CHROM. 3363

Comparative electrophoresis of cytidines, adenines, uracils and guanines*

The separation of the individual compounds of the four ribobase families is difficult and time consuming. Each of the four groups is composed of five common members ranging in complexity from ribobase to riboside triphosphate. A relatively simple and extremely rapid electrophoretic resolution appeared possible by means of a technique previously described for the separation of the adenines[1], RNA nucleotides[2], nucleosides[3] and nucleobases[4]. The uncomplicated characteristics of the separatory system along with the simple tools required for the electrophoretic step could make this a useful analytical adjunct to either chromatographic determinations or other separation procedures. The present study will illustrate the following:

(1) The separation of the five members of the cytosines, adenines, guanines and uracils at both alkaline and acid pHs.

(2) The relationship of the mobilities of the separated compounds to those of the adenines as a baseline of study.

(3) The position of uric acid in the electrophoretic separation of the adenines to see if it is a possible source of interference in the analytical scheme for free nucleotides.

Materials and methods

Reagents

Nucleobase families. The five adenines, cytosines, guanines and uracils representing the bases, nucleosides and mono-, di- and triphospho-nucleosides were prepared as four mixtures of five compounds. Ten milligrams of each of the five compounds containing the same base were dissolved in 1 ml of H_2O. Guanine represented a special problem in the study. For alkaline electrophoresis 10 mg of guanine were dissolved in 1 ml of 0.5 *N* NaOH with gentle heating. The remaining members of the guanine family were dissolved in a separate solution as described above. A sliver of Whatman No. 3MM paper was dipped into the guanine solution, dried and then 2–3 μl of the second solution were pipetted onto the sliver. Alternatively, the dried sliver containing the guanine was quickly dipped into the second solution before electrophoresis. This wetting step did not cause the guanine to dissolve off.

For acid electrophoresis, 10 mg of guanine were dissolved in 1 ml of 0.05 *N* HCl and the procedure described for alkaline electrophoresis was followed.

Alkaline buffer (4X), pH 9.6. Prepare a buffer solution to contain 3.728 g of KCl, 3.092 g of boric acid and 43.7 ml of 0.5 *M* NaOH per liter.

Electrophoresis

Agarose gel (0.3 %) was prepared with the acid buffer as previously described. Agar gel (0.3 % Ion Agar No. 2) was prepared with the alkaline buffer in the same manner as described for the agarose. Each gel when used was pipetted onto a lantern slide and allowed to solidify. The semi-solid gel was connected to the buffer in the

* Supported in part by Grants-in-aid from the Detroit General Hospital Research Corporation, The Michigan Heart Association and the Public Health Service Grant CAO 8217-01 from the National Cancer Institute.

baffle boxes with filter paper wicks (Schleicher and Schuell No. 900). Solutions of each of the four 5-membered families were transferred to slivers of Whatman 3MM filter paper by dipping (the slivers) into the respective solutions and then blotting them free of excess liquid.

The slivers were placed in the center of the 4 inch axis for the pH 3.1 gel and 1.25 in. from the cathode in the pH 9.6 gel, and 250 V was applied to the entire system for 15 min. At the end of this time the voltage was disconnected, the positions of the different compounds were observed under a 256 nm ultraviolet lamp and their distances from the starting point were recorded.

Results and discussion

The resolution characteristics of the four groups of five compounds are shown in Figs. 1 and 2 for both the acid and alkaline mediums of agar and agarose gel respectively. The uracil–uridine pair migrate at the same speed in acid mediums during the 15 min time period and represent the only coincidental mobilities shown for an individual family. As a single advantage, in alkaline medium (Fig. 2) there is no set in which the same mobility was obtained for any two compounds of a family. In general, the acid resolution was better than the alkaline in terms of reproducibility of mobility. The order of separation of nucleotides varied with the pH for it can be seen that the

