Anal Bioanal Chem (2002) 373:159–162
DOI 10.1007/s00216-002-1306-y

ORIGINAL PAPER

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Competitive interaction of the antitumor drug daunorubicin and the fluorescence probe ethidium bromide with DNA as studied by resolving trilinear fluorescence data: the use of PARAFAC and its modification

Received: 15 November 2001 / Revised: 12 February 2002 / Accepted: 28 March 2002 / Published online: 4 May 2002

Abstract The competitive interaction with DNA of daunorubicin (DR), being present in the clinical anti-tumor drug daunoblastina, and the fluorescence probe ethidium bromide (EB) has been studied by parallel-factor analysis (PARAFAC) and full-rank parallel-factor analysis (FRA-PARAFAC) of a fluorescence excitation–emission three-way data array. The PARAFAC algorithm can furnish stable resolution results for the data array studied, if the estimated number of chemical components is consistent with the real number. The FRA-PARAFAC algorithm is not sensitive to the estimated number of components of the fluorescence data array if the estimated number is not less than the real number. Both algorithms gave identical resolution for the three components concerned DR, EB, and the complex EB–DNA. Variations of the equilibrium concentrations of free DR, EB, and the complex EB–DNA were resolved by both algorithms. Experimental observation confirms the hypothesis that DR is an intercalator of DNA and that the binding interactions of DR and EB with DNA are a pair of parallel competitive intercalation reactions on same base sites of DNA. The method exemplified by this study provides a useful approach for studying competitive interactions of different drugs with DNA in the presence of interferents.

Keywords Competitive interaction · Daunorubicin · Ethidium bromide · DNA · Trilinear resolution

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Introduction

Several anthracyclines are powerful anti-tumor antibiotics, among which daunorubicin (DR) is the best known and potent antileukemic drug used widely in cancer chemotherapy [1, 2, 3]. The biological activity of DR results from its binding interaction with DNA; this inhibits cellular DNA- and RNA-dependent replication and transcription processes [4, 5, 6, 7]. Studies of binding interaction are, therefore, valuable not only for understanding how proteins recognize and bind to specific DNA sequences, but also for guiding the design of new drugs [8].

The binding interactions of small molecules with DNA have been studied extensively with a variety of techniques, including fluorescence spectroscopy [9], dichroism spectroscopy [10], and X-ray diffraction [11]. With the development of high order analytical instruments and chemometric algorithms, it becomes easier to obtain and resolve multi-dimensional data from complex systems. The combination of excitation–emission spectroscopy and trilinear chemometric algorithms could provide a powerful tool for studies of parallel competitive binding reactions of many chemical components with DNA in the presence of interferents. Such studies would be very helpful for understanding binding interactions of many drugs used in combination in the clinical treatment of some diseases. Full utilization of the so-called second-order advantage [12] makes it possible to determine the reaction pattern of different interacting pairs in a mixture medium.

In the work discussed in this paper the competitive binding interactions of the anti-tumor agent daunorubicin and the fluorescence probe ethidium bromide (EB) with DNA have been studied by excitation–emission fluorescence spectroscopy to obtain a three-dimensional excitation–emission fluorescence data array. The well-known chemometric algorithm parallel factor analysis (PARAFAC), developed and popularized by Harshman [13], was used to resolve the data array obtained. To avoid large error in the resolutions obtained, owing to inaccuracy in the estimate of the component number for the PARAFAC algorithm, a modified PARAFAC algorithm, full-rank parallel-factor

analysis (FRA-PARAFAC) [14], was also used for comparison. Compared with the traditional fluorescence method [9] which scans emission spectra with a fixed excitation wavelength, the method used in this study can simultaneously determine equilibrium concentration variations of DR, EB, and the complex EB–DNA for different initial DR concentrations; this would shed light on the intercalation interaction mechanism of DR and the identity of competitive interaction base sites of DNA with DR and EB.

