Agarose electrophoresis of adenine nucleotides*

The electrophoretic separation of mixtures of the nucleotides AMP, ADP and ATP has been carried out from time to time[1-3], but no simple process for an analytical step has been achieved as yet. A modified technique based on recent reports[4-6] furnished a simple tool for their separation and two methods useful for quantitative determinations. These mixtures could be pure preparations or any combination of the adenine nucleotides once they had been separated from tissue as a mixture by an isolation scheme[7]. The purpose of this study was to show how a mixture of any of the three nucleotides could be rapidly resolved and easily analyzed. Separations were made in agarose gel over-laying a tissue slide cover-glass held flat on a glass lantern slide, and in dilute agarose gel over-laying quartz glass held flat on a glass lantern side. Quantitative determinations were carried out by elution of the nucleotides in alkali followed by ultraviolet spectrophotometry and by ultraviolet scanning of the separated nucleotides with an integrating monochromatic densitometer developed in this laboratory[8].

Materials

Nucleotide solutions. Solutions were prepared containing 10 mg of AMP per ml of distilled water, equivalent amounts of ADP and ATP and mixtures of the various nucleotides in the same concentration range.

Sodium hydroxide eluting fluid. An 0.05 *N* solution was prepared.

Ammonium acetate–acetic acid buffer, pH 3.1. This was prepared as previously described[6].

Schleicher & Schuell No. 900 wicks ($3^1/_4$ in. by 4 in.).

Adenine standards. Standards covering the range of 0–10 mg per ml were prepared.

Electrophoresis

An agarose solution was prepared as previously described[6] for agar containing 300 mg/100 ml agarose and 0.0225 *N*, pH 3.1, ammonium acetate buffer. 8–12 ml of hot solution was pipetted on to 25 by 50-60 mm tissue cover glasses placed along the center of the 4 in. axis of a $3^1/_4$ in. × 4 in. lantern slide. The liquid must cover the entire lantern slide and the cover glasses and its surface must be at the same height as that of the liquid surface of the 0.045 *N* buffer in the baffle boxes. The edges of the lantern slide were connected to the boxes with the Schleicher and Schüll No. 900 paper wicks. The agarose is allowed to gel (5–10 min). Thin Whatman 3MM strips which have been soaked in the nucleotide solution are placed about 5 mm from the edge of the cover glass on the cathodic side and 250 V applied to the buffer box-plate system. The run is observed with a short wave ultraviolet lamp until the distance between the bands is adequate; this does not exceed 8–10 min. After separation is complete, the plate is dried with an infrared lamp placed 15 in. above the plate[10].

Ultraviolet determination

The nucleotide area on the cover slip is cut off and eluted for several minutes with 1 ml of 0.05 *N* NaOH. The absorbance is measured in a quartz micro cuvet

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against an alkali blank at 260 mμ and the concentration of the unknown is determined against an adenine standard curve. Adenine has the same molar absorptivity as the nucleotides and can be used as a direct standard.

Ultraviolet densitometry

The electrophoretic procedure was similar to the one previously described and was carried out on 1 in. by 3 in. quartz slides held on a lantern slide rather than on a cover slip held on a lantern slide. The developed pherograms were scanned in an ultraviolet densitometer[10].

Results

Using the described procedure, electrophoresis of mixtures of adenine nucleotides resulted in distinct mobility differences for AMP, ADP and ATP within 3 min. The electrophoresis time was extended to 8–10 min because a greater distance between bands made it easier to cut the glass cover slips, gave better densitometric scans, and moved AMP away from the starting line. An actual electrophoretic pattern for the individual nucleotides and a mixture of AMP, ADP and ATP are shown in the ultraviolet photograph, Fig. 1. There is no interference seen in the mobility of any component of the mixture. The densitometry of an 8 min separation of the three nucleotides from a mixture is shown in Fig. 2. A separation and scan of AMP and ADP is also

