Journal of Chromatography, 337 (1985) 110—113
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2355

Note

Development of a system to simplify identification of peptides and their catabolites in a high-performance liquid chromatographic effluent

CHARLES N.S. SOPARKAR*, ROBERT T. MALISON** and JAMES BURTON*

Biomolecular Medicine, University Hospital, 75 East Newton Street, Boston, MA 02118 (U.S.A.)

(First received February 1st, 1984; revised manuscript received August 22nd, 1984)

Increasing usage of peptides as drugs has focused attention on the in vivo fate of these labile compounds. Both endo- and exopeptidases operate on peptides to produce numerous catabolites. Frequently, the spectroscopic properties of these catabolites are similar to each other as well as to the parent peptide, making identification after high-performance liquid chromatographic (HPLC) separation difficult. If, in the course of analyzing a series of samples, the retention time of a component changes, identification of this compound may be speculative.

In response to these problems, a new configuration of equipment and software has been developed. Samples containing radioactive peptide and resulting catabolites are co-chromatographed with a mixture of unlabeled standards. The HPLC effluent is passed sequentially through a spectrophotometer and a radioactive flow monitor. Data are gathered by each instrument and transferred to a microcomputer, where elution profiles are constructed and superimposed. Superimposition of the radioactivity and absorbance data facilitate identification of catabolites in a complex mixture.

MATERIALS AND METHODS

Equipment

Components of the HPLC system are: high-performance liquid chromato-

*Present address: University of Massachusetts Medical School, Worcester, MA 01604, U.S.A.
**Present address: Yale University Medical School, 333 Cedar Street, New Haven, CT 06510, U.S.A.

0378-4347/85/$03.30

graph (Model 110A, Beckman Instruments, Palo Alto, CA, U.S.A.), spectrophotometer (Model 8450A, Hewlett-Packard, Palo Alto, CA, U.S.A.) [1], radioactive flow monitor (TRACE 7140, United Technologies, Chicago, IL, U.S.A.), micro-computer (Model 85, Hewlett-Packard), plotter (Model 7225B, Hewlett-Packard).

System design

The system is arranged as shown in Fig. 1. Effluent from the HPLC column passes through the spectrophotometer and radioactive flow monitor sequentially. This arrangement, rather than the reverse, was chosen to minimize band spreading caused by the larger volume of the flow cell in the radioactivity monitor (160 μl versus 8 μl). Two serial interfaces connect the spectrophotometer and flow monitor to the computer.

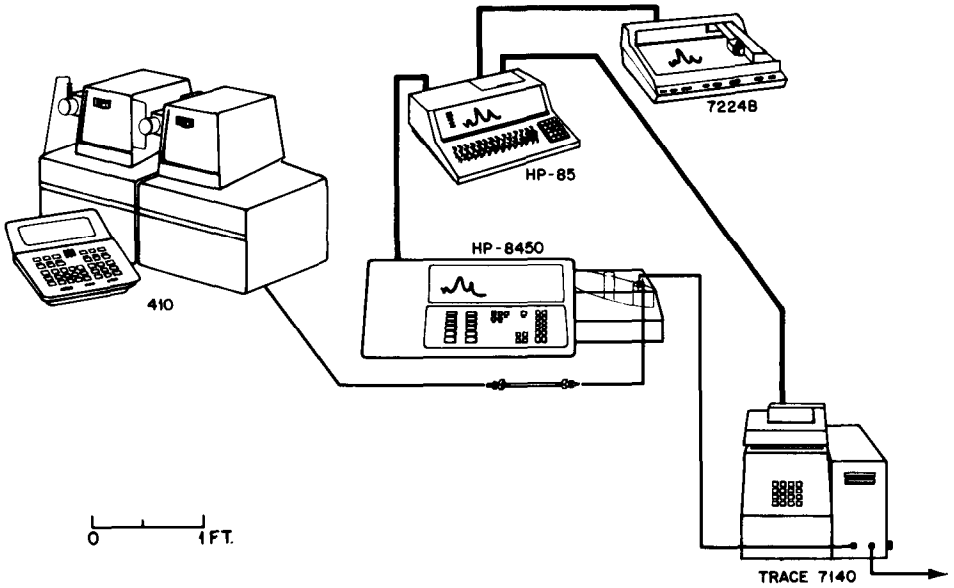

Fig. 1. Arrangement of the HPLC system which measures both absorbance (200—800 nm) and radioactivity of a column effluent and constructs superimposed elution profiles.

Programs

A series of menu-driven, linked programs converts the radioactivity and ultraviolet (UV) absorbance data into comparable elution profiles. Programs are MASTER, DATAQ, and GRAPH*. MASTER establishes the desired parameters, DATAQ controls the actual run, and GRAPH plots acquired data. The programs are written in HP BASIC and occupy 16 kilobytes of memory.

MASTER is an interactive program which prompts the operator to choose data collection start and finish times, frequency of radioactivity and absorbance readings, type of radioactivity to be monitored, spectral range, reference wavelengths, and number of wavelengths monitored. These variables

*Either HP-85 tapes containing the programs or program listings are available from the authors.

are printed and may be stored for subsequent runs involving the same parameters. MASTER provides the user with detailed operational instructions and checks all entered parameters for compatability.

