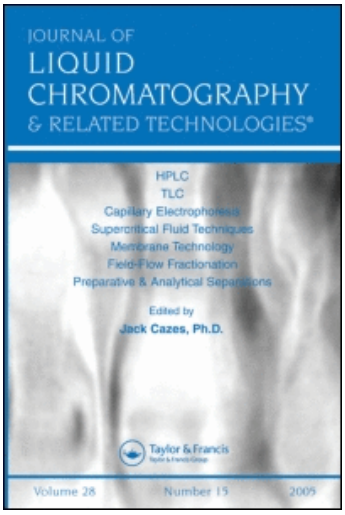

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Assessment of the Plasma-Induced Changes under Acidic Conditions in the Apparent Molecular Volume of β-Endorphin, β-Lipotropin and γ-Lipotropin by Gel Permeation and Reversed Phase High Performance Liquid Chromatography

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ASSESSMENT OF THE PLASMA-INDUCED CHANGES UNDER ACIDIC CONDITIONS IN THE APPARENT MOLECULAR VOLUME OF β-ENDORPHIN, β-LIPOTROPIN AND γ-LIPOTROPIN BY GEL PERMEATION AND REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY.

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SUMMARY

Addition of radiolabeled β-endorphin, β-lipotropin and γ-lipotropin to plasma at acid pH results in an apparent reduction in size of these molecules as evidenced by gel permeation chromatography. These acid plasma-treated molecules, however, are indistinguishable from the untreated radiolabeled polypeptides when subjected to high performance liquid chromatographic separations suggesting no differences in molecular composition. As these apparent changes in polypeptide molecular volume are prevented by addition of Trasylol or sodium azide to the plasma, a likely explanation would appear to be an enzyme-dependent production of anionic lipids in plasma which at acid pH bind to the lipotropins and endorphins reducing their molar volume.

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INTRODUCTION

Recent investigations have established that β-lipotropin (βLPH), γ-lipotropin (γLPH) and β-endorphin (βEP) are present in the peripheral circulation in man (1,2,3) and that they are secreted into the circulation from the pituitary gland. βLPH, a 91 amino acid residue polypeptide contains within its structure the sequences of γLPH (βLPH-(1-58)), β-melanotropin (βMSH) (βLPH-(41-58)), βEP (βLPH-(61-91)), γ-endorphin (γEP) (βLPH-(61-77)) and α-endorphin (αEP) (βLPH-(61-76)). Consequently, it has been suggested that βLPH may serve as a prohormone for some or all of these peptides (4). In the course of experiments designed to separate βLPH and βEP in plasma by chromatographic techniques, we noted that acidification of the plasma caused these peptides to elute at positions corresponding with smaller apparent molecular size than when the same peptides were treated and chromatographed under neutral conditions. The present study was undertaken to investigate these observations and establish the possible role of fragmentation or deamidation processess.

MATERIALS AND METHODS

Synthetic human βEP, αEP and γEP were purchased from UCB Bioproducts (Brussels, Belgium). γLPH and βLPH were kindly supplied by Dr. P. Lowry (St. Bartholomew's Hospital, London, U.K.) and by Dr. C.H. Li (Hormone Research Laboratory,

San Francisco, Ca., U.S.A.) respectively. βMSH was donated by CIBA-Geigy through the U.S.A. National Pituitary Agency (NIAMDD).

Preparation of Radiolabeled Peptides

All peptides were labeled with Na ^{125}I using chloramine T according to the method of Hunter & Greenwood (5). Purification was performed using columns packed with Sephadex G75 for βLPH, G50 for γLPH, βEP and βMSH and Sphadex G25 for αEP and γEP.

Gel Filtration

Sephadex G50 Fine columns (1.3 x 70cm) were pre-equilibrated with either 0.05M phosphate buffer (pH 7.4) or 0.1N acetic acid (pH 2.8). Both eluants contained 0.1% bovine serum albumin (fraction V, Sigma Chemical Co., St. Louis, Mo., U.S.A.). Purified radiolabeled peptides (500,000 cpm) and Na ^{125}I (100,000 cpm) were added to 1ml aliquots of either human plasma or eluant, incubated at 20° for 5 min before loading onto the column, and eluted from the columns at a flow rate of 10ml h^{-1} at 4°. Fractions (1ml) were collected and peaks of radioactivity pooled and lyophilised prior to analysis using high performance liquid chromatographic techniques (HPLC). In those experiments investigating the effects of enzyme inhibitors, Trasylol (Aprotinin, Bayer Pharmaceutical Co. N.S.W., Australia; 1000 KIU ml^{-1}), or sodium azide (0.018%) were added to the plasma and the

eluant prior to addition of the radiolabeled polypeptides.