Trilinear model and resolution algorithms

Trilinear model

K equilibrium mixtures with different initial concentrations of DR and fixed initial concentrations of fluorescence probe EB and DNA are prepared, and the excitation–emission fluorescence spectra are recorded at I excitation and J emission wavelengths. A set of fluorescence excitation–emission matrices is obtained, producing an (I×J×K) three-way data array $\underline{R}$. A trilinear model can be expressed as [15]:

$$\underline{R}_{I\times J\times K} = \sum_{n=1}^{N} x_n \otimes y_n \otimes z_n + \underline{E}_{I\times J\times K} \quad (1)$$

where N is the number of fluorescing species; the symbol ⊗ denotes a tensor product; x_n, y_n, and z_n are the excitation, emission and concentration profiles, respectively, of the nth fluorescing chemical component; and $\underline{E}$ is a three-way array of residuals.

The trilinear model can also be expressed as matrices along K-mode, i.e. sample mode for the data array studied:

$$R_{..k} = X\,\mathrm{diag}\left(z_{(k)}\right) Y^T + E_{..k}\ (k = 1, 2, ..., K) \quad (2)$$

where the superscript "T" denotes transpose of a matrix; and X and Y are the excitation and emission spectrum matrices, respectively, of N fluorescing chemical components:

$$X_{I\times N} = (x_1, x_2, ..., x_N) \quad (3)$$

$$Y_{J\times N} = (y_1, y_2, ..., y_N) \quad (4)$$

where $R_{..k}$ and $E_{..k}$ are the k-th matrix slices of $\underline{R}$ and $\underline{E}$, respectively, along K-mode; and diag($z_{(k)}$) denotes the diagonal matrix whose diagonal elements are the corresponding ones of the k-th row vector $z_{(k)}$ of the concentration matrix $Z_{K\times N}$:

$$Z_{K\times N} = (z_1, z_2, ..., z_N) \quad (5)$$

Trilinear algorithms

Based on the trilinear model, PARAFAC algorithm [13] uses alternating least squares (ALS) iteratively to perform trilinear resolution and to determine the response profiles X, Y, and Z. By using an ALS method, the PARAFAC algorithm minimizes a loss function which is a sum of squared residuals (SSR) expressed as:

$$SSS = \sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K} e_{ijk}^2 \quad (6)$$

where e_{ijk} is the (i, j, k)-th element of $\underline{E}_{I\times J\times K}$.

Actually, for trilinear resolution it is only necessary that matrices X and Y are full rank matrices for their column spaces, i.e. the trilinear model shown by Eq. (1) would be symmetrical with respect to x and y only. The FRA-PARAFAC algorithm uses the constrained condition of column full rank of matrices X and Y in the ALS procedure, which is expressed with the equations:

$$X^T P = I_N \quad (7a)$$

$$Y^T Q = I_N \quad (7b)$$

where P and Q are I×N and J×N matrices, respectively, and I_N is a N×N identity matrix. The FRA-PARAFAC algorithm minimizes the loss function Eq. (6) with the constrained condition expressed with Eqs. (7a) and (7b).

The PARAFAC and FRA-PARAFAC algorithms can both provide resolved solutions fitting the data array well under appropriate conditions. PARAFAC has the slower speed of convergence than FRA-PARAFAC. When the number of components is correctly estimated for the data array studied, both methods can give the right solutions. When the estimated number of components is more than the real number the PARAFAC algorithm would not converge and the FRA-PARAFAC algorithm could still provide the correct results. This property of FRA-PARAFAC is very valuable in the study of complex systems with unknown numbers of component; it has been used to study the competitive interactions of DR and EB with DNA. The results obtained by use of the two algorithms could be used for cross-validation.

Experimental

Daunoblastina, obtained from Pharmacia and Upjohn, Italy, is a freeze-dried power agent for clinical injection which contains 20 mg of daunorubicin hydrochloride in a phial. Daunoblastina was dissolved in re-distilled water as stock solution with a daunorubicin hydrochloride concentration of 3.55×10^{-4} mol L^{-1}. Stock solution of calf thymus DNA, purchased from Sino–American Biotechnology Company, China, was prepared with re-distilled water containing DNA of 0.21 mg mL^{-1}. Ethidium bromide from Sigma was used to prepare stock solution of 1.11×10^{-3} mol L^{-1}. These solutions were stored at 4 °C. The phosphate buffer solution used contained 5.05×10^{-3} mol L^{-1} NaH_2PO_4 adjusted to pH 7.0 with NaOH solution.

