Waters
Lab Highlights
An Internal Communication of Applications and Techniques
LAH 0133 10/83
AN/LS/MD/PR/OT

LITERATURE CORNER

PROTEIN SEPARATION ON μBONDAPAK™ C_{18}: DISCOVERY OF A VARIANT β-CHAIN IN HUMAN HEMOGLOBIN

This is a typical application of the important role which μBONDAPAK™ C_{18} reverse phase columns play in protein analysis. In this example, a variant β-globin was discovered and subsequently purified using μBONDAPAK™ C_{18} column and Waters LC equipment. This work is most significant because a number of other common analytical techniques revealed no abnormal hemoglobins. The other analytical tools which were tried included:

1) Standard electrophoresis on cellulose acetate and citrate agar.
2) Isoelectric focusing in a pH gradient from 6 to 8.
3) Anion exchange LC.
4) Cation exchange LC.

Only the μBONDAPAK™ C_{18} column in the reverse phase mode accomplished the separation of the hemoglobin. The chromatogram is shown in Figure 1.

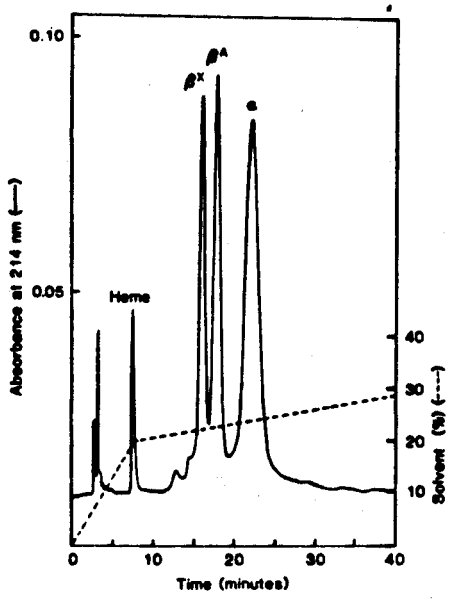

FIGURE 1: Detection of a β-globin variant (β^X) on a μBONDAPAK™ C_{18} column. Linear gradient segments from Solvent A consisting of acetonitrile and water (40:60) with 0.2 percent TFA (weight to volume) to Solvent B consisting of acetonitrile and water (60:40) with 0.2 percent TFA (weight to volume).

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This work uncovered the fact that a substitution of alanine for valine at position 126 in the β-chain of hemoglobin occurred in a hematologically normal adult male of Lebanese extraction. The variant β-globin was initially observed and subsequently purified by reverse-phase liquid chromatography (see Figure 1). Also LC on a μBONDAPAK™ column was used to isolate the variant tryptic peptide of β-T13 that had alanine replacing the valine at residue 126. This is shown in Figure 2.

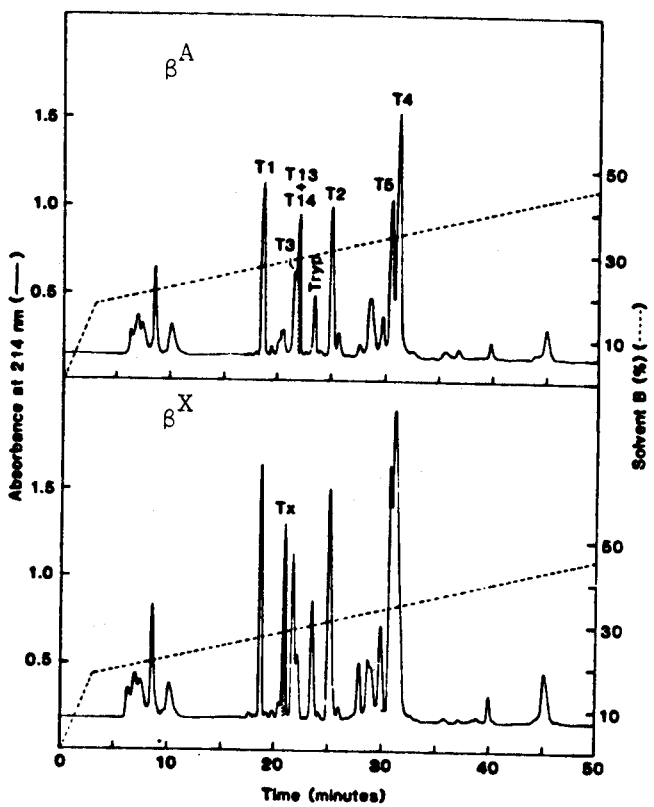

FIGURE 2: Separation of tryptic peptides on a μBONDAPAK™ C_{18} column. Linear gradient segments from Solvent A (0.1 percent TFA in H_2O, weight to volume) to Solvent B (0.05 percent TFA in acetonitrile, weight to volume).

This discovery of "hemoglobin Beirut" illustrates the usefulness of reverse-phase HPLC for the detection of neutral amino acid substitutions in proteins. Furthermore, this work has shown the ability to detect neutral substitutions in undigested proteins is pertinent to the monitoring of genetic variation in humans.

1. J. R. Strahler, B. B. Rosenbloom, S. M. Hanash, Science, 221,(1983),860-62.