High Pressure Liquid Chromatography

The HPLC techniques used in this study were based on procedures previously described by Hearn (6) and Hearn and Grego (7). In brief, radiolabeled peptide standards and plasma-treated peptides were chromatographed on a μBondapak C_{18} column (30 x 0.4cm I.D., 10um) using a 60 min linear gradient generated from aqueous 15mM orthophosphoric acid and acetonitrile (0 → 50% v/v) at a flow rate of 2ml min^{-1}. Fractions were collected every 30 sec.

RESULTS

Figure 1(a) shows the elution profiles for radiolabeled βLPH, γLPH, and βEP chromatographed on Sephadex G50 under varying conditions. Treatment of these peptides with plasma at neutral pH resulted in no significant change in the elution profiles when compared with those chromatograms obtained running the peptides in the absence of plasma at both pH 7.4 and 2.8. In contrast, when plasma-treated radiolabeled βLPH, γLPH and βEP were chromatographed under acid conditions, there was an increase in elution volume of the radioactive peak indicating that the labeled moiety was now contained in molecules of apparently smaller size. These apparently "smaller" peptides eluted in a similar position to that

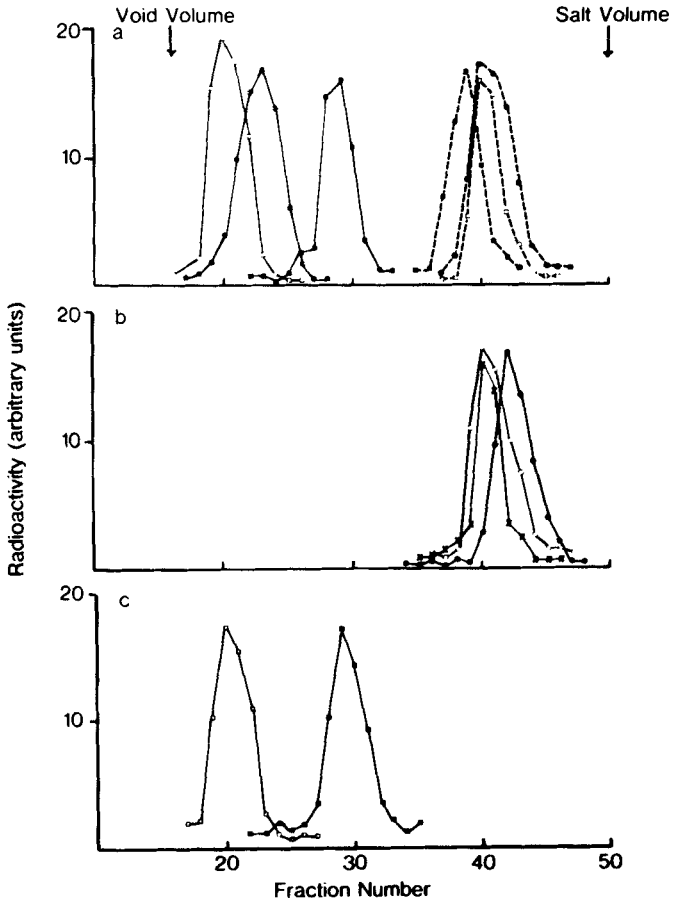

Figure 1. Gel filtration of radiolabeled polypeptides on Sephadex G50. See text for experimental details.

a. Radiolabeled polypeptides eluted + plasma, pH 7.4 or 2.8.

_____ Elution position of polypeptides prior to acid plasma treatment.

----- Elution position of polypeptides after acid plasma treatment.

b. Radiolabeled polypeptides eluted ± plasma, pH 7.4 or 2.8.

c. Radiolabeled polypeptides eluted + plasma, pH 2.8 with Trasylol or sodium azide present.

X βMSH; ● αEP; ○ γEP; ■ βEP; □ βLPH; ⊕ γLPH

found for the peptides βMSH, αEP and γEP (Fig. 1,b). However, The elution volume of these latter peptides (as well as radiolabeled insulin) was unaffected when the samples were treated with acid plasma.

Addition of the enzyme inhibitors Trasylol or sodium azide to the plasma and eluant prevented the apparent change in elution volume for βLPH and βEP when the peptides were run in acid plasma (Fig. 1,c). Furthermore, collection of the eluted βEP peak after an acid plasma run and subsequent rechromatography under neutral conditions resulted in the peak of radioactivity eluting in the same position as observed in the absence of plasma with eluants at either pH 7.4 or 2.8. This suggests that the observed change is freely reversible.

Following gel chromatography, recovered peaks of radioactivity were analysed by HPLC and the resulting chromatograms shown in Fig. 2. It should be noted that radiolabeled βLPH chromatographed as two peaks, βLPH and βLPH', an unexplained observation previously noted (M.T.W.Hearn, unpublished results). A similar result was obtained using a different batch of βLPH supplied by Dr. P. Lowry. As shown in the chromatogram, acid plasma-treated βLPH and βEP eluted in positions coincident with those of untreated βLPH and βEP respectively indicating no change in composition of these polypeptides.

