Ammonium Metavanadate Complexation with Glutathione Disulfide: a Contribution to the Inhibition of Glutathione Reductase*

MITCHELL D. COHEN, ASOK C. SEN and CHENG-I WEI**

Department of Food Science and Human Nutrition, University of Florida, Gainesville, Fla. 32611, U.S.A.

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The mechanism for the prevention of oxidative damage to cell membranes is primarily through the glutathione peroxidase/reductase cycle. Binding of cellular glutathione (GSH) [1–3], its conversion to the oxidized form (GSSG) [4–6], or a decrease in reducing equivalents [7, 8] by vanadate may be major contributing factors to an observed increase in the level of membrane peroxides after vanadate exposure [9–11]. In this study we examined the interaction of ammonium metavanadate with GSH and GSSG and the effects imparted on the activity of glutathione reductase (GSHRX).

When aqueous GSSG (2 mM, Sigma) was mixed with aqueous vanadate (0.02–0.10 M, pH 7.4; J. T. Baker Chem. Co.), the solution turned yellow. At higher levels of vanadate, an orange color was immediately formed suggesting the formation of a ligand to metal charge transfer (LMCT) complex between vanadate and GSSG. From the absorbance scans performed against an aqueous GSSG blank in a Perkin-Elmer spectrophotometer, a new broad peak at 410–430 nm was detected (Fig. 1). By varying the concentrations of vanadate and GSSG, it was noted that both the peak amplitude and position were more dependent on the level of GSSG than the metal at vanadate levels greater than 50 mM. Analysis of the changes in absorbance at 420 nm with increasing levels of GSSG allowed for Scatchard analysis of the binding. The degree of binding was low with an association constant of 1.82×10^3 M^{-1} ($pK_a = 3.26$) at room temperature.

Binding of the vanadate with excess GSH had a pK_a of 4.30 [2], but no charge transfer complexation was indicated. Vanadate formed 1:2 complexes with excess GSH through the terminal carboxyl groups [1] or the thiol and amine moieties [3] after reduction to the vanadyl ion (V^{4+}). In our studies using an excess of vanadate, no discoloration or peak formation at 410–430 nm was observed. A similar spectroscopic analysis using cysteine or cystine (2 mM) resulted in the coloration and new peak only with the latter amino acid (data not shown).

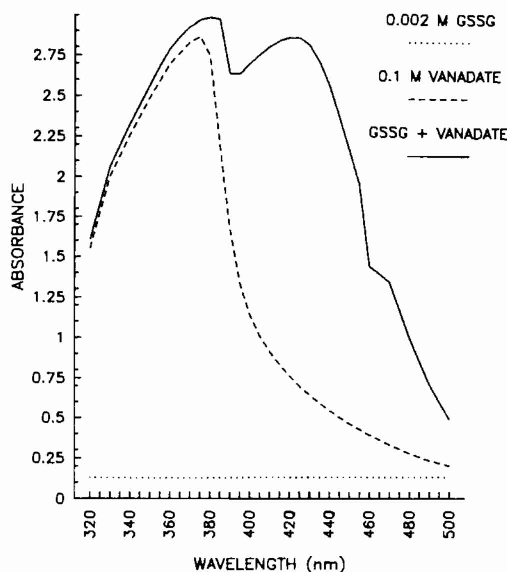

Fig. 1. Absorbance spectra of GSSG in the presence of aqueous vanadate. Spectra were constructed from measurements of absorbance at 5 nm intervals. Mixture volume (3 ml) containing aqueous vanadate (0.10 M) and 2 mM GSSG. In scans without GSSG, water was used as replacement. Each curve is the composite of 3 scans.

The LMCT transition is apparently $n \rightarrow \pi^*$ (t_{2g^*}) with the non-bonding electrons of the disulfide moiety acting as donors to the empty 3d shell ($3d^0$, $^1S_0$ non-degenerate) of vanadate. This transition is favored because vanadium in the pentavalent state has a high splitting energy (Δ_0) and therefore, low spin octahedral complexes would result; aqueous vanadate readily assumes distorted octahedral structures [12].

The interaction of vanadate with GSSG was examined for its effect on the activity of bovine mucosa GSHRX (Sigma). In the absence of vanadate, an increase in the level of GSSG resulted in increased enzyme activity. Aqueous vanadate or a vanadate/phosphate solution (0.1 M, pH 7.4) was added to a mixture containing GSSG (0.5–2.0 mM), enzyme (0.05 units in 0.01 M phosphate buffer), and Ellman's reagent (DTNB, 0.8 mM in phosphate buffer). After 5 min preincubation at 25 °C, NADPH (0.17 mM) was added and the increase in absorbance at 412 nm was monitored 3 min at 15 s intervals for the formation of the GSH:Ellman complex. The reference contained all reagents except the NADPH. While maintaining an optimal total ionic concentration of 0.1 M [13] and increasing the percentage of vanadate ions present, the activity was depressed in a dose-dependent manner (Fig. 2). The 50% inhibitory concentration (IC_{50}) was calculated to be 19 mM vanadate.