A set of the nine reaction mixture solutions was prepared with DNA and EB initial concentrations of 3.8×10^{-3} mg mL^{-1} and 2.99×10^{-6} mol L^{-1}, respectively; initial concentrations of DR varied from 0 to 2.27×10^{-6} mol L^{-1} with an interval of 2.84×10^{-7} mol L^{-1}. The binding reaction was performed at room temperature (26 °C). After the equilibrium was reached the excitation–emission fluorescence spectra were recorded by means of a Hitachi F4500 fluorescence spectrophotometer with slit widths of 5 nm, scan wavelength intervals of 5 nm, and a scan wavelength speed of 1200 nm min^{-1}. The excitation and emission wavelength ranges were 390–565 nm and 575–680 nm, respectively, for all the samples.

The background was subtracted by use of a blank sample. The PARAFAC and FRA-PARAFAC algorithms compiled in MATLAB

were used to resolve the spectra and concentrations of co-existing species in the equilibrium mixtures.

Results and discussion

The excitation and emission fluorescence spectra and equilibrium concentrations of DR, EB, and EB–DNA resolved by PARAFAC algorithm are shown in Figs. 1 and 2, respectively. Although the PARAFAC algorithm can provide resolution solutions fitting the trilinear model, it is too sensitive to the estimated number of chemical components. The FRA-PARAFAC algorithm, which utilizes the conditions of full rank of column vector spaces of excitation and emission spectrum matrices, was used for comparison and a double-check of PARAFAC results. The experimental studies proved that the resolution results obtained by use of the FRA-PARAFAC algorithm were really stable when the estimated number of components was more than the real number. This robustness in relation to the estimated number of components is very valuable in the study of a complex analytical system in the presence of unknown interferents. When the fluorescence three-way data array obtained in this study was resolved with the FRA-PARAFAC algorithm, with different numbers of components postulated, which were more than the real value, the results from resolution of the three chemical components concerned DR, EB, and EB–DNA, were very stable. The results obtained by use of FRA-PARAFAC are shown in Figs. 3 and 4. It is apparent from Figs. 1, 2, 3, and 4 that when the estimated number of components was correct PARAFAC and FRA-PARAFAC gave almost identical results for the excitation and emission spectra and equilibrium concentration profiles for the three species DR, EB, and EB–DNA, which indicates the reliability of the results obtained. From Figs. 1 and 3 it is obvious that

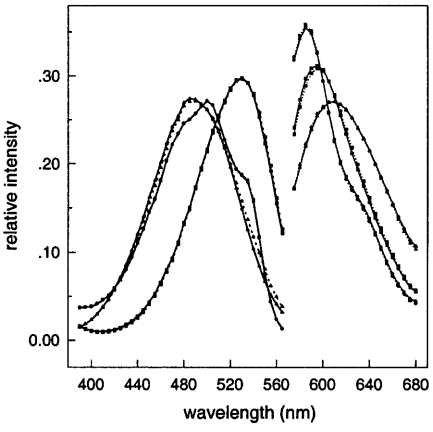

Fig. 1 Fluorescence spectra resolved by the PARAFAC algorithm for EB, EB–DNA, and DR. The excitation wavelengths were 390–565 nm and the emission wavelengths 575–680 nm. *Dashed line*, resolved spectra; *solid line*, recorded spectra; *circles*, DR; *triangles*, EB; *squares*, EB–DNA

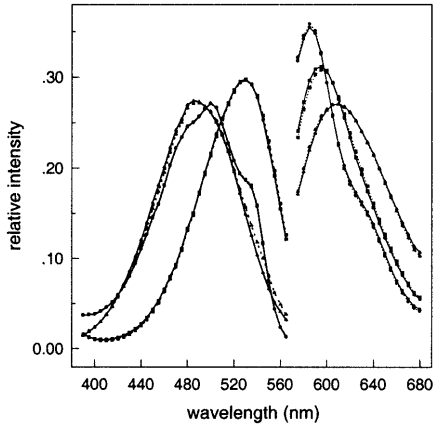

Fig. 3 Fluorescence spectra resolved by the FRA-PARAFAC algorithm for EB, EB–DNA, and DR. The excitation wavelengths were 390–565 nm and the emission wavelengths 575–680 nm. *Dashed line*, resolved spectra; *solid line*, recorded spectra; *circles*, DR; *triangles*, EB; *squares*, EB–DNA