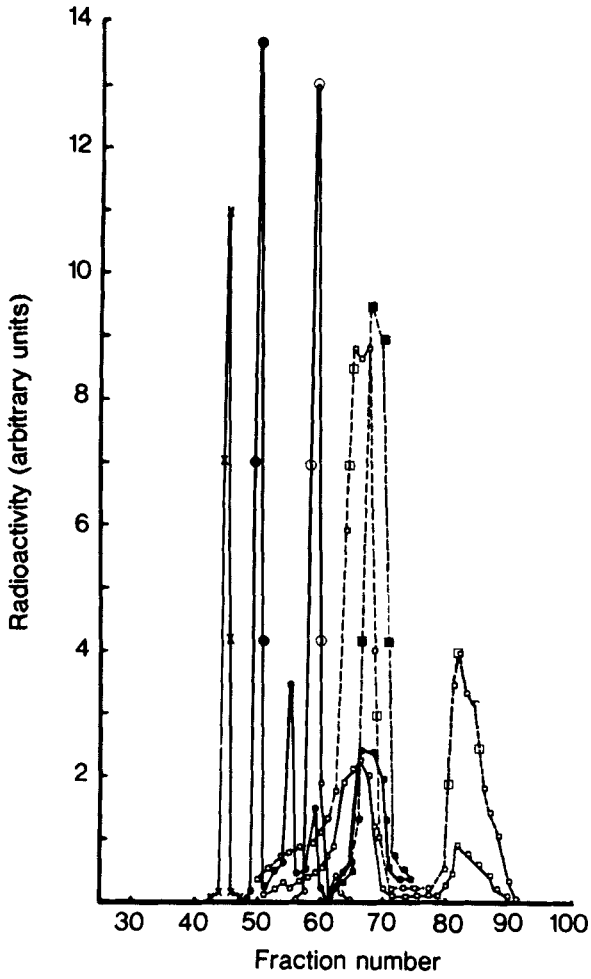

Figure 2. HPLC separation of labeled peptides using a reversed phase uBondapak C_{18} column. See text for experimental details.

_____ Elution position of polypeptides prior to acid plasma treatment.

----- Elution position of polypeptides after acid plasma treatment.

X βMSH; ● αEP; ○ γEP; ■ βEP; □ βLPH

DISCUSSION

Treatment of radiolabeled βEP, βLPH and γLPH with plasma at acid pH resulted in a considerable change in their apparent molecular volumes as assessed by changes in their gel permeation elution volumes. As all chromatograms were carried out on columns of identical dimensions with the same flow rate, gel bed and fraction size, it is unlikely that the enhanced permeation shown by the acid plasma-treated samples could be attributed to physical changes in elution volume arising from eluant viscosity or bed permeability changes. Rather, these changes are indicative of a more compact molecular form for these polypeptides, with smaller apparent Stokes radii.

From the data obtained from HPLC investigations, it can be concluded that there is no change in composition of the peptides following acid plasma treatment and therefore, the changes in elution volumes cannot be attributed to a proteolytic degradation process or fragmentation. A likely explanation is that at acid pH and in the presence of plasma, conformational changes occur in the molecules resulting in a decrease in molecular volume. There is now considerable evidence to suggest that in the presence of acidic lipids, an appreciable helical structure is formed by βLPH and βEP (8,9,10,11), resulting in these peptides occupying a smaller molecular volume. Hence, in water at neutral or acid pHs both polypeptides appear to have little, if any secondary structure whereas upon interaction with various anionic lipids these

polypeptides undergo a conformational change which includes a significant amount of α-helix. Mattice & Robinson (10) predict that it is the carboxyl terminal half of βEP and βLPH which shows high helix potential in the presence of acidic lipids and that αEP, which is the first sixteen amino acid residues of βEP, has a very low helix profile in both water and in anionic lipids. This explanation is also in accord with our observation that αEP and γEP do not undergo an apparent reduction in molecular volume when chromatographed with acid plasma. Assuming that this explanation is correct, the finding that γLPH but not βMSH exhibits changes in molecular size in acid plasma suggests that helical content is also contained between residues 1 to 41 at the amino terminal of βLPH. Furthermore, the observation that these changes in apparent molecular volume are abolished by Trasylol and sodium azide suggest either that an enzyme(s) is present in plasma that promotes rapid conformational changes of these molecules or that binding of β-LPH and β-EP to acidic lipids in plasma is enzyme and pH dependent. The latter possibility is favoured.

It has been suggested that it is the helical segment of the βLPH-related polypeptides which participates in effective receptor binding, the receptor membrane containing anionic lipids (9). Hence, these peptides must assume some ordered conformation to elicit their biological activity and explains why βEP shows significantly higher opiate activity than does αEP (10).

In summary, the present study indicates that an interaction

between β-endorphin and β- and γ-lipotropins with acidic lipids may occur in plasma at low pH. The conformational changes resulting from this interaction give rise to polypeptides with smaller apparent molecular volumes and thus greater permeation on Sephadex gel columns.

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