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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF BOVINE AND HUMAN CARBONIC ANHYDRASE, COMPONENTS A AND B

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Bovine carbonic anhydrase [carbonate hydrolyase, EC 4.2.11] has been analyzed by high performance liquid chromatography, HPLC, using a TSK column, IEX-540 DEAE, eluted with 0.01 mol dm^{-3} phosphate buffer of pH 6.5. By means of this method, components A and B of bovine carbonic anhydrase, BCA(A) and BCA(B), showed peaks at t_R=13.5 and 5.5 min, and the apo-BCA(A) and apo-BCA(B) showed peaks at t_R=15.8 and 6.0 min, respectively. Under the same conditions, human CA(B) showed a peak at t_R=2.4 min.

The authors have established conditions for analyzing carbonic anhydrase, components A and B, by high performance liquid chromatography, using a TSK column, IEX-540 DEAE. By means of this method, the metalloenzyme has been identified easily. The authors prepared bovine carbonic anhydrase, component B(=BCA(B)),[1,2] from erythrocytes. The apo-BCA(B) prepared[3] was also identified by this method. Based upon these data, the marketing BCA(A) and human CA(B) purchased from Sigma Chemical Co. were analyzed effectively. Native and apo-BCA have been confirmed by measuring the Zn contents by atomic absorption spectrometry , enzyme activity,[3] as well as protein concentrations. A part of the data obtained for the enzymes used were shown in Table I.

A high performance liquid chromatograph(Shimadzu LC-3A) was equipped with a TSK IEX-540 DEAE column(4.0 ID x 300 mm) connected to its precolumn(4.0 ID x 50 mm) and a UV detector(Shimadzu SPD-2A). One hundredth mol dm^{-3} phosphate buffer of pH 6.5 was used as an eluating solvent(flow rate 1.0 cm^3/min) and detection was made at 280 nm.

Establishment of the HPLC Conditions

In order to establish the HPLC conditions to differentiate BCA(A) and BCA(B), BCA(A) was analyzed by changing concentrations of phosphate buffer of pH 6.5. At the concentration of 0.05 mol dm^{-3} phosphate buffer, a main peak was observed at t_R=3.4 min accompanied by a small peak observed as a shoulder at t_R=4.0 min. By decreasing the concentrations, the peak at t_R=3.4 min began to separate into three or four peaks and the best separation was achieved by adopting 0.01 mol dm^{-3} phosphate buffer of pH 6.5 as an eluate. Under the conditions, the marketing BCA(A) was separated into four peaks at t_R=5.5, 6.6, 7.7, and 13.5 min. In order to assign these peaks, BCA(B) prepared in authors' lab. was chromatographed and it showed one

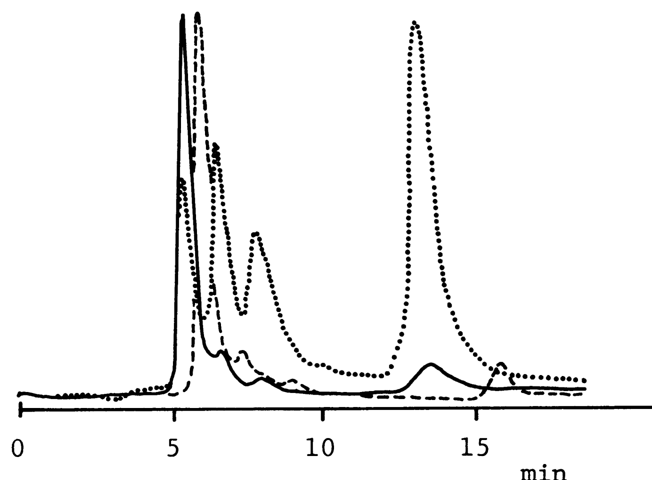

Fig. 1 Chromatograms illustrating the separation of native and apo-BCA

······· BCA(A), —— BCA(B) ---- apo-BCA(B)

Table I Retention Times Carbonic Anhydrase

Enzymes	t_R(min)	Zn (x 10^{-4} mol dm^{-3})	Protein (x 10^{-4} mol dm^{-3})
BCA(B)	5.5	1.91	1.78
apo-BCA(B)	6.0	0.12	1.08
BCA(A)	13.5	1.09	0.85
apo-BCA(A)	15.8	-	-
HCA(B)	2.4	0.88	0.90
apo-HCA(B)	2.8	-	-

main peak at t_R=5.5 min, besides very small peaks at t_R=6.6, 7.7, and 13.5 min as illustrated in Fig. 1. The apo-BCA(B) prepared with 2,6-picolinic acid[3] showed two peaks at t_R=6.0 and 15.8 min with elimination of the peak at t_R=13.5 min, both of whose Zn contents were checked to be none by AAS. Therefore, the peaks at t_R=5.5, 6.0, 13.5, and 15.8 min were assigned to BCA(B), apo-BCA(B), BCA(A), and apo-BCA(A), respectively, as shown in Table I. The peaks at t_R=6.6 and 7.7 min observed for BCA(A) were assigned due to the impurities. That is, the marketing BCA(A) contains not only component B, but also other contaminants.

Human carbonic anhydrase, component B, was also chromatographed under the same conditions and peaks at t_R=2.4 and 2.8 min were assigned to the component B and apo-HCA(B), respectively, but their separation was not sufficient with observation of the latter as an shoulder when both of them were chromatographed.

Application of HPLC is considered to be one of the simple and rapid tools to identify the purity of metalloenzymes, and this method may also be applicable to approach the studies of enzyme mechanisms, as well as properties.

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