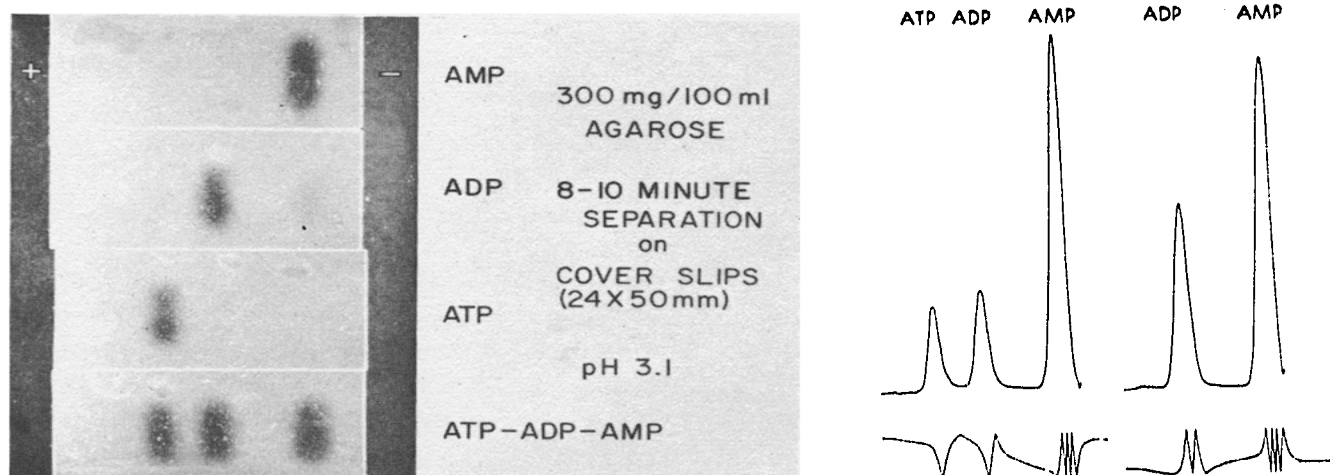

Fig. 1. Ultraviolet photography of electrophoretic separation of individual and mixed nucleotides.

Fig. 2. Ultraviolet densitometric scan of mixtures of nucleotides after electrophoresis in agarose on quartz.

shown. The resolution of either mixture was excellent, even though mild electrophoretic conditions were employed and a small distance for a short time period was involved. The disproportionation in concentration of buffer between baffle boxes and plate seemed to minimize excessive heating of the gel during the electrophoretic process.

Table I shows the results obtained for known quantities of AMP, ADP and ATP when analyzed for their adenine content by ultraviolet spectrophotometry. Similar amounts eluted from dried agarose plates gave identical results. The calculation of the

TABLE I

RECOVERY OF ABSOLUTE QUANTITIES OF AMP, ADP AND ATP FROM SOLUTION AND ELUTED FROM AGAROSE GEL

Sample	*Present* (μg)[a]	*Found* (μg)
AMP	5.5	6.0[b]
AMP	11.0	11.0
AMP	16.5	16.0
AMP	22.0	20.8
ADP	5.0	4.8
ADP	10.0	10.0
ADP	15.0	14.3
ADP	20.0	18.8
ATP	5.5	5.5
ATP	11.0	10.0
ATP	16.5	15.3
ATP	22.0	20.8
AMP	5.0	4.6[c]
AMP	10.0	9.6
ADP	5.0	5.2
ADP	10.0	10.5
ATP	5.0	5.0
ATP	10.0	10.4

[a] Present as adenine.
[b] Recovered from solution.
[c] Recovered after elution from dried agarose plates.

estimating equation by the method of least squares gave a y intercept of 0.556, a slope of 0.92 and a standard error of estimate of $\pm$ 0.39 μg of adenine content[11].

Discussion

The ability to separate compounds in any given biological material by electrophoretic techniques is dependent upon several prime considerations. The pH, ionic strength, chemical make-up of the buffer system and the anti-convection material chosen are important factors in electrophoresis. No one of the many electrophoretic matrices represent a general ultimate for all electrophoretic procedures. In the present procedure, agarose gel was selected although no advantage for agarose over agar was found in earlier methods for the immuno-electrophoresis of serum proteins[9] or for the electrophoresis of RNA nucleotides[4], nucleosides[5], and nucleobases[6]. Adenine nucleotides showed a somewhat different picture. They were not difficult to resolve in agar, but gave a starting line artifact due to the method of sample application. This was overcome by the decreased endosmosis concomitant with a decrease in sulfonic acid groups by the use of agarose gel matrix. The slow-moving AMP moved away from the starting line during the 8–10 min time period prescribed.

The surface on which agar gel electrophoresis takes place is an often overlooked variable. Vegetable parchment paper[10] was an excellent base for serum protein separation, and teflon-coated glass paper[6] worked well for nucleobase separations, but both gave marred resolution when underlaying agarose gel in the attempted separation of adenine nucleotides. Perhaps the interference was due to charge characteristics at the gel–paper or gel–teflon interfaces, which differ from the glass–gel interface.

Some evidence of this peculiar phenomenon had previously been noted for RNA nucleosides[5]. In the present study, the best separations were obtained with agarose gel on glass. For solution analysis, cover slip glass which could be cut easily with a diamond point cutter was used as the carrier for the gel. The nucleotides resolved well and were eluted easily into dilute alkali. For densitometric analysis, the use of quartz glass was mandatory.

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Thin-layer partition chromatography of benzenecarboxylic and hydroxybenzenecarboxylic acids

Destructive oxidation of peat, lignites, bituminous coal and humic acids leads to the formation of complicated mixtures of low molecular weight polycarboxylic acids. Various chromatographic methods have been proposed for the separation of these acids[1–4].

We have shown[5,6] that large quantities of water soluble organic acids are formed during the natural weathering of coals at temperature below zero (in the eternally frigid zone of the arctic area).

These water soluble acids contained many hydroxy compounds and could not be separated by previously mentioned methods. Satisfactory results were obtained by using partition chromatography on a thin non-adhering layer of silica gel.

The present report describes the separation conditions for thirteen synthetic polycarboxylic acids; eleven of them were detected in water soluble fractions of weathering coals.