COMMUNICATIONS TO THE EDITOR

ANION EXCHANGE STUDIES. XVI. ADSORPTION FROM LITHIUM CHLORIDE SOLUTIONS[1,2]

Sir:

A study was undertaken of the adsorbability of a number of metal ions from LiCl solutions by a quaternary amine polystyrene-divinylbenzene anion exchange resin to compare with earlier similar studies of the adsorbability of these elements from HCl solutions on the same batch of resin (Dowex-1). Since the activity coefficients of LiCl and HCl in aqueous solutions do not differ greatly,[3] it was anticipated that most metals would adsorb similarly from these two media. It was found, however, quite unexpectedly, that for all metals studied, adsorption from LiCl solutions was very much greater than from HCl solutions of the same concentration. A typical case is Mn(II). In 12 *M* solutions the distribution coefficient *D* (amount per kg. dry resin/amount per liter solution) of Mn(II) is approximately 550 from LiCl solutions,[4] while *D* is only *ca.* 4 from HCl solutions.[5a] The effect is still pronounced in 6 *M* HCl solutions, although the ratio of the distribution coefficients for the two media is less.

Similar effects have been so far observed for Sc(III), Fe(III), Co(II), Zn(II), Ga(III) and Au(III). In general the distribution coefficients in 12 *M* LiCl solutions were more than a factor of 100 greater than in 12 *M* HCl solutions. This is particularly striking since the adsorbabilities of these elements vary widely (in 12 *M* HCl solutions the distribution coefficients are: *ca.* 2 (Sc(III)),[6] *ca.* 4 (Mn(II)),[5a] *ca.* 80 (Co(II), Zn(II)),[5a] *ca.* 6000 (Au(III))[7] and *ca.* 50,000 (Fe(III),[5b] Ga(III)[6]). Even Be(II) shows considerable adsorption from concentrated LiCl solutions (*D* = *ca.* 8 in 13 *M* LiCl) while it had essentially negligible adsorption from concentrated HCl.[6] This adsorbability of Be(II) in concentrated LiCl is sufficient to permit anion exchange separation from non-adsorbable elements (*e.g.*, alkali metals, Mg(II), etc.) and in general the use of LiCl–low acidity media may permit anion exchange separations which are only marginal or ineffective with HCl solutions.

At the present time the reason for this pronounced effect is not clear. Qualitatively the results might be explained by assuming that the negatively charged chloride complexes of these elements form essentially non-adsorbable undissociated acids at very high acidities. However, this appears to be an unlikely explanation since the effect is so general.[8] More probably it is due to differences in the activity coefficients in the resin phase when the resin is immersed in these two media.

(1) This document is based on work performed for the U. S. Atomic Energy Commission at the Oak Ridge National Laboratory.

(2) Previous communication: K. A. Kraus and G. E. Moore, THIS JOURNAL, **77**, 1383 (1955).

(3) H. S. Harned and B. B. Owen, "Physical Chemistry of Electrolytic Solutions," Second Edition, Reinhold Publishing Corp., New York, N. Y., 1950, pp. 577, 601.

(4) All lithium chloride solutions contained a small amount (0.1 *M*) of HCl to avoid complications from possible hydrolytic reactions.

(5) K. A. Kraus and G. E. Moore, THIS JOURNAL, (a) **75**, 1460 (1953); (b) **72**, 5792 (1950); and unpublished results.

(6) K. A. Kraus, F. Nelson and G. W. Smith, *J. Phys. Chem.*, **58**, 11 (1954).

(7) K. A. Kraus and F. Nelson, *J. Am. Chem. Soc.*, **76**, 984 (1954).

(8) A similar, though less pronounced, effect had earlier been found for HNO_3 and NH_4NO_3 solutions, F. Nelson and K. A. Kraus, THIS JOURNAL, **76**, 5916 (1954).

