A demonstration of Uncorrelated Component Analysis for estimating nitric oxide ESR spectra

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INTRODUCTION

Electron spin resonance (ESR) spin trapping technique has been widely used to measure short-lived nitric oxide (NO) free radical as a direct assay with high sensitivity and specificity. However, although the utilization of spin trap agent have greatly improved the specificity of this assay, the spin-traps are not ideally species-specific usually, hence the identification and quantification of NO by ESR, is still problematic when there are spectra of other paramagnetic species overlying on the spectra of NO-adduct. For instance, as shown in Fig.1A, the ESR signal recorded from venous blood of rats, the typical triplet hyperfine structure of spin adduct HbNO is superimposed on the line of another paramagnetic species.

METHODS

NO ESR spectral signals were obtained by using a Bruker ESP 300E spectrometer to record the frozen venous blood and kidney tissue from rats at 77 K. The rats from which blood samples were taken were subjected to left renal artery occlusion for 1 hour, and samples were taken during periods of reperfusion. Hemoglobin in erythrocytes automatically trapped NO in blood to form HbNO (nitrosyl hemoglobin) for ESR detection. The rats from which kidney tissue samples were taken were preprocessed by injecting spin trap agents DETC and iron sulphate/sodium citrate to form NO-adduct $Fe^{2+}(DETC)_2NO$ for detection. The ESR measuring conditions were: 10 mW microwave power, 9.34/9.45 GHz microwave frequency, and 0.52 mT modulation amplitude.

Uncorrelated component analysis (UCA) method was introduced to estimate pure NO-adduct ESR signals from mixture signals [1]. This method assumes the component signals in the detected mixture are temporally uncorrelated to each other, and exploits second-order statistics of the received signal mixture to separate signals. When two or more mixed signals (channels) each with same components but with distinct component proportion were provided, the designated number of component signals can be estimated. The codes were written in MATLAB according to Chang et al. [2].

RESULTS AND DISCUSSIONS

For two detected HbNO signals similar to Fig.1A, the separated two component are shown in Fig.1B,C. Fig.1B exhibits an explicit triplet hyperfine structure assigned to HbNO. Fig.2c depicts the remaining background line which originally overlaid the HbNO signal. For two mixed signals similar to Fig.2A, the estimated component in Fig.2B demonstrates typical triplet spectrum of $Fe^{2+}(DETC)_2NO$ and Fig.1C mainly the spectrum of $Cu^{2+}(DETC)$. This result further manifests that, in the original recorded mixture signals, the existing of $Cu^{2+}(DETC)$ altered the shape of $Fe^{2+}(DETC)_2NO$ spectrum, and affected the latter's signal intensity. Using this easy performed method, we need not any extra animal experiments for acquiring background signal. Also, any previous knowledge of the hidden components and mixing ways of the components are not necessary. After this procedure, the double integration of the NO triplets became possible and allows a quantitative determination. Although this estimation is not so perfect, mostly because the requirement of uncorrelation was only mildly met in the two components, we believe that after further improvement this method could provide a useful tool for ESR mixture spectral analysis, and could be extended to other spectroscopy analysis.

REFERENCES:

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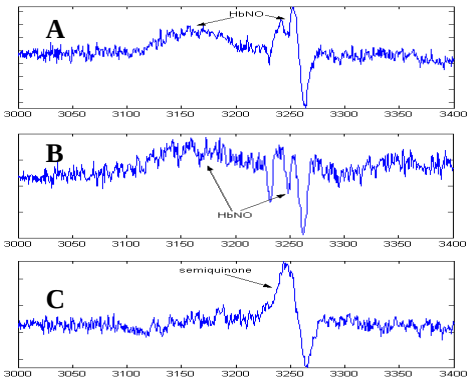

Fig.1. (A) Detected ESR spectra of HbNO from rat venous blood. (B,C) Separated component signals from two signals similar to A., (B) is assigned to HbNO.

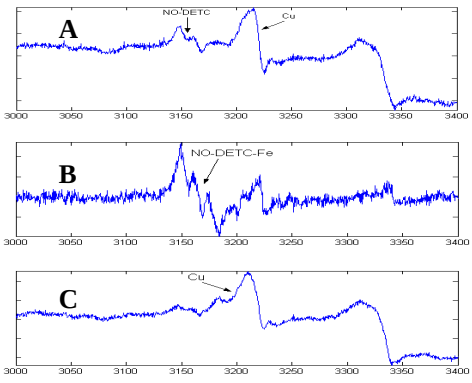

Fig.2. (A) Representative ESR spectra of DETC spin adduct recorded from rat kidney. (B,C) Retrieved component signals from two signals similar to A, (B) is assigned to NO-DETC-Fe.