# ACTIVATION OF DIOL DEHYDRASE BY FORMAMIDINIUM OR GUANIDINIUM ION, POLYATOMIC MONOVALENT CATIONS HAVING $sp^2$ NITROGEN

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SUMMARY

Coenzyme $B_{12}$-dependent diol dehydrase was activated by formamidinium or guanidinium ion. These polyatomic monovalent cations having $sp^2$ hybrid atomic orbitals and trigonal orientation were much more effective in activating the enzyme than methylammonium ion, but less active than $NH_4^+$ or $K^+$. Formamidinium and guanidinium ions were also effective both in forming and maintaining the binding of coenzyme $B_{12}$ to the apoenzyme. There is a close relationship between the effectiveness in activating the enzyme and those in forming and maintaining the holoenzyme, suggesting that these polyatomic monovalent cations play the same role in the diol dehydrase system as alkali metal monovalent cation such as $K^+$.

Diol dehydrase (D,L-1, 2-propanediol hydro-lyase, EC 4.2.1.28), a coenzyme $B_{12}$-dependent enzyme from Aerobacter aerogenes, absolutely requires a monovalent cation (e. g., $K^+$) for catalytic activity (1, 2). Our previous study on the role of a monovalent cation in the diol dehydrase system has shown that a certain monovalent cation is necessary for the binding of coenzyme $B_{12}$ to the apoenzyme (2). The relative effectiveness of various monovalent cations in activating the enzyme as well as in forming and maintaining the apoenzyme-coenzyme $B_{12}$ complex correlates closely to their ionic radii. Furthermore, we found that methylammonium ion is the only alkylammonium ion that activates the enzyme. On the other hand, ethyl- and dimethylammonium ions behaved inhibitory. This would be the first observation for an activation of an enzyme by a polyatomic substituted ammonium ion.

In this communication, we report a new type of monovalent cation activation of diol dehydrase by formamidinium or guanidinium ion, each of which has $sp^2$ hybrid atomic orbitals and trigonal orientation.

## MATERIALS AND METHODS

The crystalline coenzyme $B_{12}$, α-(5,6-dimethylbenzimidazolyl)-Co-5'-deoxyadenosylcobamide, was purchased from Glaxo Ltd., England. Other

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chemicals were obtained commercially. Partially purified apoenzyme of diol dehydrase was prepared from Aerobacter aerogenes (ATCC 8724) and assayed as described before (2). $K^+$-free apoenzyme was obtained by gel filtration on Sephadex G-25 using Tris•HCl buffer as mentioned in a previous paper (2). Effects of substituted ammonium ions on the diol dehydrase system were investigated in the following three ways: The effect on activating this enzyme (cofactor activity) was examined by adding varying concentrations of a monovalent cation to the $K^+$-free diol dehydrase system. The effect on forming the holoenzyme was studied based on the oxygen-sensitivity of the holoenzyme in the absence of substrate. The effect on maintaining the holoenzyme was estimated by measuring the degree of resolution of the holoenzyme, when this holoenzyme was subjected to gel filtration on a column of Sephadex G-25 (fine) previously equilibrated with 0.05 M Tris•HCl buffer (pH 7.0) containing 0.10 M 1,2-propanediol and each monovalent cation at the concentration of 50 times $K_m$ value (2).

## RESULTS

### Activation of Diol Dehydrase by Polyatomic Monovalent Cation.

Methylammonium ion was shown in our previous paper to activate diol dehydrase (2). We have therefore designed to test the cofactor activity of formamidinium and guanidinium ions which have $sp^2$ hybrid orbitals. Initially, the effectiveness of the polyatomic monovalent cations in activating diol dehydrase was examined. Table I summarizes $K_m$ and $V_{max}$ values for these ions determined by the Lineweaver-Burk plots (3). Formamidinium and guanidinium ions were not so effective as $NH_4^+$, but far more effective than methylammonium ion. The following order of the cofactor activity was obtained with respect to various monovalent cations tested hitherto.

$NH_4^+ > K^+ >$ formamidinium $\simeq Rb^+ >$ guanidinium $> CH_3NH_3^+ > Cs^+ > Na^+ > Li^+ \sim 0$.

### The Effect on Maintenance of Holoenzyme.

A certain monovalent cation is essential for the binding of coenzyme $B_{12}$ to apodiol dehydrase (2). The effect of formamidinium and guanidinium ions on the maintenance of the apoenzyme-coenzyme complex was studied. Table II shows the degree of resolution of the holoenzyme by gel filtration on Sephadex G-25 column using Tris•HCl buffer containing both the substrate and a polyatomic monovalent cation at the concentration of 50 times $K_m$. Both formamidinium and guanidinium ions were effective in maintaining the binding of coenzyme $B_{12}$ to the apoenzyme. Thus, the efficiency of various mono-

Table I Effect of Various Polyatomic Monovalent Cations on Diol Dehydrase Activity

Experimental procedure is identical with that described before (2). About 0.1 unit of the enzyme was used per tube. $K_m$ and $V_{max}$ values were determined kinetically. Relative cofactor activity is expressed in relation to the activity with $K^+$ taken as 100.

