# Interaction of Sodium, Lithium, Caesium, and Potassium Ions with Ascorbyl Radicals

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The influence of the concentration of sodium, lithium, caesium, and potassium ions as well as of the ionic strength of the solutions used on the dismutation rate of ascorbyl radicals has been investigated. While the dismutation rate was not influenced by $Li^+$, it decreased, however, with increasing concentrations of the other ions investigated. The largest effect was obtained with $Na^+$. This change in dismutation rate indicates a stabilizing effect on ascorbyl radical by these ions.

## Introduction

Oxidation of ascorbic acid by ascorbate oxidase is a one-electron process which results in the formation of the ascorbyl radical [1]. These radicals can be investigated by electronspin resonance (ESR) technique [2] or by spectrophotometric method [3]. The biological function of these radicals is not exactly known. Lohmann *et al.* have discussed not only their significance in acute lymphatic leukemia [4] but also their possible involvement in $Na^+$ and $K^+$ transport across membranes of erythrocytes [5, 6], in which case an electroneutral 1:1 complex between these ions and the ascorbyl anion radical seems to be a prerequisite. In addition, they have pointed out that the ascorbyl radical forms preferably a complex with $Na^+$. This complex is considerably stronger than the one formed with $K^+$.

The purpose of this paper was, therefore, to determine the influence of lithium, sodium, caesium, and potassium ions on the stability of the ascorbyl radical.

## Material and Methods

Ascorbate oxidase (EC 1.10.3.3) was prepared according to a method described by Avigliano [7]. The following reagents were used: lithium chloride, caesium chloride (Merck, Darmstadt); sodium chloride, potassium chloride, hydrochloric acid (POCh Gliwice czda); ascorbic acid (Sigma). Collidine (Fluka, Buchs) was distilled twice and passed through a Chelex X-100 column. Bidistilled, deionized water was used.

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The reaction was performed in collidine-HCl buffer at pH 7.32 in order to eliminate the involvement of other cations. At this pH, the buffer exhibits its greatest capacity and the ascorbyl radicals are most stable [5].

The buffers were prepared in the following way: a 0.2 M solution of collidine-HCl buffer, pH 7.32, was diluted with different amounts of water resulting in buffers with different ionic strengths. Thereafter, different concentrations of LiCl, NaCl, CsCl, and KCl were added to a 0.025 M collidine buffer resulting in buffers with increasing ionic strengths. Ascorbyl radicals were produced and their concentrations were measured by the method proposed by Skotland *et al.* [3] slightly modified, however, in this way that the concentration of the reagents was changed.

The reaction mixtures consisted of 1 mM of ascorbic acid dissolved in collidine-HCl buffer, pH 7.32, of different ionic strength to which 4.6 U ascorbate oxidase were added. In a different experiment, different concentrations of the ions mentioned above were added to this solution. Mixing of the reagents took place in flow cuvette located in the spectrophotometer SPECORD M40 (Carl Zeiss, Jena).

## Results and Discussion

With an increase in ionic strength of the collidine buffer an increase in the dismutation rate of the radicals can be observed indicating a decrease in radical stability (Fig. 1). This agrees well with results reported by Bielski [8], who, however, observed no modification of the dismutation rate whenever the radicals were in the electroneutral form.

The effect of LiCl, NaCl, CsCl, and KCl on the dismutation rate of the ascorbyl radicals in shown in

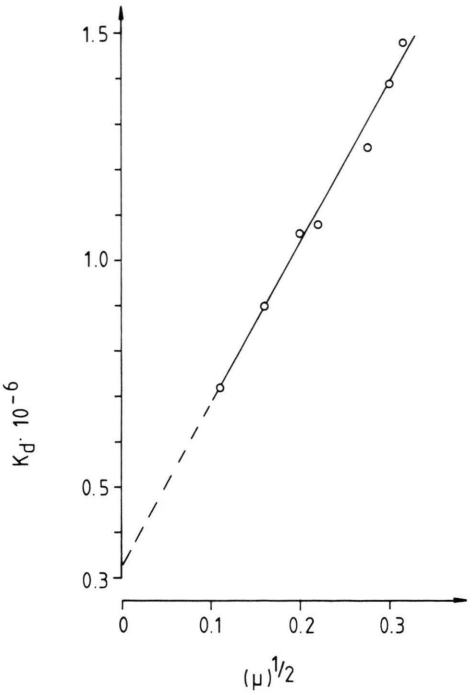


Fig. 1. Effect of the ionic strength ($\mu^{1/2}$) of collidine buffer on the dismutation rate ($K_d$) of the ascorbyl radical. Explanation s. text. SD ≤ 5%.

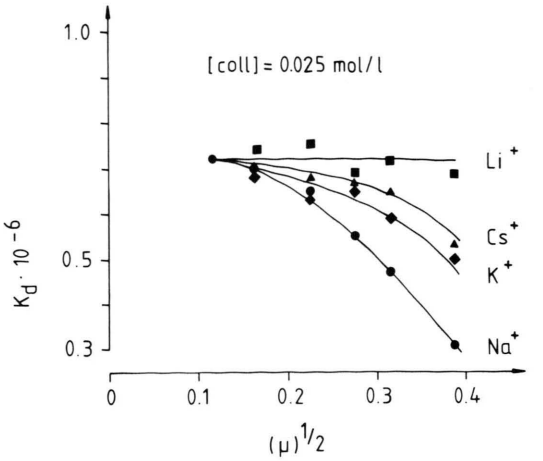


Fig. 2. Effect of the ionic strength ($\mu^{1/2}$) of several ions ($Li^+$, $Cs^+$, $K^+$, $Na^+$) on the dismutation rate ($K_d$) of the ascorbyl radical. The concentration of the collidine (coll) buffer is 0.025 mol/l. SD ≤ 8%.

Fig. 2. As can be seen, $Li^+$, in the concentration range used, doesn't seem to exert any influence on the dismutation rate of the ascorbyl radical. Thus, the complex formed between $Li^+$ and the ascorbyl anion radical seems to be electroneutral making it insensitive to the increase in ionic strength of the buffer.

In the case of $Na^+$, $K^+$, and $Cs^+$, there is a reduction of the dismutation rate with increasing ionic strength. It is interesting to note that the degree of the change of the dismutation rate is of the following order: $Na^+ > K^+ > Cs^+$. It might be possible that the stabilizing effect is due to an incorporation of these ions into the structure of the ascorbyl radical.

A similar dependence for $Na^+$ and $K^+$ was reported by Lohmann [6]. He suggests that, in the case of the radical, a hydrogen bond is formed between C(3)−O$^-$···HO−C(6) resulting in a ring closure of the free side chain. A sodium cation is, then, attached to the anionic cyclic structure which might cause a stabilization of the radical.

Considering the ionic radius of the ions added, one might conclude that the size of the sodium ion is most suitable for being built in into the ascorbyl radical structure. With increasing ionic radii ($K^+$, $Cs^+$) the incorporation of these ions into the radical structure is more and more hindered resulting in a reduction of the stabilization of the radical. It is also obvious (s. Fig. 2) that $Li^+$, the ionic radius of which is the smallest one of all ions studied [9], cannot be built in into the structure of the radical.

It should be emphasized that especially the sodium ion is prevailing in the body fluids of living organisms. Its stabilizing action upon the stability of the ascorbyl radical might be of biological significance.

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