EPR SPECTRAL SIMULATION ON CLUSTER N-1b IN NADH-UBIQUINONE OXIDOREDUCTASE OF BOVINE HEART MITOCHONDRIA

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1. Introduction

It is now generally agreed that NADH-ubiquinone oxidoreductase (complex I) from beef heart mitochondria (and presumably also from related tissues) contains 4 to 6 iron-sulfur clusters which can be differentiated by low temperature EPR spectroscopy. One of these clusters is readily detected at liquid nitrogen temperature and has been given the name cluster 1 or N-1b [1,2]. An additional iron–sulfur cluster, called N-1a, was reported by Ohnishi [3]. Its oxidation–reduction midpoint potential is so low that it cannot be reduced by NADH [4]. Albracht and his associates, in their efforts to simulate the EPR spectra of mitochondrial iron–sulfur clusters and quantitatively determine their concentration, reported that cluster 1 of NADH dehydrogenase is composed of two components *a* and *b*, which are present each at 0.25-times the concentration of the bound flavin of the enzyme [5,6]. It is to be noted that Albracht's clusters *a* and *b* are subcomponents of what Ohnishi [3] calls N-1b. N-1a was not observed by Albracht. Since the occurrence of iron–sulfur clusters at 0.25-times the concentration of flavin raises a number of questions bearing on the homogeneity, stoichiometry, and molecular mass of complex I, it was important to find independent support for Albracht's observations. More recently, Ohnishi et al. [7] were able to simulate the EPR spectrum of their cluster N-1b from bovine heart complex I as a single component with rhombic EPR features, whereas Albracht had reported best fits with two axial components. It was, therefore, desirable to obtain an independent verification of these observations by a laboratory experienced in the simulation of iron–sulfur cluster spectra using the experimental data of Ohnishi et al. and Albracht et al. By using computer minimization techniques [8], we have studied the published EPR signals from NADH–UQ oxidoreductase (complex I) from beef heart mitochondria. We find that, with the present signal-to-noise ratio, these spectra can be satisfactorily fit by a set of parameters describing a single rhombic paramagnetic center. Thus, it is not necessary to invoke a 'two-site' model for cluster N-1b.

2. Experimental

The Michigan computer programs are similar to those written by the groups in Amsterdam and Philadelphia except for two main differences:

(i) EPR simulating subroutines are attached to a sophisticated minimization program [8];

(ii) *g*-Strain [9] is modeled statistically as fluctua-

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tions in the 3 principal g-values, each with its expectation value and variance, and correlation coefficients [10].

The program generates the same spectra as those in Amsterdam and Philadelphia if the correlation coefficients are fixed at value 1. These coefficients are included because they are necessary for an exact duplication of the results reported below.

3. Results and discussion

Original data from both groups were digitized and are shown in fig.1. Because of the high noise level in both data sets, an attempt was made to fit the 'two axial' g-tensor simulation, provided by Albracht, with a 'single rhombic' g-tensor simulation. This approach was followed because:

