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Determination of DNA protein affinity constants using chemometric analysis of tryptophan fluorescence quenching data

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Abstract

In this work we present a sensitive method to determine DNA protein affinity constants by analyzing tryptophan fluorescence quenching data. Chemometric analysis of the entire fluorescence emission spectra is used in combination with the equilibrium expression. This way all spectral changes are taken into account, which considerably enhances the accuracy compared to traditional methods where only the overall quenching is studied. In addition to the affinity constant, the concentrations in each sample and the spectral profiles of free and bound protein are determined.

Keywords: Chemometrics; DNA protein interaction; Affinity constant

1. Introduction

A common way to determine protein affinity for DNA is by measuring the fluorescence quenching of tryptophan residues, often at a single wavelength [1]. We have previously shown that chemometric analysis of entire spectra can be utilised in studies of chemical equilibria [2]. In this work we show how changes in protein fluorescence emission spectra upon binding to DNA can be combined with the equilibrium expression to determine the affinity constant. The method is demonstrated by studying the binding of the C-terminal part of the origin binding protein of herpes simplex virus type 1, ΔOBP [3], to a single stranded 65-mer of oligodeoxythymidylate (dT_{65}).

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2. Method

Fluorescence emission spectra recorded at different proteins: DNA ratios are arranged as rows in a matrix $\boldsymbol{A}$. These are linear combinations of the components' concentrations, $\boldsymbol{C}$, and their spectral profiles, $\boldsymbol{V}$, (cf. Ref. [4]):

$$\boldsymbol{A} = \boldsymbol{CV} + \boldsymbol{E} \approx \boldsymbol{CV} = \sum_{i=1}^{r} \boldsymbol{c}_i \boldsymbol{v}_i, \tag{1}$$

where r is the number of fluorescent species and $\boldsymbol{E}$ is an error matrix. Matrix $\boldsymbol{A}$ can also be decomposed into an orthonormal basis set [5]:

$$\boldsymbol{A} = \boldsymbol{TP}' + \boldsymbol{E} \approx \boldsymbol{TP}' = \sum_{i=1}^{r} \boldsymbol{t}_i \boldsymbol{p}_i', \tag{2}$$

where the target matrix, $\boldsymbol{T}$, and the projection matrix, $\boldsymbol{P}'$, have the same dimensions as $\boldsymbol{C}$ and $\boldsymbol{V}$, respectively. These are related [5]:

$$\boldsymbol{V} = \boldsymbol{RP}', \tag{3a}$$

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$$C = TR^{-1}, \tag{3b}$$

where R is a square $(r \times r)$ rotation matrix. It is determined by calculating the amount of free and bound protein for various trial values of the affinity constant, K, which are fitted to the calculated target vectors. The trial value that generates the best fit, determines R and K.

3. Experimental

Fig. 1 shows fluorescence emission spectra of ΔOBP at different ΔOBP:dT_{65} ratios. ΔOBP binds DNA as monomer [3] and forms a 1:1 complex with dT_{65} (Fig. 1, inset). The concentrations c_p and c_{DP}, of free and bound ΔOBP, respectively are given, by:

$$c_p = c_p^{TOT} - c_{DP}, \tag{4a}$$

$$c_{DP} = x \pm \sqrt{x^2 - c_D^{TOT} c_P^{TOT}}, \tag{4b}$$

where $x = \frac{1}{2}(K^{-1} + c_D^{TOT} + c_P^{TOT})$ and c_D^{TOT} and c_P^{TOT} are the total concentrations of dT_{65} and ΔOBP.

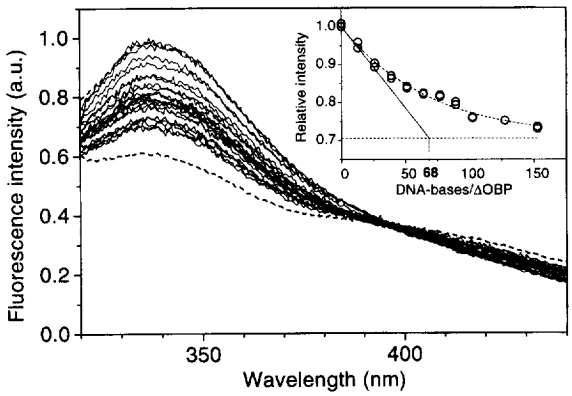

Fig. 1. Fluorescence emission spectra of 80 nM ΔOBP (purified as described in Ref. [3]) in 50 mM Tris (pH 7.4), 50 mM NaCl and in the presence of 0 to 188 nM dT_{65}-strand (solid lines). Spectra are corrected for the inner filter effect [4]. The calculated profiles of free and bound ΔOBP are shown as dashed lines. *Inset*: Total fluorescence intensity as a function of the number of dT_{65}-bases per ΔOBP-monomer (o). The apparent binding size is determined from the intercept of the asymptote and the initial slope to 68 bases/ΔOBP.

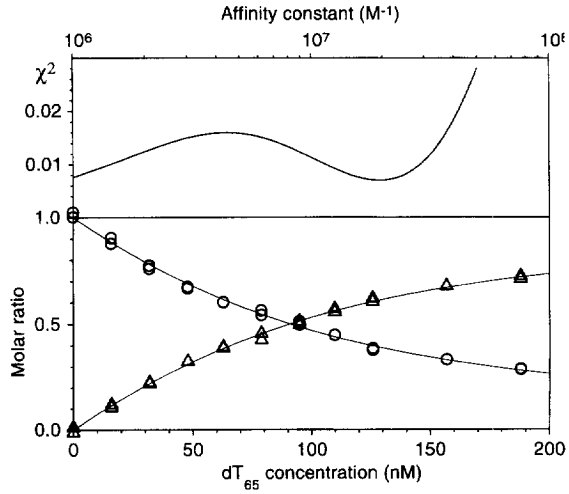

Fig. 2. *Top*: Dependence of χ^2 (squared residual) on trial values of the affinity constant. *Bottom*: Best fit of molar ratios of free (o) and bound (Δ) ΔOBP calculated by using Eq. 3(b) to the molar ratios predicted by the equilibrium equation (lines).

The best fit of the experimental data was obtained with an affinity constant of 2.0×10^7 M^{-1} (Fig. 2). Values below 2×10^6 M^{-1} give lower χ^2 values, but these solutions can be ruled out since they give negative spectral features.

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