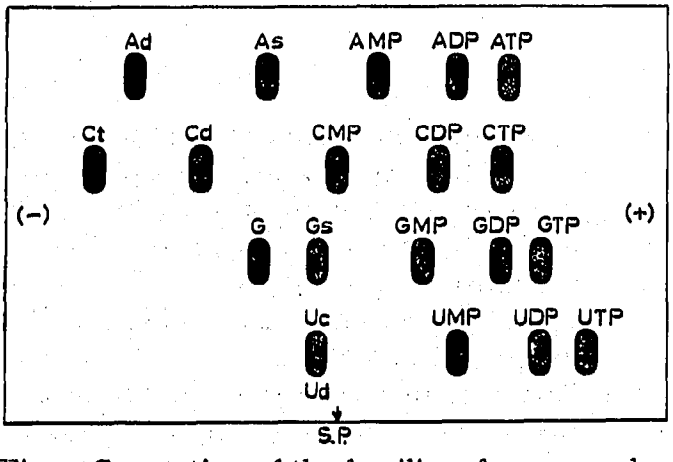

Fig. 1. Separation of the families of compounds at pH 3.1. Potential applied: 250 V; time: 15 min; 0.3 % agarose.

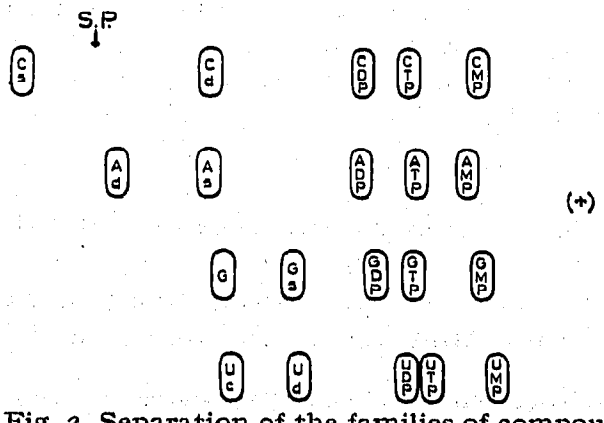

Fig. 2. Separation of the families of compounds at pH 9.6. Potential applied: 250 V; time: 15 min; 0.3% agarose.

triphosphate, and monophosphate of the pH 3.1 system change places in the alkaline system in every group while the others maintain their same relative positions.

A practical probability for the described procedure is the separation of free nucleotides such as adenine compounds in biological systems. But a potential interference could be uric acid, a fact which has been previously reported[5]. Therefore, an experiment was carried out to test this possibility. Solutions of the individual adenine compounds and uric acid were prepared as well as the mixture which was contaminated with uric acid. The results are shown in Fig. 3.The individually electrophoresed

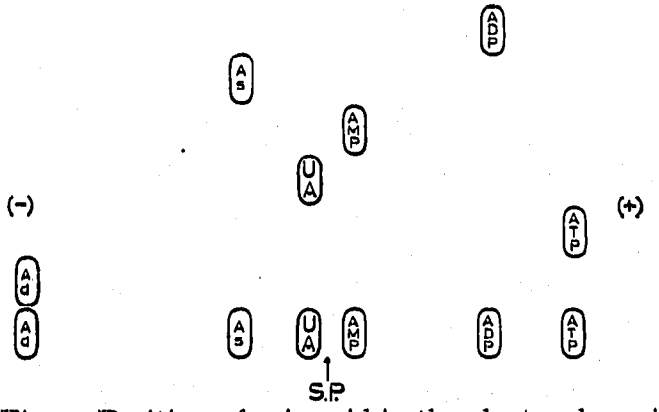

Fig. 3. Position of uric acid in the electrophoresis schematic of the pH 3.1 separation of the adenine family. Potential applied: 250 V; time: 15 min; agarose 300 mg/100 ml.

compounds had the same mobility characteristics as did the same compounds in a mixture and the uric acid moved very slightly toward the cathode. There is obviously no interference from uric acid in this particular separatory scheme.

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Received December 11th, 1967