Metal-dependent activity of the 8-17 DNAzyme studied by resolution of rank deficient multi-way fluorescence data

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Abstract

The recent advances in multi-way analysis provide new solutions to traditional enzyme activity assessment. In the present study enzyme activity has been determined by Hard-modeling and restricted-Tucker3. In Tucker3 analysis of three-way data array obtained from a chemical or biological system, it is sometimes possible to use a priori knowledge about the system to specify what is called a restricted Tucker3 model. Often, the restricted Tucker3 model is characterized by having some elements of the core forced to zero. The method relies on monitoring spectral changes of reaction mixture at specific time points during the course of the whole enzyme catalyzed reaction and employs multi-way analysis to detect the spectral changes. Parameters of chemical model (rate constants), interaction (for core of Tucker3) and initial concentrations (enzyme, substrate and metal ion) are estimated during optimization process by Replacement Method (RM). The work presented in this paper was undertaken to test the hypothesis that FRET and chemometric multiway analysis can be used as a universally applicable approach for rapid assessment of enzyme activity without using any external standards. A simulated chemical equilibrium data set is used to evaluate the applicability of this hypothesis. The obtained information from our study by using chemometrics methods will be helpful for prediction of DNAzyme behavior during processes and determination of substrate yield that is cleaved.

Key words: Three-way data, Restricted Tucker3, Rank deficiency, Hard modeling, Replacement method

Introduction

One of the most important discoveries in the last decades is that DNA molecules are not only materials for genetic information storage, but also catalysts for a variety of biological reactions, and therefore DNA molecules with catalytic properties are called catalytic DNAs or DNAzymes. DNAzymes that catalyze the cleavage of RNA are by far the largest class of catalytic DNA molecules [1] and almost is applied as quantitative analysis of cations[2] (Figure1). A wide variety of techniques have been developed to investigate metal-dependent activity of DNAzyme [3]. In this study, three-way data array was recorded by measuring excitation-emission fluorescence during the titration of DNAzyme-substrate complex with metal ion.

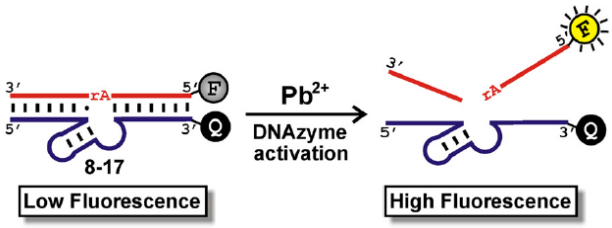

Figure 1. A Pb2+ Biosensor Based on 8-17.[Ref. 1]

Smilde and Kiers [4,5] have pioneered the work on restricted Tucker3 models. Based on the idea that PARAFAC may be too restricted and simple for many problems and that Tucker3 is too flexible, they developed an idea of using prior chemical/physical knowledge of the system studied to fix certain elements in the Tucker3 core array to zero and thereby in the end develop new models; restricted Tucker3 models, which are unique and chemically meaningful.

In kinetic hard-modelling concentration profiles are calculated by numerical integration of the rate laws describing the postulated kinetic hard-model. For some kinetic models and experimental conditions, however, the concentration matrix is rank deficient and the pure component spectra cannot be computed, as the linear regression step cannot be performed. Different solutions have been proposed in order to circumvent this rank deficiency problem and to allow the fitting of pure component spectra [6]. Here we used some independently known component spectra to the analysis by restricted Tucker3.

A (P, Q, R) component Tucker3 model on a data array $X \in RI \times J \times K$ can be represented in matrix notation as in Eq. (1), $X=AG(C \otimes B)T + E$ (1)

Where $A \in RI \times P$, $B \in RJ \times Q$ and $C \in RK \times R$ are the component matrices in the first, second and third mode, respectively, and G represents the core array ($G \in RP \times Q \times R$) matricized in the first mode. X is the metricized data array and E holds the residual variation not explained by the model. The symbol $\otimes$ denotes the Kronecker product.

In order to optimize the parameters (rate constants (k1:k5), interaction paramters at core of Tucker3 (g5:g13) and nitial concentrations (c1:c3)) replacement method (RM) was used. RM is a very simple method with prediction ability in a simple, rapid, high performance and reproducible way.

In the present case study,we apply this systematic method to sets of kinetic data, monitored by fluorescence spectroscopy, recorded during the course of the reaction of 8-17 DNAzyme with substrate catalysed by metal ion (see Fig. 1). FRET ocurrs between Enzyme and substrate strand. Therefore, rank deficiency of FRET is excitation and emission modes. Kinetic mechanism of this reaction will discussed here by hard modeling. First step of reaction is second order and rank deficiency because of closure will be possible. We applied independently known component spectra to the analysis by restricted Tucker3 to this catalytic reaction in order to resolve of chemical system with the rank deficiency in three mode (concentration, excitation and emission). Parameters optimized by replacement method.

The obtained information from our study by using chemometrics methods will be helpful for prediction of DNAzyme behavior during processes and determination of substrate yield that is cleaved.

Results and discussion

We supposed chemical model for cleavage activity of DNAzyme at Figure 2.

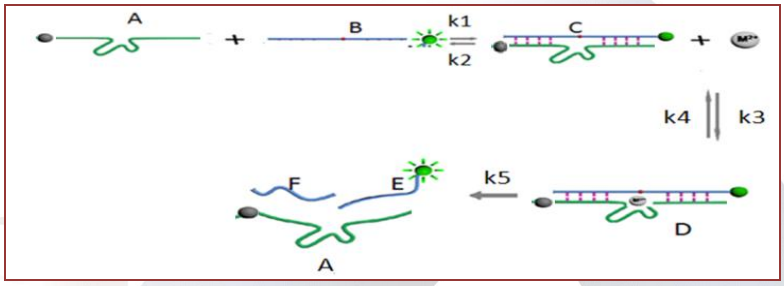

Figure 2. Mechanism of metal-dependent activity of DNAzyme

Simulated (81time×21em×31ex) fluorescence data array from complexometric titration of DNAzyme-substrate complex C with metal ion M (a cleavaging agent) is considered. The concentration profiles of each species of considered analyte (A, B, M, C, D, E) can be expressed as a function of five parameters (k1:k5) and initial analytical concentrations (c1:c3 for enzyme, substrate and metal ion respectively). F is non-absorbing species.Restricted-Tucker3 is applied for analysis of three-mode rank deficient chemical system. Dimentions of core array G is (6×3×3) for concentration, excitation and emission modes, respectively. Because we need six concentration profiles for explain chemical model and three independent excitation and emission profiles for A, B and M. Interaction parameters (g5:g13) at core of G are very informative, they estimated during optimization process. Other interaction prameters supposed are known. Replacement Method (RM) is applied to optimized all of parameres (3initial concentration+5rate constants+9interaction). Optimization algorithm is replicated until estimated parameters is converged and residual error for reconstruction of data be minima. Mentioned algorithm can estimate enzyme dosage and substrate concentration without needing calibration samples. In biological systems, RNA-cleaving DNAzymes can be used as therapeutic agents for treatment of cancer and a number of viral and microbial diseases. The obtained information from our study by using chemometrics methods will be helpful for prediction of DNAzyme behavior during processes and determination of substrate yield that is cleaved.

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