

## NUMBER OF FLUORIDE IONS BINDING TO SUCCINATE DEHYDROGENASE DURING MIXED INHIBITION

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**SUMMARY:** We have measured the number of fluoride ions bound to one molecule of succinate dehydrogenase (SDH) of submitochondrial particles from porcine kidney cortex. Enzyme activity was studied according to the method of Dixon and the number of fluoride ions bound was established arithmetically. A mixed competitive and noncompetitive inhibition of SDH by fluoride was confirmed. At high levels of succinate (~20 mM) that saturate the active site of the enzyme, the binding of one fluoride ion outside the site results in noncompetitive inhibition. At lower levels (~1 mM), the site is not saturated and inhibition is competitive, with two fluoride ions binding to each enzyme molecule.

Keywords: Competitive inhibition, Fluoride binding, Mitochondrial membrane, Noncompetitive inhibition, Succinate dehydrogenase.

### INTRODUCTION

One of the most important effects of fluoride is inhibition of cellular energy production.<sup>1</sup> It has been shown that fluoride passes through the inner mitochondrial membrane and inhibits two enzymes of the respiratory chain – cytochrome oxidase and succinate dehydrogenase.<sup>2,3</sup> In 1952 Slater<sup>4</sup> reported that fluoride is a competitive inhibitor of the succinic oxidase system in cardiac mitochondria. This finding was later extended to the liver and kidney.<sup>4,5</sup> However, when the effect of fluoride was studied in submitochondrial particles, a mixed inhibition pattern was revealed.<sup>6</sup>

Succinate dehydrogenase (SDH) [E.C.1.3.99.1] is a mitochondrial enzyme encoded on the 1p22.1-q ter fragment of chromosome 1.<sup>7</sup> In the mitochondrial membrane, SDH and the electron acceptor ubiquinone form succinate-ubiquinone reductase (SA-Q reductase), the so-called complex II of the respiratory chain.<sup>8-11</sup> SA-Q reductase is composed of two subunits of iron-sulfur flavoprotein (succinate dehydrogenase) and two peptides (membrane anchoring proteins) responsible for interaction between ubiquinone and one of the iron-sulfur clusters of the enzyme.<sup>12,13</sup>

Our goal in the present work was to measure the number of fluoride ions binding to succinate dehydrogenase in submitochondrial particles, depending on the concentration of succinate as substrate.

### MATERIALS AND METHODS

Submitochondrial particles (SMP) were prepared by sonication of the mitochondrial pellet (from porcine kidney cortex) and ultracentrifugation.<sup>14,15</sup> Oxaloacetate, a strong competitive inhibitor of the enzyme, was removed from SMP by incubation in 0.02M Tris-cacodylate buffer (pH 7.8) containing 0.01 M potassium malonate.<sup>6,16</sup> The pellet was resuspended in a minimal volume of

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0.25 M sucrose, split into aliquots, and frozen in liquid nitrogen.<sup>6</sup> Protein was measured according to the method of Gornall.<sup>17</sup> Each experiment was preceded by testing SMP to ensure the absence of oxaloacetate.<sup>16</sup> Enzyme activity in the presence of fluoride ions was followed by measuring oxygen consumption with an oxygen electrode. Each experimental series started with measurement of basic enzyme activity. A given amount of protein was introduced into the reaction chamber containing 0.25 M sucrose and 0.01 Tris-cacodylate buffer, supplemented with 0.2% BSA (bovine serum albumin fraction V), 20 mM succinate, 1 mM KCN, 1 mM rotenone, and 1.4 mg/mL antimycin A. Following 5-min incubation at 25° C, electron acceptors – 2 mM phenazine methosulphate (PMS), and 0.05 mM 2,6-dichloroindophenol (DPIP) – were added. SDH activity was measured according to the method of Dixon<sup>18</sup> at five concentrations of fluoride ranging from 10 to 100 mM. The final concentration of succinate ranged from 0.2 to 20 mM. Reaction rate was calculated in nanoatoms O/min. The active fraction of succinate dehydrogenase was measured in 0.01 M Tris-cacodylate buffer containing 20, 2, 1, and 0.5 mM succinate and 0.1, 0.075, 0.05, and 0.025 M fluoride. The number of fluoride ions bound to one enzyme molecule was calculated using the following formula:<sup>19</sup>

$$\frac{1}{2 - n_B} = \frac{1}{n_S} + \frac{K_S}{n_S} \times \frac{1}{[\text{succ}]}$$

