An ion-exchange chromatographic technique for the purification of renin has been described. The specific activity of the preparation which was applied to the column was 4.3. The eluted renin had a specific activity of 43.2. This constitutes a 10-fold purification of renin by use of cellulose ion-exchange chromatography, with a recovery of 85 % of the enzyme activity.

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A simplified method for the purification of mushroom polyphenol oxidase

Purification of mushroom polyphenol oxidase has been sought in this laboratory in order to pursue structural studies on this enzyme[1] and to explore the role of tyrosine in the secondary and tertiary structure of proteins[2–5]. While this work was in progress, KERTESZ AND ZITO[6] reported the preparation of homogeneous mushroom polyphenol oxidase. Taking advantage of the excellent early steps of this procedure*, we have used chromatography on diethylaminoethyl-cellulose (DEAE-cellulose)[7] as the basis for a highly simplified method to prepare the purified enzyme in good yield. A similar chromatographic procedure for the preparation of a soluble mammalian tyrosinase was also recently reported by BROWN AND WARD[8].

The yields and steps in the method are summarized in Table I and in the experimental section. After extraction and preliminary purification through Step 4, the crude enzyme is prepared for direct chromatography by an extensive dialysis

* We are very grateful to Professor D. KERTESZ for details of his method in advance of publication.

(Step 5, including dialysis against 1.0 M NaCl) which removes $^5/_6$ of the nitrogen and $^3/_4$ of the u.v.-absorbing material. In Step 6, illustrated in Fig. 1, the crude dialyzed enzyme is adsorbed on DEAE-cellulose and selectively eluted with 0.080 M phosphate buffer, pH 8, after an extensive wash with 0.040 M phosphate buffer, pH 8. The elution pattern corresponds, at least qualitatively, with that of BROWN AND WARD[7] for crude mammalian tyrosinase.

TABLE I

ISOLATION OF MUSHROOM POLYPHENOL OXIDASE

1 kg frozen mushrooms used. Polyphenol oxidase activity determined essentially as described by ASHRAF AND FRIEDEN[4]. A unit is defined as the change in absorbance at 265 mμ produced in the first 2 min at 25° in a 3.0 ml reaction vol. containing $3.3 \cdot 10^{-4}$ M catechol, $6.7 \cdot 10^{-5}$ M ascorbate, $2 \cdot 10^{-5}$ M ethylenediaminetetraacetic acid, and 0.050 M phosphate buffer, pH 7.1.

Step No.	*Vol. (ml)*	*Activity ml (10^3 units/ml)*	*Total activity (10^3 units)*	*Nitrogen (mg/ml)*	*Absorbance* ($A_{280\ m\mu}$)*	*Specific activity (units/ml $A_{280\ m\mu}$)*	*Yield (%)*
(1, 2)	200	1.52	304	3.6	90.3	15.3	100
(3, 4)	230	1.22	281	2.1	51.0	23.9	92
(5)	250	1.06	266	0.31	18.5	57.3	88
(6)	36	2.90	104	—	1.10	2650	34
(7)	1.50	59.2	89	—	15.5	3800	29

* It is recognized that the absorbance at 280 mμ is strongly affected by chromogenic impurities at the early stages of isolation as suggested by EIGER AND DAWSON[8]. This chromogenic material is eliminated by chromatography.

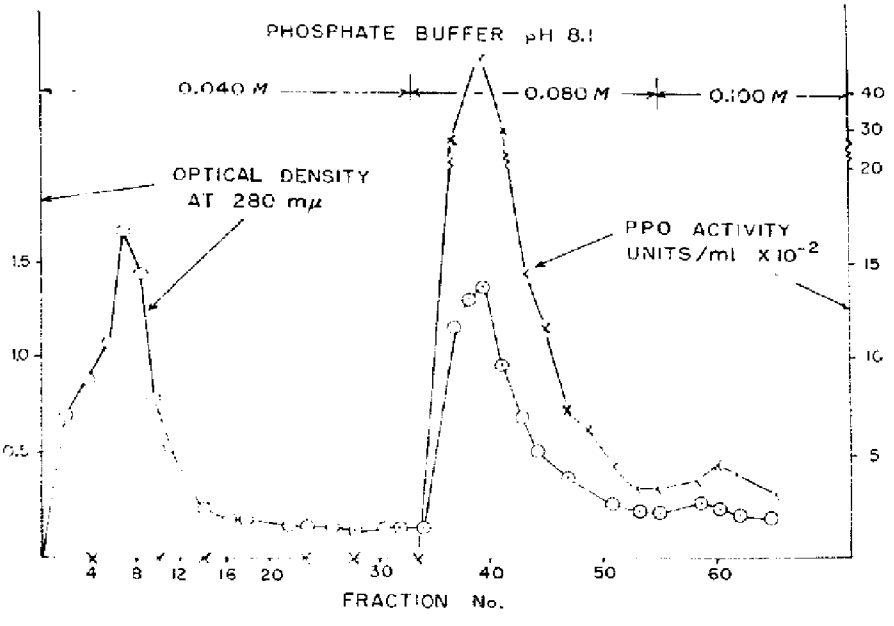

Fig. 1. Chromatography of polyphenol oxidase from Step 5. In this experiment, 135,000 units were chromatographed on DEAE-cellulose and eluted as indicated at 5°. Enzyme (PPO) activity was determined as described in Table I. The vol. of each fraction was 3.0 ± 0.5 ml.

