ACTIVATION OF BOVINE CARBONIC ANHYDRASE BY ETHLYENEDIAMINE TETRAACETIC ACID

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We have recently found (Ho and Sturtevant, 1960) that the activity of bovine carbonic anhydrase (CA) at 25° is markedly increased by the addition of chelating agents such as ethylenediamine tetraacetic acid (EDTA), nitrilo-triacetic acid, and 1, 10- phenanthroline (OP). Our results indicated that this activation is not due to the removal of inhibitory metal ions, but is the result of a more subtle change produced in the enzyme by the activator. We have now obtained further support for the view that a true activation is involved, based on the important finding of Lindskog and Malmström (1960) that the zinc can be reversibly removed from CA by dialysis at pH 5 in the presence of chelating agents.

Experimental

Bovine CA, obtained from Worthington Biochemical Company (lot 5513), was used without further purification. All solutions were prepared using analytical grade reagents and glass distilled water. Activity determinations were based on measurements of the rate of hydration of CO_2 in a stopped-flow apparatus (Spencer and Sturtevant, 1959), using an indicator (10^{-4}M p-nitro-phenol or m-cresol purple) to follow the liberation of hydrogen ions. All rate

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measurements were made in 0.025 M triethanolamine buffer, pH 7.5, 0.1 M in KCl, with an initial CO_2 concentration of 0.005 M, and are reported on a relative basis with the non-enzymic rate deducted. The time resolution of the stopped-flow apparatus is such that initial rates can be determined in less than 0.1 sec., and the rates reported in this paper are initial rates, except for those determined for the zinc-containing enzyme in the presence of EDTA. As previously found (Ho and Sturtevant, 1960), if solutions of CO_2 and CA, both containing EDTA, are mixed, the initial rate of hydration of CO_2 is that observed under the same conditions in the absence of EDTA, but the rate then increases to the activated value according to apparent first order kinetics within one second or less. The rates given here are those observed after completion of the activation process.

Dialysis experiments were carried out as described by Lindskog and Malmström (1960), with 10^{-3} M OP as chelating agent, in 0.05 M acetate buffer, pH 5, at about 5° C. Control samples were dialyzed under identical conditions in the absence of OP. During dialysis the enzyme concentration was approximately $5 \cdot 10^{-5}$ M. The enzyme was regenerated by adding $2 \cdot 10^{-3}$ M $Zn(C_2H_3O_2)_2$ to the concentrated dialyzed material. All assays were performed at enzyme concentrations of $1 \cdot 10^{-7}$ M to $4 \cdot 10^{-7}$ M, the lower concentration being used for native and regenerated enzyme. Thus the concentration of free Zn^{++} during assay did not exceed $2 \cdot 10^{-6}$ M. Activation was tested with EDTA at a concentration of 10^{-4} M in the assay solution.

Results and Discussion

The results of the dialysis experiments are summarized in Table I. Comparison of the activities of the control sample without (A) and with (B) EDTA indicates activation by EDTA by a factor of approximately 6, as

previously observed. The data for sample C show a loss of activity somewhat more rapid than reported by Lindskog and Malmström, followed by an apparent recovery of activity at longer dialysis times, which was not observed by Lindskog and Malmström. We can offer no satisfactory explanation for this recovery of activity, and are giving further attention to it. Whatever its cause, it is evident that this active enzyme is very different from the original enzyme, since its activity is practically completely removed by addition of EDTA (sample D).

Table I

Effect of dialysis and chelating agents on the activity of bovine carbonic anhydrase at 25°, pH 7.5

	Relative activities			
Dialysis time, days	0	6	10	16
A. Dialyzed against acetate at pH 5	1.0	0.9	0.8	0.7
B. A + EDTA	5.8	5.1	5.0	4.6
C. Dialyzed against acetate + OP at pH 5	---	0.2	0.7	1.0
D. C + EDTA	---	0.09	0.03	0.03
E. C + $Zn(C_2H_3O_2)_2$	---	1.0	1.1	1.0
F. E + EDTA	---	5.6	5.9	5.9

The observations which are of greatest significance are that the enzyme inactivated or altered by dialysis against OP can be restored to full activity by addition of Zn^{++} (sample E), as found by Lindskog and Malmström, and that this regenerated enzyme is identical with the original material as judged by its activation by EDTA. The material obtained after 16 days' dialysis against OP is particularly interesting; enzyme having full activity is

essentially completely inactivated by EDTA, but is activated by a factor of 6 by Zn^{++} plus excess EDTA. These results give added support to the conclusion reached on the basis of our earlier work that EDTA produces a more active form of the enzyme, and does not simply remove an inhibiting impurity. The fact that the presence of Zn^{++} in the enzyme is essential for activation would suggest that the chelating agent is bound to the enzyme by the metal ion. However, we have previously shown that full activation requires the binding of two molecules of the activator per molecule of the enzyme, although only one atom of metal is present.

We are continuing our study of this interesting phenomenon.

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References

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