The Determination of Free Amino Acids in Blood Plasma

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The importance of the determination of free amino acids and amines in small quantities of blood plasma is evident, e.g. in cases of inborn errors of metabolism. In several routine methods the proteins of the blood plasma are precipitated and subsequently the free amino acids are determined in the remaining solution. The disadvantages of precipitation techniques may be losses of amino acids due to inclusion or absorption or both. In an attempt to avoid these complications we decided to apply a method (Tommel, Vliegenthart, Penders & Arens, 1968) recently described for the separation of α -amino acids and peptides. The latter separation procedure utilizes the difference in charge of the cupric chelates of peptides and α -amino acids respectively.

In anion-exchange chromatography at pH 8.0 the peptide chelates are retarded on the column; the amino acid chelates, however, are eluted. The experiment was performed as follows. To 1 ml. of human blood 0.2 mg. of heparin was added. (Citrate cannot be applied, as it interferes during the anion-exchange chromatography.) The blood plasma was obtained by centrifugation. Plasma (250 μ l.) was diluted with an equal volume of 0.01 M-2,4,6-collidine. Thereafter 50 mg. of cupric carbonate was added and the mixture was magnetically stirred for 2 hr. at room temperature. After removal of the excess of cupric carbonate by centrifugation the blue supernatant was applied to a column (10 cm. $\times$ 0.6 cm.) of TEAE-cellulose in the acetate form, which was equilibrated with 0.01 M-2,4,6-collidine-acetate buffer, pH 8.0. The column was eluted with the 2,4,6-collidine-acetate buffer. The first 7 ml. of eluate, containing the amino acid chelates, was collected in bulk. Cu^{2+} was removed from the eluate by extraction with a solution of 8-hydroxyquinoline in chloroform (concentration 6 mg./ml.). Subsequently, the eluate was washed with chloroform to avoid a possible contamination with 8-hydroxyquinoline. In the remaining solution the amino acids can be quantitatively determined, e.g. by paper chromatography or by means of an automatic amino acid analyser. It is noteworthy that aspartic acid and glutamic acid remained partially on the column with the proteins. The content of these amino acids in plasma could not be determined. The amino acid fraction turned out to be completely free of proteins and of peptides.

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The Biological Half-life of Pulmonary Surface-Active Agent

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Pulmonary surface-active agent is remarkable for the large amount of highly saturated (mainly dipalmitoyl) phosphatidylcholine that it contains (Abrams, 1966; Tierney, Clements & Trahan, 1967). To try and measure its turnover rate and to determine the possible synthetic pathway of this phosphatidylcholine, the incorporation of [U^{14}C]-glucose into pulmonary surface-active agent has been studied. [U^{14}C]Glucose was injected intravenously into New Zealand White rabbits (wt. 2.5–3 kg.), and the animals were killed with intravenous pentobarbital at various times up to 116 hr. The lungs were removed rapidly and used (a) for extraction of surface-active agent (Abrams, 1966) and (b) after homogenization in chloroform-methanol (2:1, v/v), for extraction of total lipid (Folch, Lees & Sloane-Stanley, 1957). The lipid extracts were further fractionated by column chromatography on silicic acid and thin-layer chromatography (silica gel G or H). The ^{14}C content and weight of the various fractions were determined and specific radioactivities were calculated. The results indicated a biological half-life for the phosphatidylcholine fraction of about 28 hr. in surface-active lipid and about 35 hr. in total lung lipid. Some of the non-phospholipid constituents of the total lung lipid were also examined and the following biological half-lives found: 1,2-diglycerides, 40 hr.; 1,3-diglycerides, 70 hr.; triglycerides, 35 hr.; free fatty acids, 40 hr. A phospholipid present in surface-active agent in small amount (approx. 3% of the total phospholipid) was found to have a high specific radioactivity and a half-life of about 15 hr. This phospholipid has been tentatively identified as one of the lysobisphosphatidic acid compounds described by Body & Gray (1967) and thus lends support to their suggestion that 'these phospholipids are metabolically "highly active" and are acting as "carriers" for the palmitic acid at some stage in a reaction sequence involving lung lecithin, lipolytic enzymes and acyl CoA-acyltransferases, the end product being dipalmityllecithin.'