$n_S$  – number of ion-binding sites

$K_S$  – enzyme-substrate dissociation constant

$n_B$  – number of ions bound

## RESULTS

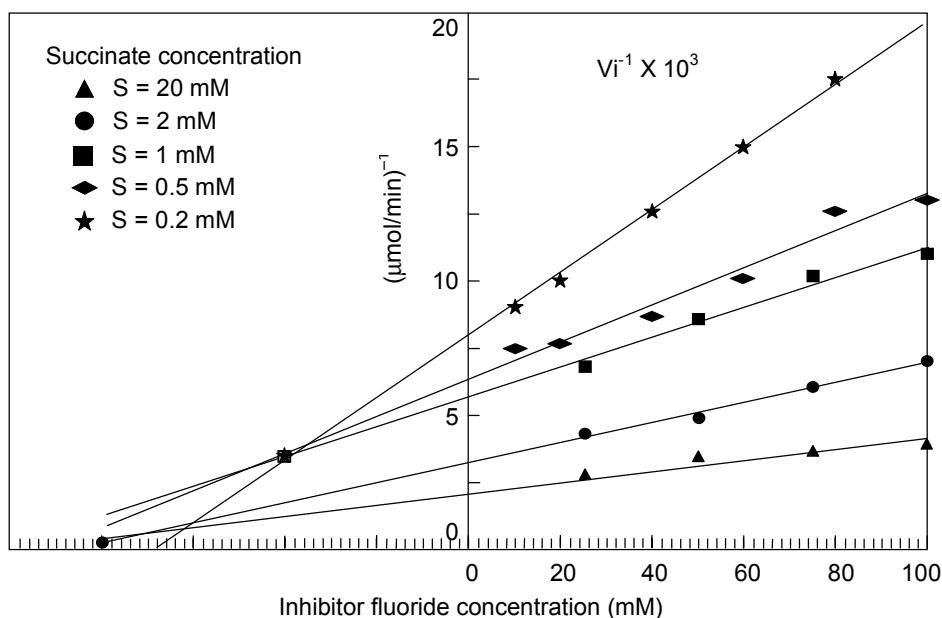
Figure 1 presents changes in enzyme activity with rising fluoride concentration (from 10 to 100 mM) at varying succinate levels. The final concentration of succinate ranged from 0.2 to 20 mM.

Figure 2 shows the active fraction of succinate dehydrogenase as a function of fluoride and succinate concentrations in the medium. Enzyme activity was measured in Tris-cacodylate buffer containing 20, 2, 1 and 0.5 mM succinate and 0.1, 0.075, 0.05 and 0.025 M fluoride.

The calculated number of fluoride ions bound to one enzyme molecule is presented in Figure 3.

## DISCUSSION

We have found a mixed type of inhibition by fluoride of succinate dehydrogenase (Figure 1). Using 80 mM fluoride, the inhibition was noncompetitive at succinate concentrations ranging from 20 to 2 mM (Figure 1). Under these conditions the active site of the enzyme is saturated with succinate, with a calculated  $K_m$  of  $6.6 \times 10^{-4}$  M.<sup>6</sup> At succinate concentrations of 1, 0.5, and 0.2 mM the site is not saturated. In this case, competitive inhibition was observed at 40 mM fluoride (Figure 1).

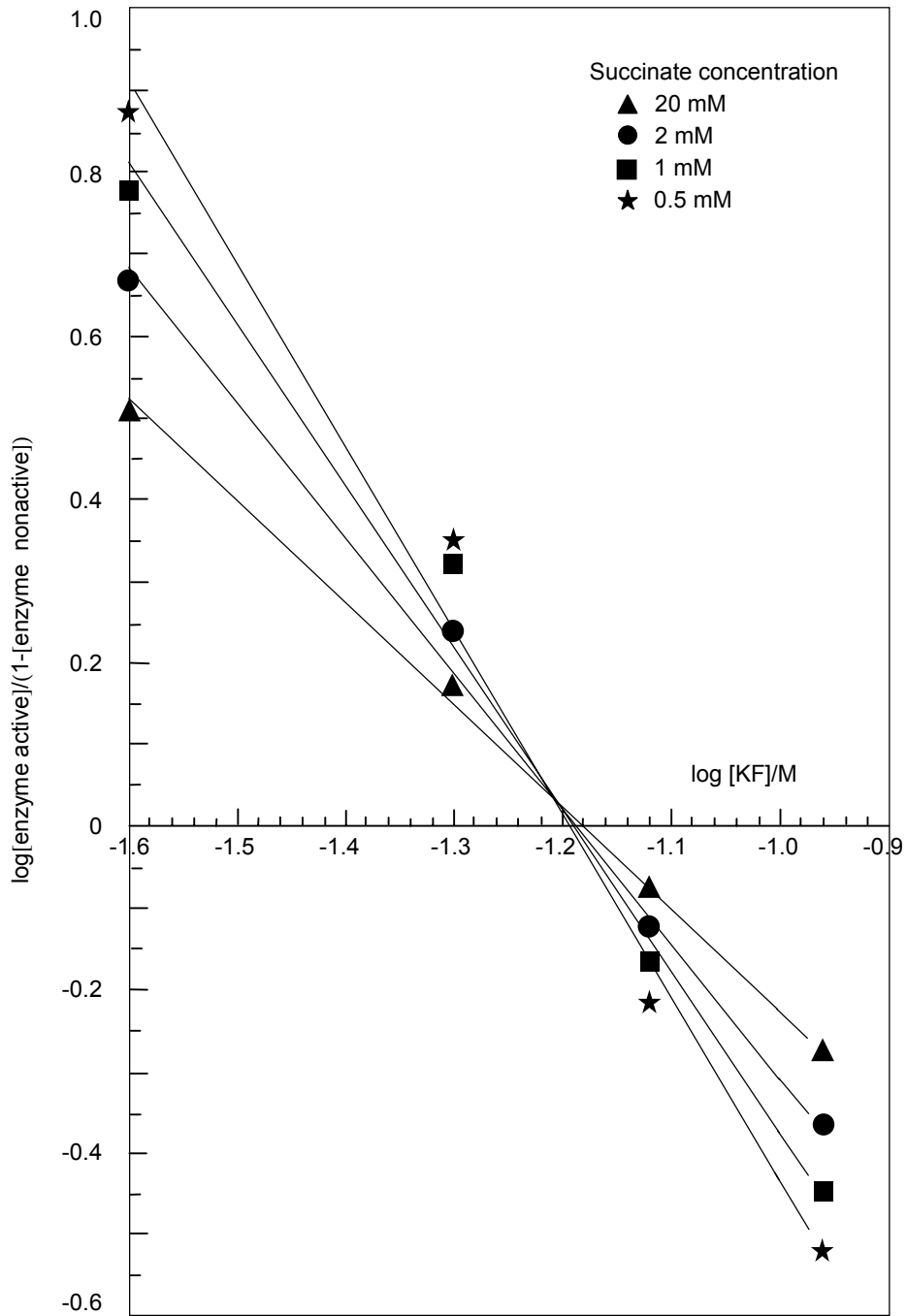
**Figure 1.** Dixon plot for the inhibition of succinate dehydrogenase by fluoride