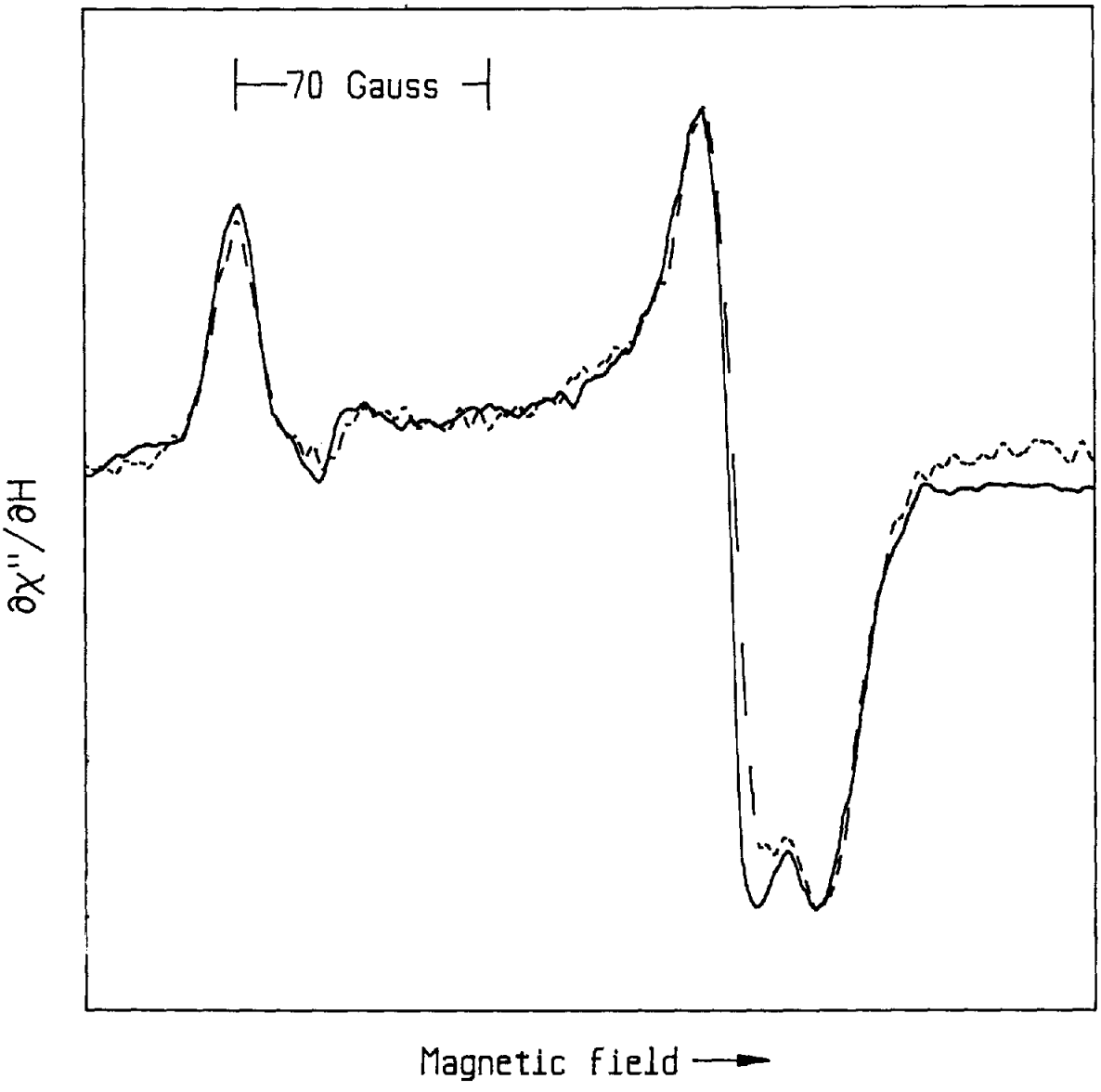

Fig.1. The dashed line is the spectrum provided by T. Ohnishi. Details are provided in fig.2 and 5 of [7]. The solid line is the spectrum provided by S. P. J. Albracht, details may be found in fig.3 and 5 of [5].

(i) The minimization program converges more rapidly with less noise in the test spectrum (the simulation has no noise);
(ii) If the sum of two axial g-tensors has the appearance of a single rhombic g-tensor then the question becomes academic.

A satisfactory fit to the 'two axial' simulation was obtained (see fig.2).

The g-values are 2.023, 1.941, 1.924, with linewidths (halfwidths in Gauss) 5.2, 6.3, 8.75 and correlation coefficients 1.0, 0.27 and −0.62. It should be stressed that since simulation fits the 'two axial' g-tensor simulation, these g-values and linewidths are not to be taken to the precise values for the spectral parameters. The 'single rhombic' g-tensor simulation is in very good agreement with the 'two axial' g-tensor