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Influence of Metal Ions on the Orthophosphatase and Inorganic Pyrophosphatase Activities of Human Alkaline Phosphatase

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It is now recognized that mammalian alkaline phosphatases (EC 3.1.3.1) are able to hydrolyse both pyrophosphate and orthophosphate substrates (Cox & Griffin, 1965; Moss, Eaton, Smith & Whitby, 1967; Fernley & Walker, 1967), and it seems probable that a single type of active centre is responsible for activity towards both classes of substrate (Eaton & Moss, 1967a). However, Mg^{2+} ions have a differential effect on the two activities of alkaline phosphatase, activating the hydrolysis of orthophosphates but inhibiting that of pyrophosphates (Fernley & Walker, 1967; Eaton & Moss, 1967a,b). The influence of Zn^{2+} and Mg^{2+} ions on the activities of purified human intestinal alkaline phosphatase (Moss *et al.* 1967) has now been examined in more detail, and the results indicate that the differential effects of these ions are consistent with the suggestion that both catalytic activities are properties of a single type of active centre.

Both the inorganic pyrophosphatase and orthophosphatase (substrate, *p*-nitrophenyl phosphate) activities of the enzyme were measured at pH 9.0 in tris-HCl buffer; at this pH both substrates are hydrolysed at measurable rates over a wide range of concentration. Addition of Zn^{2+} ions to the pyrophosphatase assay system almost completely inhibits release of inorganic phosphate when the concentration of Zn^{2+} is greater than that of the substrate, inorganic pyrophosphate. As the concentration of pyrophosphate is increased above that of Zn^{2+} , competitive inhibition of the pyrophosphatase activity is seen. These observations can be explained by assuming that, when Zn^{2+} ions are in excess, all the substrate is in the form of a Zn^{2+} -pyrophosphate complex which is not broken down by the enzyme, or is only slowly attacked. As the substrate concentration is increased free pyrophosphate appears and is hydrolysed, but the Zn^{2+} -pyrophosphate complex is a competitive inhibitor of this hydrolysis. The Zn^{2+} -pyrophosphate complex is also a competitive inhibitor of the orthophosphatase activity when Zn^{2+} and pyrophosphate are added to this assay system in proportions calculated to produce the complex.

A similar explanation seems to be valid for the effect of Mg^{2+} ions on the pyrophosphatase activity of alkaline phosphatase. However, the inhibition of pyrophosphate hydrolysis by the Mg^{2+} -pyrophosphate complex is less marked than that by the Zn^{2+} complex, and the stoichiometry of the interactions of the two metal ions with pyrophosphate appears to be different.

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The Role of Endogenous Cations in the Na^+ - K^+ - Mg^{2+} -Effected Adenosine Triphosphatase of Cerebral Microsomes

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Hydrolysis of [^{32}P]ATP by cerebral microsomes is associated with a Na^+ -dependent and K^+ -inhibited binding of ^{32}P to a protein component in the membrane (Rodnight, Hems & Lavin, 1966, and references cited therein). Assuming that the Na^+ - K^+ - Mg^{2+} -effected ATPase* proceeds by a multistage reaction, the action of K^+ can be considered as one of increasing the turnover of a phosphorylated intermediate, this resulting in a lower steady-state content of intermediate when ATP is limited. With $2.5\mu M$ -ATP and twice-washed microsomes, however, appreciable Na^+ -dependent hydrolysis of ATP occurs in the absence of added K^+ and Mg^{2+} , the steady-state content of bound ^{32}P declining to zero as hydrolysis approaches completion. This suggests that sufficient endogenous K^+ and Mg^{2+} are present to permit the Na^+ - K^+ - Mg^{2+} -effected ATPase to hydrolyse very low concentrations of ATP.

The above observations were made with a microsomal preparation prepared by a modification of an earlier method (Rodnight & Lavin, 1966) involving several centrifugal washes of the crude microsomes to remove free cations. The preparation contained ($m\mu$ moles/mg. of protein): Mg^{2+} , 20 ± 2 ; Na^+ , 90 ± 20 ; K^+ , 27 ± 3 . By further washing with EDTA-containing buffers these levels have now been decreased to: Mg^{2+} , 3 ± 1 ; Na^+ , 24 ± 12 ; K^+ , 7 ± 2 . The exhaustively washed preparations showed a definitive requirement for Mg^{2+} for full phosphorylation, but turnover of bound ^{32}P still continued, presumably due to the residual bound

* Abbreviation: ATPase, adenosine triphosphatase.