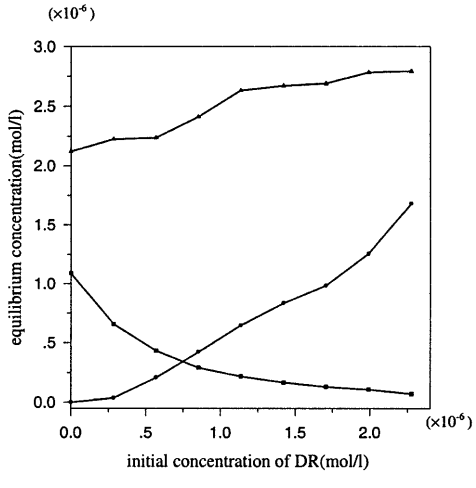

Fig. 2 Equilibrium concentrations resolved by the PARAFAC algorithm for EB, EB–DNA, and DR: *circles*, DR; *triangles*, EB; *squares*, EB–DNA

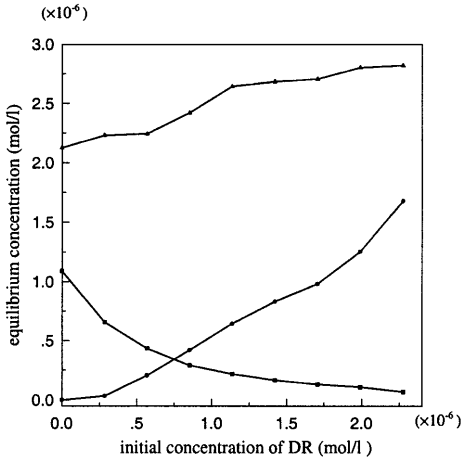

Fig. 4 Equilibrium concentrations resolved by the FRA-PARAFAC algorithm for EB, EB–DNA, and DR: *circles*, DR; *triangles*, EB; *squares*, EB–DNA

the resolved spectra are very consistent with the recorded spectra of pure compounds. One might assume that the resolved concentration profiles shown in Figs. 2 and 4 also reflect the real equilibrium concentrations of the reactive fluorescing species in the system studied.

It is well known that EB interacts with DNA by intercalation and that EB is an intercalator [16]. EB and the EB–DNA complex are both fluorescing species and the latter fluoresces more strongly. It is used as a standard agent for probing the structure of DNA and the interaction of small molecules with DNA. In the study of drugs, in particular, EB has been widely used as a fluorescence probe [17, 18]. In this study EB was used as a fluorescence probe to study the interaction of DR with DNA. The nine samples measured contained the same initial amount of DNA and EB, only the initial concentrations of DR were varied from 0 to 2.27×10^{-6} mol L^{-1}. In Figs. 2 and 4 the equilibrium concentrations of the complex EB–DNA and the free EB decrease and increase gradually, respectively, with increasing initial concentration of the anti-tumor drug DR. The replacement of EB in the EB–DNA complex by free DR can clearly be visualized from Figs. 2 and 4. One can assume that DR intercalates into the same base sites of DNA releasing the bound EB. The binding reactions are a pair of parallel competitive reactions. The interaction of DR with DNA is also assumed to belong to the intercalation model, the DR molecules intercalating between the base pairs of double-stranded DNA. This observation is consistent with the results obtained by X-ray diffraction [11]. The resolution of excitation–emission fluorescence data can provide a more direct indication of the equilibrium state of the system studied than the traditional treatment using the Scatchard plot [16].

Conclusion

The competitive interactions of the anti-tumor drug DR and fluorescence probe EB with DNA have been studied by PARAFAC and FRA-PARAFAC resolution of fluorescence excitation–emission three-way array. Compared with the PARAFAC algorithm, the FRA-PARAFAC algorithm is robust for estimation of the component number of the system studied. The excitation and emission spectra resolved by the two algorithms are very consistent with the real spectra of the three pure chemical components DR, EB, and EB–DNA. The variations of the equilibrium concentrations of the three chemical components in the nine samples were obtained directly, which further confirms X-ray diffraction results indicating the intercalation model of DR–DNA. The system studied in this research shows that trilinear chemometric methods can be useful tools for resolution of complex spectral data of reacting mixtures.

Acknowledgments This research was supported by grants from the National Natural Science Foundation of China (Grant Nos. 29735150, 20075006 and 29975007).

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