A rapid lipase purification

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Pancreatic lipase (glycerol ester hydrolase, EC 3.1.1.3) has been used in the investigation of the distribution of fatty acids in natural fats[1-3]. This investigation requires a preparation exhibiting the characteristic positional specificity of this enzyme, but a very high specific activity is not necessary. We have found that several commercial preparations are not satisfactory for these studies, but lipase purified by the method of DESNUELLE *et al.*[4] to the end of Stage 2 meets these requirements; and we have employed this material in previous investigations[3,5]. However, this method involves high-speed centrifugation, which limits the amount of material which may be processed at a time. We have modified this purification procedure, so that larger amounts of material may be handled at one time.

An alumina gel was first prepared by dissolving 161 g of $Al_2(SO_4)_3 \cdot 16H_2O$ in 2 l of warm water, and adding 50 g of $(NH_4)_2SO_4$, also dissolved in 2 l of warm water; 150 ml of NH_4OH (sp. gr. 0.880) were added, and water to a total volume of 5 l. After settling, sufficient (3 ml) of the NH_4OH was added until no further precipitation occurred. The alumina was then washed six times by decantation, suspended in 1330 ml of citrate buffer (pH 5)[6] and stored for at least 3 months at 2°.

An acetone powder was prepared in the following way. Fresh pork pancreas, removed immediately after slaughter, was frozen with solid CO_2. This material (800 g) was broken into small lumps, and the fat removed by hand. It was macerated briefly (2–3 min) in an 'Atomix' in 200-g portions, with 200 ml of acetone for each portion. Each macerate was centrifuged for 5 min at 1500 rev./min and the supernatant discarded. The combined residues (approx. 1 l) were stored overnight at 0°. The material was then subjected to two extractions with 800-ml portions of acetone, two of acetone–ether (1 : 1, v/v), and two of ether, and the residue was dried in air. The yield at this stage was approx. 100 g; and after sieving to remove fibrous material, 65 g of a fine powder were obtained.

This powder was extracted with 300 ml of ice-water for 30 min with gentle stirring, and the suspension centrifuged at 3000 rev./min and 0° for 20 min. The supernatant was stored at 0°, whilst the centrifugate was re-extracted with a further 300 ml of ice-water. The combined supernatants amounted to 500 ml.

The previously aged alumina gel (183 ml) was diluted to 500 ml with citrate buffer (pH 5) and this suspension was added to the combined supernatants. After standing for 5–10 min the mixture was centrifuged at 5000 rev./min and 0° for 30 min and the centrifugate discarded. To the 855 ml of supernatant, 690 ml of ice-cold, satd. $(NH_4)_2SO_4$ solution were added, slowly, with stirring. The precipitate was collected by centrifuging for 10 min at 5000 rev./min and 0°. This was suspended in 60 ml of citrate buffer (pH 5) and dialysed for 48 h at 2° against the same buffer. When the dialysate was freeze-dried, 9 g of the soluble lipase preparation were obtained.

The total amount of lipase activity extracted in this way was the same as that obtained by the method of DESNUELLE[4]; but the specific activity was lower (75%). However, for use in the study of natural fats the lower specific activity is of little consequence. For this purpose, the positional specificity is of paramount importance. When a synthetic specimen of 2-oleodistearin was hydrolysed with 50 mg of this preparation, the monoglycerides produced contained 98% oleic acid. The 2% stearic

acid present may well have resulted from isomeric 1-oleodistearin present as impurity in the original 2-oleodistearin. Hence the positional specificity of this preparation is very high, if not indeed absolute. The advantage of this modification lies in the elimination of high-speed centrifugation, and this makes it possible to prepare eight times as much material in a single batch as the amount prepared by the original method.

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Human-erythrocyte reduced triphosphopyridine nucleotide oxidase

TPNH oxidase which is present in the mature human erythrocyte has been purified by KIESE *et al.*[1,2] and HUENNEKENS *et al.*[3,4]. The enzyme studied by HUENNEKENS *et al.*[3,4] was found to be devoid of flavin at all stages of its purification, could utilize as terminal acceptors either oxygen, methemoglobin, or cytochrome *c*, and by spectrophotometric examination contained a rather large Soret peak at 406 mμ. As a result of their investigations HUENNEKENS *et al.* proposed that an iron-porphyrin moiety served as a prosthetic group for the enzyme.

As part of a study of the metabolism of the mature human erythrocyte, we have had occasion to purify this enzyme according to the method of HUENNEKENS *et al.*[3]. Starting from an acetone-powder extract prepared from 200 ml of packed washed human erythrocytes, the enzyme was carried through an ethanol chloroform fractionation, lyophilization, fractionation at pH 5.4 and ammonium sulfate fractionation. In accordance with the findings of HUENNEKENS *et al.* a 180-fold purification was achieved. Further purification was attained by use of DEAE-cellulose chromatography. The ammonium sulfate fraction with the highest specific activity, which in our hands precipitated between 70–80% saturation, was dialyzed against 0.005 M potassium phosphate buffer at pH 7.0 and then placed on a DEAE-cellulose column which had been equilibrated against the same buffer. One active fraction was eluted with 0.005 M potassium phosphate buffer at pH 7.0, while another more active fraction was eluted with 0.02 M potassium phosphate at pH 7.0. The fraction eluted with the higher concentration of potassium phosphate buffer showed as 5–10-fold increase in specific activity over that of the previous step and resulted in an