Fractions with high specific activity obtained from several chromatograms were concentrated by precipitation with 50% $(NH_4)_2SO_4$ and examined in the ultra-

centrifuge. As shown in Fig. 2, two peaks were obtained, but the larger, faster peak, comprising 90 % of the total area, had over 99 % of the polyphenol oxidase activity as indicated in an additional experiment in a separation cell. This major component has an S_{20} of $7.3 \cdot 10^{-13}$, when extrapolated to zero concentration*. For the preparation of a completely homogeneous enzyme, more selective use of chromatographic fractions or the $(NH_4)_2SO_4$-fractionation procedure of KERTESZ AND ZITO[5] may be used.

Fig. 2. Sedimentation pattern of polyphenol oxidase in 0.050 *M* Na_2HPO_4. The enzyme solution had an absorbance at 280 mμ of 15.5 and an activity of 59,200 units/ml. Temp. 20°, rotor speed, 59,780 rev./min. Exposure times 0, 16, 32, 48 and 82 min.

Experimental

The first four steps correspond to those of KERTESZ AND ZITO[5] and will be described elsewhere.

Step 1. An acetone powder of frozen mushrooms is prepared as rapidly as possible at −10° to −20°. The 30 % acetone extract is saved and the residue discarded.

Step 2. The polyphenol oxidase is precipitated from the 30 % acetone extract with 1.5–2 vol. acetone at −20°. The washed solid is dissolved using 200 ml water/kg mushroom. This solution may be stored frozen or lyophilized.

Step 3. The aqueous solution is made up to 0.48 % in calcium acetate, frozen overnight, thawed, and the insoluble material discarded after centrifugation.

Step 4. The supernatant from Step 3 is made up to 0.95 % in calcium acetate, frozen overnight, thawed, and the insoluble material discarded after centrifugation.

Step 5. The supernatant from Step 4 is transferred to a large dialysis bag and dialysed in the cold with stirring as follows (for each solution): twice overnight against 10 l 0.001 *M* Na_2HPO_4, twice over two nights against 0.005 *M* Na_2HPO_4, 1.0 *M* NaCl, and finally twice overnight against 0.005 *M* Na_2HPO_4. (The data listed in Table I for step 5 were obtained at this point.) Sufficient $(NH_4)_2SO_4$ is added to 35 % saturation and the solid discarded after standing overnight in the cold. The material insoluble at 50 % saturation with $(NH_4)_2SO_4$ is centrifuged down, dissolved in a minimum volume of water, and dialysed against several changes of 0.010 *M* phosphate buffer, pH 8.

Step 6. The concentrated polyphenol oxidase preparation (using about 200,000 units for a 1.0-cm diameter column) is placed on a column of DEAE-cellulose (about 2.0 g for a 1.0-cm diameter, 10-cm high column). The dark material remains at the top of the column throughout elution, but does cause a reduced flow rate. The column is next subjected to an extensive wash with 0.040 *M* phosphate buffer, pH 8.1. 100 ml

* In a private communication, Professor KERTESZ has reported that a subsequent preparation of polyphenol oxidase in his laboratory had a sedimentation coefficient of $6.2 \cdot 10^{-13}$, when extrapolated to zero concentration.

decreases the absorbance of the effluent to less than 0.2 after a large peak with no polyphenol oxidase activity is eluted with the first 50 ml. Most of the activity (up to 50 %) is eluted with 0.080 *M* phosphate buffer, pH 8, as a colorless peak which trails off after the first 50 ml. A small additional elution is obtained with 0.10 *M* phosphate buffer, pH 8.

Step 7. The fractions containing a specific activity greater than 2000 units/ml/absorbance at 280 mμ are pooled and treated with $(NH_4)_2SO_4$, discarding the fraction insoluble at 35 % saturation and saving that insoluble at 50 % saturation. The solid is taken up in about 1.5 ml water and dialysed free of $(NH_4)_2SO_4$ against 0.005 *M* Na_2HPO_4. This solution may be used directly for studies in the ultracentrifuge.

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It has been subsequently observed that extremely slow elution can result in polyphenol oxidase preparations of even higher specific activity than reported here. A further complication arises from the finding of an inhibitor of this enzyme which accompanies it and appears in the 0.04 *M* phosphate eluate on chromatography (E. FRIEDEN AND Y. KARKHANIS, unpublished data).

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A method of desalting certain polypeptides

Corticotropin is an example of a polypeptide that has given difficulty in desalting because it can pass through cellophan membranes on dialysis. Extraction into organic solvents, precipitation, dialysis under special conditions and adsorption from solution have all been used to recover it in a salt-free state from buffer solutions, but each procedure has disadvantages and most are laborious. DIXON AND STACK-DUNNE[1] used the adsorption of corticotropin onto a carboxylic resin, but they displaced the corticotropin with alkali (ammonia) which converted some of the corticotropin A_1