corresponding to [^{32}P]ITP or [^{32}P]GTP, which would be expected to occur if these were precursors of [^{32}P]PEP. The stimulation of endogenous formation of PEP by uncouplers is reminiscent of the observation of MUDGE *et al*[3] that in liver mitochondria 2,4-dinitrophenol stimulated PEP formation in the presence of α-ketoglutarate or its immediate precursors but not in the presence of fumarate, malate, or oxaloacetate. The stimulation of PEP formation appears to be more related to the specific action of 2,4-dinitrophenol and pentachlorophenol rather than to the result of uncoupling of endogenous oxidative phosphorylation, since Ca^{++}, which is a potent uncoupler in intact mitochondria, did not exhibit the effect characteristic of the substituted phenols.

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A new approach to the separation and purification of peptides

The isolation of pure peptides is an important step in the study of protein structure. Mixtures of peptides are often difficult to fractionate because they display only small differences in physico-chemical properties. This communication describes a new approach to the chromatographic fractionation of peptides which is based on small differences in the firmness of binding by the free-base form of cellulosic anion exchangers.

The general procedure is simple and rapid. Peptides in a minimal volume of distilled water are added to a cellulosic anion-exchange column[1,2]. The chromatogram is developed with distilled water followed by distilled water saturated with 1 atm. CO_2 (ref. 3). Certain peptides are retarded only slightly and are eluted with the distilled water. Those which are bound more firmly are eluted with the CO_2 solution*.

Abbreviation: DEAE-, diethylaminoethyl-.

* It would be expected that peptides containing dicarboxylic amino acids would be bound very strongly. This was the case with L-Glu-L-Phe, which was not eluted with CO_2 solutions. On the other hand, the tetrapeptide L-Glu-L-His-L-Phe-L-Arg was eluted with CO_2 solutions.

In general, amino acids are not retarded appreciably by the exchanger*, and therefore are separated ahead of the peptides.

A commercial sample of "chromatographically pure" L-Leu-L-Tyr subjected to the procedure was separated from approx. 10 mole-% tyrosine with which it was contaminated (Fig. 1A)**. D-Leu-L-Tyr, from another source, showed no evidence of tyrosine. A mixture of these two diastereo-isomers was separated on DEAE-cellulose[1], as shown in Fig. 1B. BLACKBURN AND TETLEY[5] reported this separation on Dowex-50, using citrate buffer as eluent, but the present procedure is simpler, provides better separation, and yields salt-free preparations.

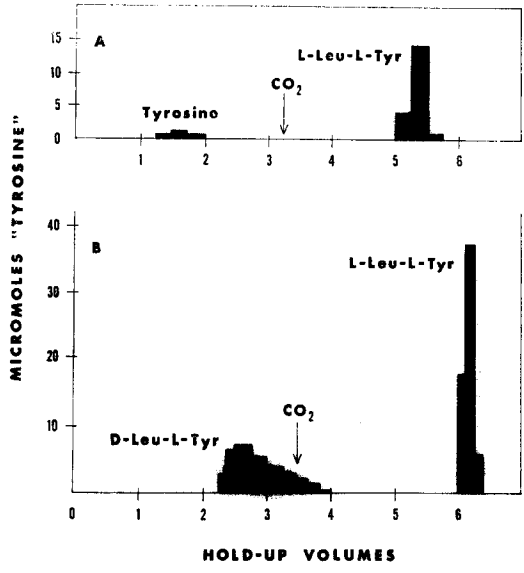

Fig. 1A. Purification of L-Leu-L-Tyr. 20 μmoles of a L-Leu-L-Tyr preparation in 2.0 ml water were added to a DEAE-cellulose column, 37.5 × 0.7 cm. The flow rate was 0.43 ml/min; the fraction volume 3.0 ml; and the hold-up volume, 12 ml.
Fig. 1B. Separation of D-Leu-L-Tyr and L-Leu-L-Tyr. 64.5 μmoles of each diastereoisomer in 5.85 ml water were added to a DEAE-cellulose column 44.5 × 1.1 cm. The flow rate was 1.1 ml/min; the fraction volume, 5.0 ml; and the held-up volume, 40 ml.

The separation of glycine from the di- and tripeptides of glycine on DEAE-cellulose is shown in Fig. 2. Similar separation of glycine and glycylglycine was obtained with Ecteola-cellulose[2].

The binding of a dipolar ion by the free-base form of DEAE-cellulose evidently depends upon the equilibrium between the tertiary amine of the exchanger and the amino group of the dipolar ion.

$$\underset{\text{not bound}}{\overset{+}{H_3N}\text{–R–COO}^-} + R'R''_2N \rightleftarrows \underset{\text{bound}}{H_2N\text{–R–COO}^-R'R''_2NH^+}$$

The relative basicities of the two amines determine the position of this equilibrium

* On a DEAE-cellulose column, asparagine is retarded slightly, cysteine is held to a greater degree, and dicarboxylic amino acids are strongly bound.
** Tyrosine and its derivatives were identified by u.v. spectrophotometry, polarimetry, paper chromatography, and the characteristic ninhydrin reaction rates and maximal color yields[4].

which, in turn, determines the degree of binding of the dipolar ion. Studies indicate that the degree of binding of dipolar ions, in general, increases with decreasing basicities of their amino groups. Dipolar ions may be classified into four categories with respect to their relative ease of elution from DEAE-cellulose: (1) those which are not retarded, (2) those which are retarded, but are eluted with distilled water, (3) those which are eluted by a CO_2 solution, and (4) those which are not eluted with CO_2 solutions. The first three of these categories are represented in Fig. 2.

Certain strong anion exchangers, *e.g.*, the methyl quaternary of DEAE-cellulose[6], may be suitable for the chromatography of those dipolar ions which are too readily

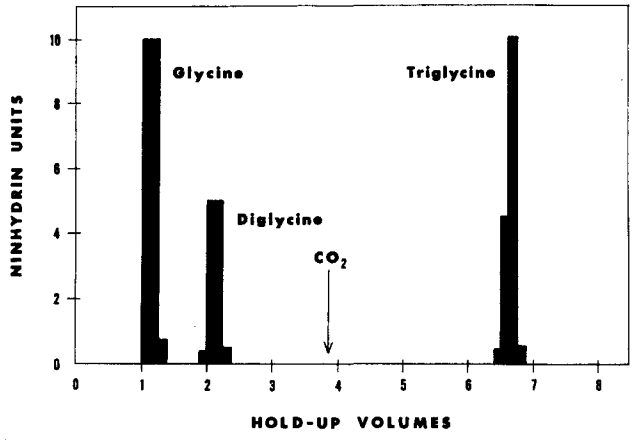

Fig. 2. The separation of glycine, diglycine, and triglycine. 25 μmoles of each compound were mixed in 1.5 ml water and added to a DEAE-cellulose column, 83 × 0.7 cm. The flow rate was 0.15 ml/min; the fraction volume, 3.0 ml; and the hold-up volume, 26 ml. (Glycine and its derivatives were chromatographed singly in order to identify the components in this chromatogram.)

eluted from DEAE-cellulose for adequate separation, whereas weak anion exchangers, *e.g.*, *p*-aminobenzylcellulose[7], may be used for those which are not eluted from DEAE-cellulose with CO_2 solutions.

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