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**Author to whom correspondence should be addressed.

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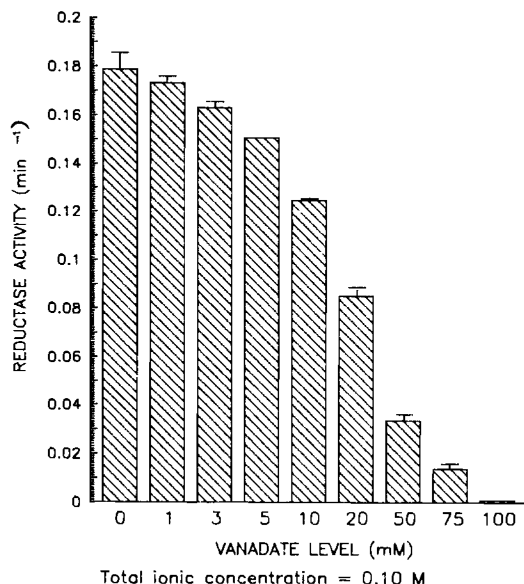

Fig. 2. Glutathione reductase activity determined by the change in absorbance at 412 nm/min over a 3 min period. The final reaction volume in a 1 cm quartz cell was 3 ml: 0.1 M phosphate/vanadate mixture, 2 mM aqueous GSSG, 0.8 mM Ellman's reagent, 0.05 units GSHRX, and 0.17 mM aqueous NADPH. Activities represent the average of 4 runs at each vanadate level.

At this point, of the many inhibitory anions studied [13, 14], iodide was the most potent with an IC_{50} of 90 mM. The vanadate IC_{50} value exemplified the potency of vanadium as an enzyme inhibitor as has been previously observed [15–17]. Ammonium ions, like most cations [18], had no effect. In our study, when levels of GSSG were increased in an attempt to overcome inhibition, the degree of inhibition was increased relative to the controls without vanadate (Fig. 3). At high levels of vanadate (>10 mM), the enzyme rate was minimal for all GSSG levels tested.

A similar study using GSH (1–4 mM; Sigma) in the place of GSSG reflected not only the oxidative

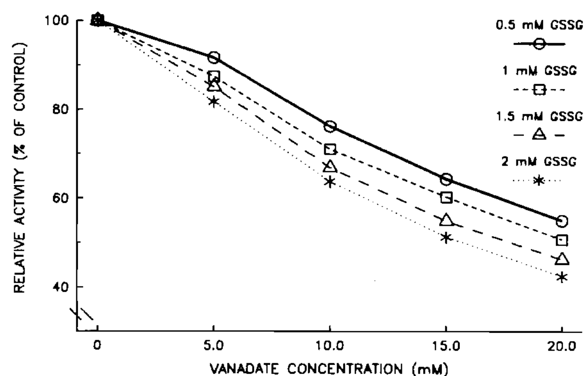

Fig. 3. Relative reductase activity as a function of GSSG and vanadate levels. All reaction conditions are as described in Fig. 2.

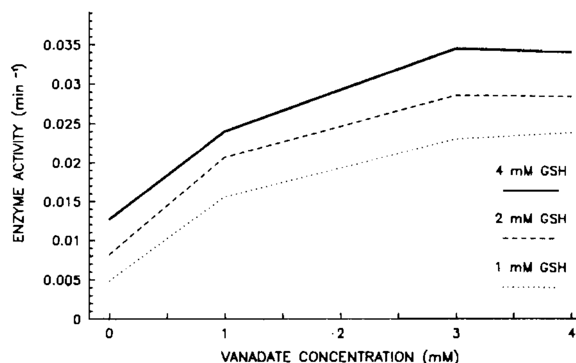

Fig. 4. Reductase activity in the presence of GSH. Activity measured as the rate of disappearance of NADPH/min at 340 nm following initiation of reaction with 0.17 mM NADPH. All conditions, excluding the Ellman's reagent, are as described in Fig. 2.

effect of vanadate on GSH (by its conversion to utilizable GSSG for the enzyme), but a similar inhibition of the newly-enhanced activity at higher levels of vanadate (Fig. 4). In this study, DTNB was not used and the activity was determined from the rate of NADPH oxidation monitored at 340 nm.

The active site of glutathione reductase has been well described [13, 19, 20]. Activation requires an NADPH-providing $2e^-$ reduction of an active site disulfide group which, mediated by a prosthetic flavin, yields thiols that bind the GSSG substrate. We concluded that the complexation of vanadate with GSSG was via the disulfide linkage, and so it is reasonable to assume that a similar interaction might occur at the enzyme active site. Metal oxidation and/or binding of the activated thiols may also contribute to enzyme inactivation [21].

A recent study from our laboratory described in detail the interaction of vanadate with NADPH [15]. The interaction of vanadate with both the NADPH and GSSG substrates contributed to the observed decrease in reductase activity when the vanadate was present although the exact mechamism at the enzyme level was not readily discernable.

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