EVIDENCE OF HPLC-INDUCED CONFORMATIONAL CHANGES IN β-ENDORPHIN

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It is now evident that reversed-phase HPLC can induce conformational change of apparently homogenous polypeptides, the chromatographic conditions, particularly the stationary phase, leading to polypeptides undergoing slow dynamic interconversion (Andrade and Hlady 1986). This results in broad asymmetrical and even multiple peaks, which correspond to the native and denatured forms, if the kinetic processes of conformational change are slow or irreversible during elution. This phenomenon has been observed with a variety of proteins. While no evidence is available for the chromatographic behaviour of ß-endorphin, preliminary investigation using standards demonstrated the appearence of deformed peaks. It was thus anticipated that ß-endorphin, like other polypeptides, undergoes denaturation during HPLC. This work details the elution characteristics of ß-endorphin when subjected to altered environmental conditions prior to analysis, changes in flow rate and elevated column temperature.

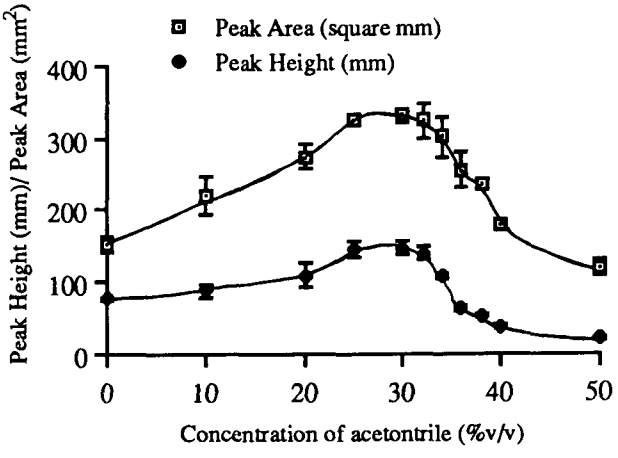
Standardised chromatographic conditions were utilised for all experimentation. A reversed-phase, guard and analytical column (Hichrom UK), packed with Nucleosil 300-7 C_{18} (250mm x 4.6mm) was used with mobile phase comprising of acetonitrile - 0.1M KH_2PO_4 adjusted to pH 2.3 with Aristar H_3PO_4 (32 : 68 v/v). Detection was obtained by means of UV absorption at 210nm.

To investigate the effect of solvent environment on the subsequent chromatograpy of ß-endorphin, lyophilised analyte (5µg/mL) was reconstituted with varying ratios of acetonitrile to 0.1M KH_2PO_4 adjusted to pH 2.3. An increase in both peak height and peak area was observed from 0 to 30% acetonitrile but a decrease noted thereafter (Figure 1). Furthermore, at 38 and 40 % acetonitrile, asymmetrical peaks appeared which had progressed to be split peaks at 50%. In addition, it was noted that when the solvent ratio of a sample was altered i.e. from 30:70 → 50:50 → 30:70 a variation in peak area of 328 mm^2, 108 mm^2 and 333 mm^2 resulted respectively, demonstrating that the apparent molecular manipulation is reversible.

Table 1

Flow Rate (mL/min)	Reconstitution of ß-endorphin (%Acetonitrile)							
	32%		36%		40%		50%	
	Area	R.T	Area	R.T	Area	R.T	Area	R.T
1.0	337	6.3	255	6.2	204	6.5	110	6.6
0.8	439	7.7	300	7.5	252	7.6	120	7.8
0.6	517	10.1	485	10.0	275	10.1	147	10.2
0.4	821	15.2	720	14.9	336	14.6	150	15.4

Figure 1.



A reduction in flow rate from 1mL/min, demonstrated an alteration in the chromatographic behaviour of ß-endorphin. As flow decreased a corresponding increase in peak area was observed being substantially higher for acetonitrile ratios of 32 and 36 % than for 40 and 50%. If this increase in peak area is as a consequence of a greater number of UV absorbing amino-acids being exposed at the time of detection, as previously alluded to (Monger and Olliff 1989), then these findings suggest that ß-endorphin assumes a more unstructured form at lower flow rates. In an attempt to verify this effect thermal denaturation was utilised. An increase in peak area with an increase in column temperature was obtained, implying denaturation of ß-endorphin. The presence of extra small peaks, however, could not be explained by the classical two state model of kinetic processes. Nevertheless it may be proposed that HPLC induces conformational change of ß-endorphin, which may be modifed by both solvent environment prior to analysis and flow rate, a slight alteration in either parameter being manifested in a major change in chromatographic behaviour.

Andrade, J. and Hlady, V. (1986) Advances in Polymer Science 79, Springer-Verlag, Paris, 1-63
Monger, L. and Olliff, C. (1989) J. Pharm. Pharmacol. **41** 153P