Nitrite–methemoglobin inadequate for hypoxic vasodilation

To the editor:

Basu *et al.*[1] recently suggested a new mechanism for the involvement of nitrite bound to hemoglobin in physiological hypoxic vasodilation. This physiological response, which is distinct from the weak pharmacological effects of nitrite, entails modulation of blood flow that is promptly responsive to oxygen saturation as red blood cells (RBCs) pass from arterial to venous circulation on a timescale of ~1 s. No plausible kinetic mechanism has been advanced through which nitrite reactions with hemoglobin could support this physiological function by direct and prompt release of NO (ref. 2).

Basu *et al.* now posit a critical role for N_2O_3 formed by the reaction of NO with a nitrite–methemoglobin complex. In support of this idea, Basu *et al.* measured the affinity of methemoglobin for nitrite by an indirect electron paramagnetic resonance (EPR) technique, and reported affinities that are substantially higher than previously determined by UV-Vis spectroscopy[3]; they did not detect the EPR spectrum of the complex. This outcome is surprising, as EPR spectra have been previously reported both for low-spin nitrite complexes with metmyoglobin[4] and for RBCs treated with nitrite[5]. We therefore re-investigated the EPR spectroscopy of the nitrite–methemoglobin complex.

We examined samples with hemoglobin (APEX Biochemicals) concentrations ranging from 0.1 to 2 mM (in heme) in aqueous solutions (HEPES or phosphate-buffered saline (Na or K)) at pH 7.4, and with a series of [$NaNO_2$]/[heme] molar ratios of 0, 1, 5, 10 and 20. In each series, the spectra obtained (at sample temperatures of 5–150 K) showed both the loss of the methemoglobin spectra (high-spin and low-spin components) and the gain of a spectrum essentially identical to those noted above[4,5], and thus attributable to the nitrite–methemoglobin complex (**Fig. 1**).

By detailed simulations of the EPR spectra[6], we quantified the amounts of each species in each sample. The trend in absolute amounts of each species for the series of spectra in **Figure 1** is shown in the inset. Within experimental error (~10%), the total amounts are constant; the detected nitrite–methemoglobin species account for all reaction products. Our EPR data yield an effective dissociation constant of 1.8 ± 0.6 mM, which is in good agreement with values determined previously by UV-Vis spectroscopy[3].

The idea of an unexpectedly strong affinity of methemoglobin for nitrite, which could lead to N_2O_3 by reaction of methemoglobin–nitrite with NO, is inconsistent with our results, in which we, in contrast to Basu *et al.*[1], directly detected the complex. Evidence for the proposed role of nitrite–methemoglobin complexes in physiological hypoxic vasodilation[7] thus remains lacking.

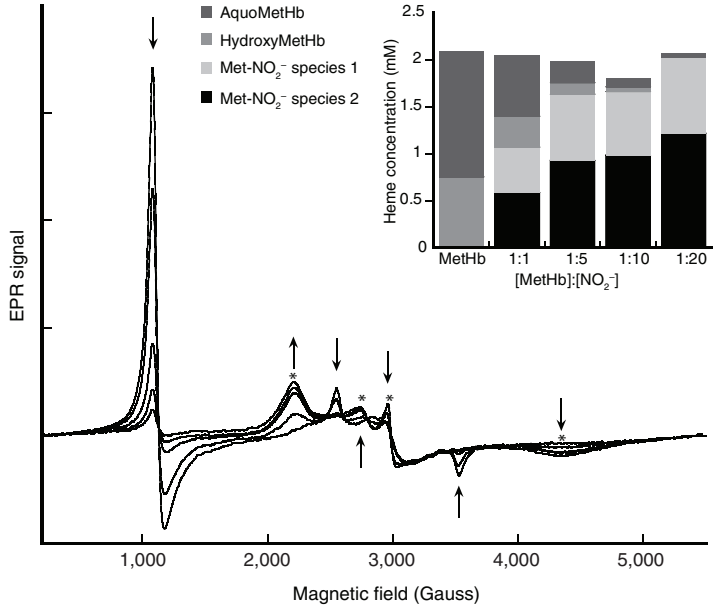

Figure 1 EPR spectra of solutions of nitrite–methemoglobin in HEPES buffer, pH 7.4. Spectra of five samples with [NO_2^-]/[methemoglobin] of 0, 1, 5, 10 and 20, and with protein concentration of ~0.5 mM, are shown. Methemoglobin was prepared, following Basu *et al.*, by treatment with ferricyanide. The EPR spectra in the figure were obtained with the samples in boiling nitrogen (76 K) and with 10 mW microwave power, frequency of 9.11GHz, 5 G modulation amplitude, 0.128 s time constant and a sweep rate of 16.67 G s^{-1}. Spectral features from nitrite–methemoglobin–provisionally assigned as two species with principal *g* values (g_x, g_y, g_z) of 1.41, 2.12, 2.98 and 1.41, 2.29, 2.89 (values typical for type II hemichromes[8])—are marked with asterisks; the other features are from methemoglobin. Amounts (in mM, on a per heme basis) of each of these species determined for each of the EPR spectra are shown in the inset.

[1]Department of Chemistry and Biochemistry, Montana State University, Bozeman, Montana, USA. [2]Department of Medicine, Duke University Medical Center, Durham, North Carolina, USA.
e-mail: dsingel@chemistry.montana.edu

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