DATAQ obtains the parameters designated in MASTER and allows for repeated background checks on both the radioactive flow monitor and the spectrophotometer. While running, DATAQ plots either absorbance or radioactivity versus time on the cathode-ray tube and stores both data types. Upon completion of data acquisition, DATAQ insures that all apparatus are turned off.

GRAPH gathers the stored data collected by DATAQ, displays data parameters available for plotting, and allows for alteration of plotting parameters such as run time and maximum ordinate. The finished plot includes radioactivity data, absorbance up to five wavelengths, and the eluent gradient. GRAPH accommodates a variable dead volume for determination of the effluent gradient and radioactivity/absorbance lag-times.

RESULTS

An HPLC system, in which both the absorbance and radioactivity (^{3}H, ^{14}C, etc.) of a column effluent are measured, the elution profiles constructed, superimposed and plotted, has been developed to simplify identification of labeled catabolites by co-chromatography with unlabeled standards. The program, MASTER, is written in BASIC and initiates interfacing between a computer, spectrophotometer, and radioactivity flow monitor. MASTER establishes and stores run parameters and then chains the program DATAQ

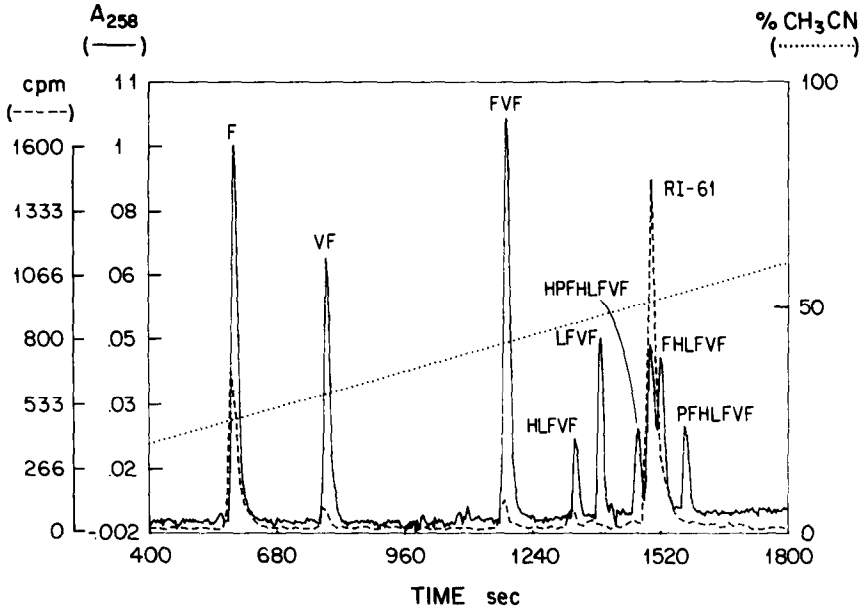

Fig. 2. Co-chromatography of partly catabolized RI-61 (Pro-His-Pro-Phe-His-Leu-Phe-Val-[^{3}H]Phe, 30 Ci/mol) with unlabeled catabolites that could arise from cleavage of the peptide chain.

to acquire data. DATAQ reads the desired values, checks for baseline consistency on the spectrophotometer and radioactivity flow monitor, gathers and stores the specified data, and plots either radioactivity or absorbance on the computer's cathode-ray tube during chromatography. The program GRAPH interfaces the computer and plotter to superimpose and plot the data along with the eluent gradient of the HPLC effluent.

An example of HPLC in which the UV absorbance (258 nm) of unlabeled peptides is used to identify radioactive catabolites is plotted in Fig. 2. The labeled peptide renin inhibitor RI-61 (Pro-His-Pro-Phe-His-Leu-Phe-Val-[^{3}H]Phe) [2] was exposed to the mucosal side of rabbit jejunum for 15 min [3]. Unlabeled RI-61 and the eight possible labeled catabolites which can arise by cleavage of the peptide chain have been co-chromatographed with the labeled inhibitor (100 Ci/mol). As can be seen, inactivation of the renin inhibitor occurs largely by removal of the C-terminal phenylalanyl residue, although small quantities of other cleavage products are also observed. Detailed catabolic patterns for the renin inhibitor in the presence of various inhibitors have been determined and will be reported elsewhere [4].

With minor changes in the interfacing portions, these programs can be adapted to function in any system able to communicate in BASIC.

ACKNOWLEDGEMENT

This research was supported by Grant No. DE-06048 from the National Institutes of Health.

REFERENCES

1 J.C. Miller, S.A. George and B.G. Willis, Science, 218 (1982) 241.
2 J. Burton, in K. Blaha and P. Malon (Editors), Peptides - 1982, De Gruyter, Berlin, 1983, p. 629.
3 R.E. Drews, K. Takaori, J. Burton and M. Donowitz, in V.J. Hruby and D.H. Rich (Editors), Proceedings 8th American Peptide Symposium, Pierce, Rockford, IL, 1984, in press.
4 Takaori et al., in preparation.