Isoelectric Fractionation, Analysis, and Characterization of Ampholytes in Natural pH Gradients. VI. Isoelectric Spectrum of Bovine Carbonic Anhydrase B

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By the method of isoelectric focusing [1-3] native bovine carbonic anhydrase B has been investigated with respect to heterogeneity and isoelectric point. The investigation has been made in cooperation with the Department of Biochemistry, University of Gothenburg. The enzyme was prepared and the enzyme activity measurements were made there by Nilsson and Lindskog.[4] The electrolysis runs were performed according to Vesterberg and Svensson [5] with some modifications. We used a density gradient device (to be published by H. Svensson and S. Pettersson) giving a constant density gradient. The enzyme solution was mixed with the dense solution. The concentration of the carrier ampholytes was 2 %. After a steady state had been reached at +4°C, a final focusing at +22°C was performed for 5 h at a voltage of 450 V. The pI values obtained then will be those at +22°C.

On draining the column, pH (+22°C) and UV absorption (280 nm) were measured continuously by means of flow through cells. The pH measurements were performed with a Beckman Expandomatic pH meter, equipped with a Beckman 97633 pH electrode assembly and a Beckman ten-inch linear, potentiometric recorder. The UV absorption measurements were made with a modified Vitatron (address: Vitatron N. V., Dieren, Holland) photometer UFD, equipped with a flow through cell and a Vitatron linear/logarithmic integrating recorder. The recorders give the pH and UV absorption courses of the column solution. The pI value belonging to a UV peak could easily be derived from the pH curve. 0.75 ml fractions were collected for the enzyme activity measurements.

In a preliminary run on a pH gradient ranging from 3 to 10, only one enzyme zone was obtained, at a pH of about 5.9. This isoelectric homogeneity of carbonic anhydrase is remarkable and indicates a very good biochemical preparation since the extreme resolving power of the isoelectric method in general reveals heterogeneity even for proteins considered to be very well purified. However, another run on a shallower pH gradient ranging from 5 to 8 gave the isoelectric spectrum shown in Fig. 1, in which the UV absorption peak is found to be somewhat broader than the activity peak. This is certainly due to the presence of inactive protein, probably inactivated carbonic anhydrase, isoelectric in the vicinity of the active enzyme. The amount of enzyme in this run was 16 mg,

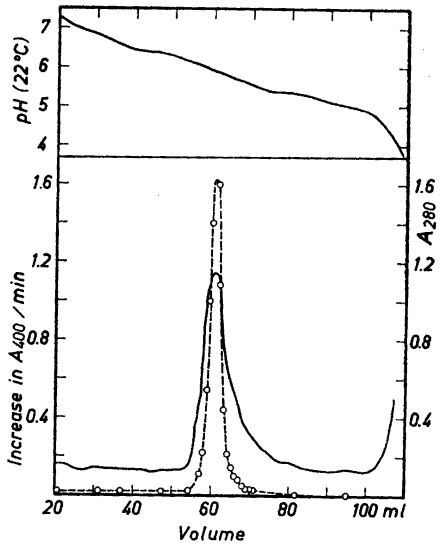

Fig. 1. pH (upper solid curve), UV absorption (280 nm) (lower solid curve), and enzyme activity (—o— —o—) as functions of the level in the column. The enzyme activity was measured on 0.75 ml fractions, taken from a column after isoelectric focusing. The background UV absorbancy of about 0.15, which can be seen on the UV absorption curve, is due to the carrier ampholytes.

and a more accurate isoelectric point of 5.89 could be derived from the experiment. According to arguments presented by Vesterberg and Svensson,[5] this should also be considered as the isoionic point of carbonic anhydrase. It is to be compared with the value 5.65 found by Nilsson and Lindskog [4] by extensive dialysis against water and subsequent removal of dissolved carbon dioxide.

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Glucoaubrietin, A New Crystalline Glucosinolate*

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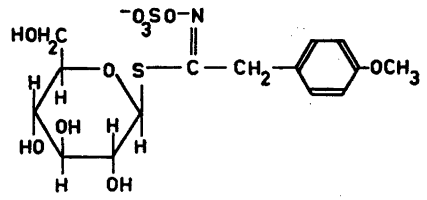
In a previous communication of this series,[1] the natural occurrence of a glucosinolate (glucoaubrietin), affording *p*-methoxybenzyl isothiocyanate on enzymatic hydrolysis, was demonstrated; no efforts were made at that time, however, to isolate the parent compound. We now wish to report the isolation of the latter, (1), in the form of the crystalline tetramethylammonium salt.



(1)

In the preceding study [1] the occurrence of (1) in a number of species of the crucifer genus *Aubrietia* was demonstrated by chromatographic methods. In the present isolation, seed material of a cultivated species of *Aubrietia* * was employed.

The glucosinolate was isolated by the usual procedure involving grinding of the seed, defatting, extraction with 70 % methanol, ion-exchange on anionotropic alumina, and elution with tetramethylammonium hydroxide. It crystallized from 85 % ethanol as colourless needles, and an analytical specimen, m.p. 181°, possessed the expected composition ($(CH_3)_4N^+$-salt of (1)), UV- (*cf.* Ref. 1) and IR-data. The rotation, $[\alpha]_D^{27}$ −19°, similar in sign and magnitude to that of several other glucosinolates (*cf.* Ref. 2), signifies its character as a β-thioglucoside. In agreement with the structure (1), acid hydrolysis afforded, *inter alia*, hydroxylamine and glucose, whereas enzymatic hydrolysis resulted in the production of *p*-methoxybenzyl isothiocyanate, characterized, upon reaction with ammonia, as the previously described *p*-methoxy-benzylthiourea.[1]

Upon exchange of potassium for $(CH_3)_4N^+$-ions, and subsequent acetylation, potassium *p*-methoxybenzylglucosinolate tetraacetate was obtained as a nicely crystalline monohydrate.

* Part LIX of a series of papers on isothiocyanates and their natural progenitors. Part LVIII: *Phytochem.* **7** (1968). *In press.*

* Many cultivated varieties of *Aubrietia* afford popular flower garden subjects. The seed employed in the present investigation was commercially obtained as '*Aubrieta cultorum* Graeca' from the seed company Ernst Benary, 351 Hann. Münden, West Germany.