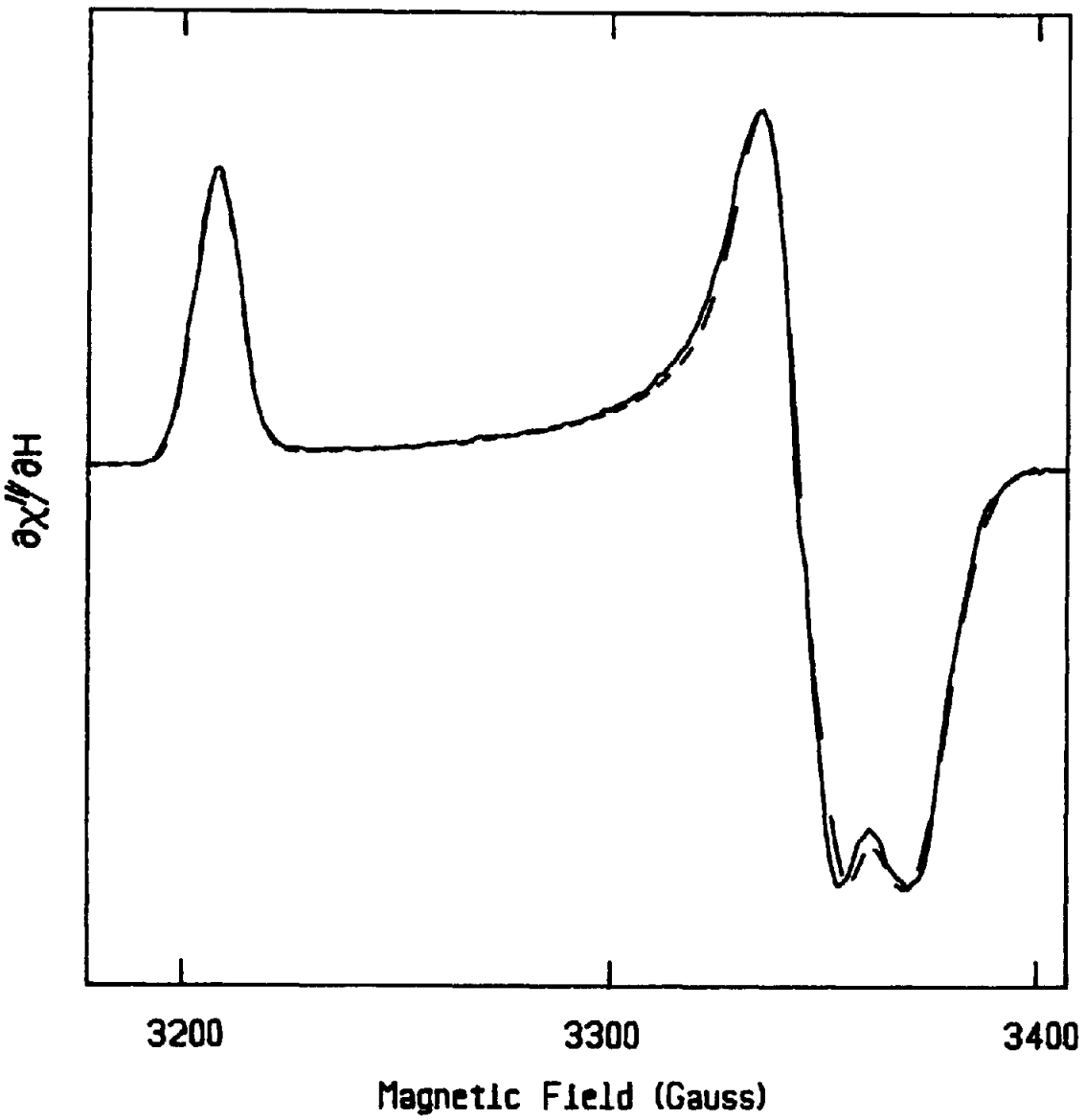

Fig.2. The solid line is the two axial g-tensor simulation provided by S. P. J. Albracht (see fig.5c in [5]). The dashed line is the single rhombic g-tensor simulation (see text for parameter values).

simulation except for a slight discrepancy in the high field region.

Even with this discrepancy, it is our conclusion that it is not necessary to use a 'two axial' *g*-tensor model in this case. There are two reasons for this conclusion:

(i) The single center simulation approaches the experimental spectrum to within approximately the same error as the two center simulation;
(ii) There is disagreement between the data from each group in the precise area where the two simulations disagree (see fig.1).

Though a two axial *g*-tensor model cannot definitely be ruled out, it does not appear to be necessary at this time. Higher signal-to-noise ratio data with reproducible features in the high field region are needed to ultimately resolve this question.

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