Fluoride is a known competitive inhibitor of SDH.<sup>4,5</sup> In these experiments, however, the active site of SDH could have contained oxaloacetate, a strong inhibitor of the enzyme.<sup>20</sup> We have removed this inhibitor as described in Materials and Methods.

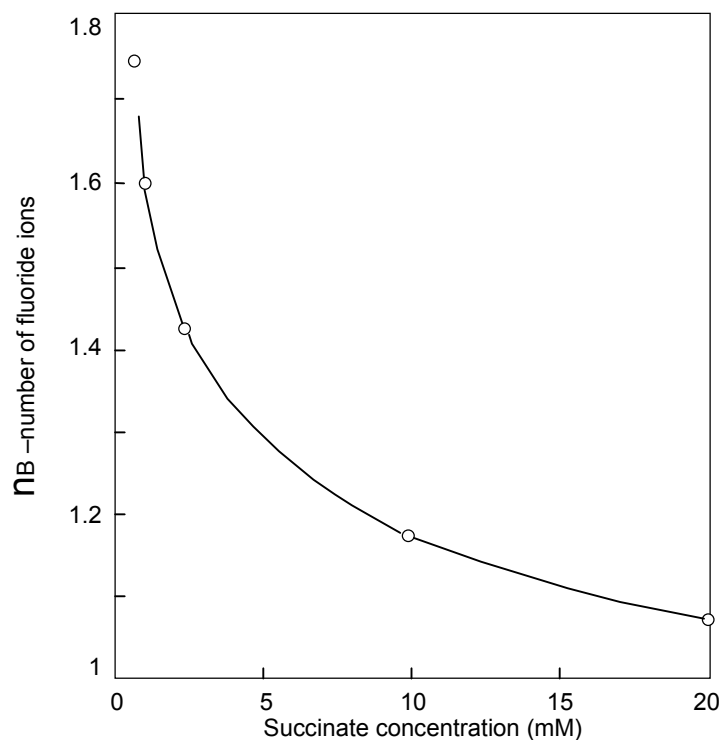
To obtain the number of fluoride ions bound with the enzyme, we analyzed the data shown in Figure 2 representing the active fraction of succinate dehydrogenase under varying saturation with inhibitor (fluoride) and substrate (succinate). In the presence of high succinate concentrations (20 mM) only one fluoride ion was bound with each molecule of enzyme, while at a lower concentration of 1 mM the number of ions was two (Figure 3).

It appears that the reaction of fluoride with SDH is based on two separate mechanisms, resulting in the binding of one or two ions. In the presence of high succinate concentrations (~20 mM), when the active site is saturated with substrate, noncompetitive inhibition (Figure 1) is caused by one fluoride ion (Figure 3) that binds elsewhere on the enzyme molecule. When the concentration of succinate is reduced to 1 mM (partial saturation of active site), inhibition is competitive (Figure 1), with two fluoride ions now binding to SDH (Figure 3).

**Figure 2.** Active fraction of succinate dehydrogenase as function of fluoride and succinate concentrations in the medium



**Figure 3.** Number of fluoride ions bound to succinate dehydrogenase molecule



Our results are in line with the study of Bonomi on the interaction of bromide with SDH.<sup>19</sup> As in the case of fluoride, bromide showed mixed inhibition at neutral pH. The proposed binding site for bromide involves basic lysine residues residing outside the active site.<sup>19</sup> Consequently, we believe that fluoride also binds to these residues during noncompetitive inhibition of succinate dehydrogenase.

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