| Monovalent cation | $V_{max}$ (μmoles/min) | Relative cofactor activity (%) | $K_m$ (mM) |
|---|---|---|---|
| None | 0.0000 | 0 | |
| $K^+$ | 0.0871 | (100) | 0.543 |
| Formamidinium | 0.0842 | 96.7 | 1.15 |
| Guanidinium | 0.0790 | 90.7 | 1.25 |
| $CH_3NH_3^+$ | 0.0750 | 86.1 | 6.25 |

Table II Effect of Formamidinium and Guanidinium Ions on Resolution of Holodiol Dehydrase by Gel Filtration

Experimental procedure is identical with that described before (2). Tris.HCl buffers (0.05 M) used for gel filtration on Sephadex G-25 contained 0.10 M 1,2-propanediol and each monovalent cation at the concentration of 50 times $K_m$.

| Monovalent cation | Specific activity (units/mg) | | Resolution (%) |
|---|---|---|---|
| | -Coenzyme $B_{12}$ | +Coenzyme $B_{12}$ | |
| Formamidinium | 0.70 | 0.75 | 7 |
| Guanidinium | 0.60 | 0.68 | 12 |

valent cations in maintaining the holoenzyme followed the pattern:

$NH_4^+ > K^+ >$ formamidinium $>$ guanidinium $> Rb^+ > CH_3NH_3^+ > Cs^+ > Na^+ > Li^+ \sim 0$.

This pattern is in a close analogy with the order of the cofactor activity mentioned above.

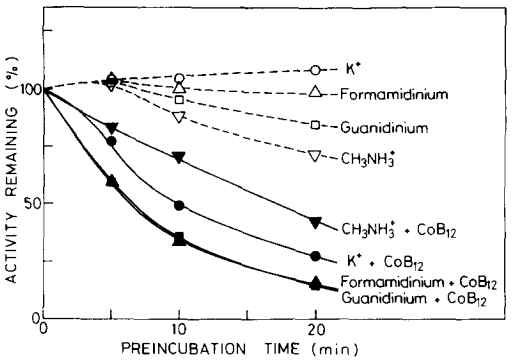


Fig. 1 Comparison of effects of polyatomic monovalent cations on inactivation of diol dehydrase in the presence and absence of coenzyme $B_{12}$

Experimental procedure is essentially identical with that mentioned before (2), except that each monovalent cation was added at the concentration of 10 times $K_m$.

The Effect on Holoenzyme Formation.

The efficiency of the polyatomic monovalent cations in forming the apoenzyme-coenzyme $B_{12}$ complex was investigated based on the marked sensitivity of holodiol dehydrase to oxygen in the absence of substrate. Aerobic incubation of the apoenzyme and coenzyme $B_{12}$ in the absence of substrate leads to inactivation accompanying irreversible cleavage of cobalt-carbon $\sigma$ bond of the coenzyme. This inactivation rate can be considered as an indication of the rate of the holoenzyme formation mediated by an appropriate monovalent cation. Figure 1 depicts the inactivation rate when the apoenzyme was incubated aerobically with and without coenzyme $B_{12}$ in the presence of the polyatomic monovalent cation in question at the concentration of 10 times $K_m$. Coenzyme $B_{12}$-dependent inactivation, that is, the difference in activity between with and without coenzyme reflects the extent of the holoenzyme formation. The result obtained indicates that formamidinium ion is as effective as $K^+$ in forming the apoenzyme-coenzyme complex, and that guanidinium ion is, though not so effective as formamidinium ion, much more efficient than methylammonium ion.

## DISCUSSION

Formamidinium and guanidinium ions behave as an effective monovalent cation activator in the diol dehydrase reaction. This is the first observation for an activation of an enzyme by a polyatomic monovalent cation having $sp^2$ hybrid orbitals and a trigonal geometry. It is interesting because guanidine is usually used at a much higher concentration as a protein-denaturing agent. There is a close relationship between the effectiveness of

formamidinium or guanidinium ion in activating diol dehydrase and those in forming and maintaining the holoenzyme, indicating that these polyatomic monovalent cations play the same role as $K^+$. In the case of the alkali metal cation which has a noble gas type of electronic configuration, the cofactor activity in the diol dehydrase system seems to be expressed as a function of ionic radius, whereas the situation is much more complicated in the case of polyatomic monovalent cations. Although the actual structures of formamidinium and guanidinium ions are visualized as a resonance hybrid, somewhere between two and three extremes respectively, it appears likely that extensive localization of a positive charge at one nitrogen atom would occur when these ions are bound to the enzyme. The cofactor activity of the polyatomic monovalent cations may be affected by three factors: The size and shape of the cationic center; the strength of the positive charge; the bulkiness of the side chains.

Recently, Eisenman and Krasne (4) have reported that formamidinium and guanidinium ions form complexes with carrier antibiotics such as nonactin and valinomycin. It is much interesting that the monovalent cation selectivity of diol dehydrase appears similar to those of these model carriers. It would be expected that a study on the model system provides valuable information regarding the mechanism of the interaction between monovalent cation and the enzyme.

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