, concentration (M) of free antigen; F, total spectral fluorescence (a.u.); F_0, total spectral fluorescence without antigen (a.u.); ΔF, change in total spectral fluorescence (a.u.); ΔF_{max}, maximum change in total spectral fluorescence (a.u.); Glc, glucose; ΔG^o, standard Gibbs free energy change; ΔH^o, standard enthalpy change; Ig, immunoglobulin; K_a, association constant (M^{-1}); K_d, dissociation constant (M); PBS, phosphate buffered saline; R, gas constant (8.314 J mol^{-1} K^{-1}); r^2, correlation coefficient from linear regression; R^2, correlation coefficient from non-linear regression; SPR, surface plasmon resonance; ΔS^o, standard entropy change; T, temperature (K); WAC, weak affinity chromatography.

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thermodynamics of an interacting antigen–antibody pair is simple and accurate since measurements are performed in solution and no immobilization or fluorophore labeling of the components is required. Introduction of fluorescence techniques will be a useful complement to current procedures to measure interaction of antibody with antigen and in particular they will offer solutions to detect transiently binding antigens.

Keywords: Carbohydrate; Fluorescence; Monoclonal antibody; Specificity; Transient binding; Weak affinity

1. Introduction

Many important biological interactions involve transient binding (K_d>10^{-6} M) and are subject to rapid dissociation events. Numerous examples that illustrate the importance of weak biological interactions including protein–protein (Causey and Dwyer, 1996), protein–carbohydrate (MacKenzie et al., 1996) and carbohydrate–carbohydrate interactions (Eggens et al., 1989) can be found. Specific recognition can be achieved either by repeated monovalent binding (Karjalainen, 1994) or by polyvalent interactions (Mammen et al., 1998). In the laboratory, procedures developed to study and analyze weak biological interactions are in high demand. Techniques based on affinity chromatography (Strandh et al., 2000), affinity electrophoresis (Ljungberg et al., 1998), fluorescence (Jolley and Glaudemans, 1974), nuclear magnetic resonance (Kronis and Carver, 1982) and surface plasmon resonance (SPR) (Nicholson et al., 1998) have all contributed to current knowledge and applications of transient biological interactions.

Antibodies with weak affinity have not received wide attention as they have been considered to be non-specific and therefore non-relevant for developing e.g. immunoassays. However, we have demonstrated in a number of papers (see e.g. Zopf and Ohlson, 1990) that they can indeed be specific and useful for bioanalytical purposes. We believe that weakly binding antibodies of both IgM and IgG classes acting upon antigens with an affinity of less than 1 μM are abundant in the immune system. However, their role in the immune response is still obscure, although it can be speculated that they are important for the identification of foreign antigens in

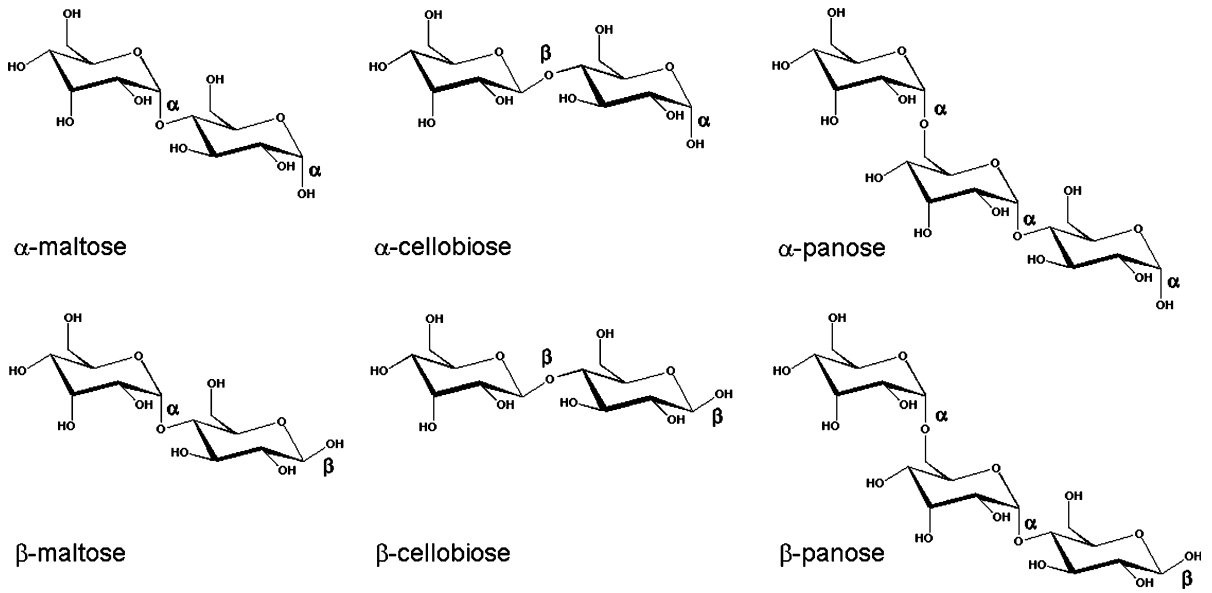

Fig. 1. Structures and anomeric forms (α- and β-) of the carbohydrates used in the present report, namely maltose (Glc α1–4Glc), cellobiose (Glc β1–4Glc) and panose (Glc α1–6Glc α1–4Glc).