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BIOSYNTHESIS OF PENTOSE PHOSPHATE FROM ERYTHRULOSE 1-PHOSPHATE AND FORMALDEHYDE[1]

Sir:

A soluble enzyme system from the liver, kidney or muscle of the rat catalyzes the anaerobic utilization of formaldehyde in the presence of erythrulose 1-phosphate. Although some formaldehyde is utilized by the system, the addition of erythrulose phosphate stimulates increased utilization of formaldehyde 10–15-fold. The enzyme can be obtained in a soluble state from a homogenate of liver in isotonic potassium chloride, by centrifugation at 38,000 r.p.m. for one hour on the Spinco Ultracentrifuge using rotor no. 40. The clear supernatant fluid contained all the activity. Using erythrulose 1-phosphate-4-C^{14} (specific activity 13,300 c.p.m. per micromole) and unlabeled formaldehyde the products of this reaction were identified by paper chromatography of the phosphate-free sugars according to published methods.[2] Ribose was identified as the major radioactive product by chromatography in three different solvent systems.[2] Upon spraying with aniline oxalate the characteristic cherry-pink spot for aldopentoses was obtained. Elution of the spot from large scale chromatograms yielded a compound which in the orcinol test gave a spectrum characteristic for ribose. The specific activity of the eluted pentose was 9300 c.p.m. per micromole and the total radioactivity incorporated ranged from 3 to 6% in eight different experiments. Smaller amounts of radioactivity were detected in the areas corresponding to xylulose or arabinose. Using both C^{14}-erythrulose phosphate and C^{14}-formaldehyde (specific activities 13,300 and 30,000 c.p.m. per micromole, respectively) the specific activity of the isolated pentose was 34,760 c.p.m. per micromole. In these experiments the specific activities of the recovered erythrulose phosphate and formaldehyde at the end of the experiment did not vary significantly from the initial values of these compounds. Incubation of either substrate alone yielded no labeled pentose (Table I). The specific

(1) Supported by grant No. C-2228 from the United States Public Health Service.

(2) F. C. Charalampous and G. C. Mueller, *J. Biol. Chem.*, **201**, 161 (1953).

activity of the isolated pentose was 70 and 80% of the expected maximum value in the two experiments mentioned above. This indicates that dilution of the synthesized pentose occurred, most likely, at a stage subsequent to the interaction of erythrulose phosphate and formaldehyde. The evidence indicates a direct condensation of erythrulose phosphate with formaldehyde as the first step in this biosynthesis. The phosphate bond in the pentose phosphate is stable to acid hydrolysis (1 *N* HCl at 100° for 30 minutes releases 5–10% of the organic phosphorus), thus excluding ribose 1-phosphate and favoring ribose 5-phosphate. No addition of any cofactor was required by the enzyme systems used.

TABLE I

Substrates Unlabeled	Substrates Labeled	Spec. act. of substr.[a]	Spec. act. of pentose	Theoret. spec. act.[b]
Formald.	Erythrul. phosph.	13,300	9,300	13,300
.....	Erythrul. phosph.	13,300	<100	None
.....	Formaldehyde	30,000	Zero	None
.....	Formaldehyde + Erythrul. phosph.	30,000 13,300	34,760	43,300

[a] All specific activities are expressed as c.p.m. per micromole. [b] Assuming a utilization of one mole each of formaldehyde and erythrulose phosphate per mole of pentose formed.

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KINETIN, A CELL DIVISION FACTOR FROM DEOXYRIBONUCLEIC ACID[1]

Sir:

Haberlandt's early concept of a specific cell division hormone (wound hormone) in plants has been strengthened gradually by evidence both for the specific need and for its satisfaction by extracts or substances of natural origin. For example, a factor required for cell division is practically lacking in pith but is present in limited amounts in vascular stem tissue and leaves of tobacco and in various plant products.[2] Yeast is a rich source, the further exploration of which now has led to deoxyribonucleic acid (DNA) as the starting material[3] for the isolation of a physiologically highly active chemical. The name *kinetin* is suggested for this substance.

For the bioassay tobacco "wound" callus tissue was used. It was obtained from stem segments grown on White's agar medium with 2 mg./l. indoleacetic acid (IAA) added. This tissue cultured on the same medium will undergo cell enlargement and limited increase in weight in response to the added IAA, but is incapable of cell division and continued growth unless a cell division factor is supplied. Rapid (5–10 day) assays of kinetin therefore are based on visual estimates of increased cell division activity as well as continuous growth of these cultures. The validity of the test has been established by cytological examinations and by determinations of weight increases.

For example, in one experiment determinations after six weeks showed that callus pieces had an average fresh weight of 1340 mg. in the presence of 100 μg./l. of kinetin, as compared with 1320 mg. in its absence. The corresponding dry weights were 101 and 73 mg. per piece, but the ratio of cell numbers treated/controls was 31/1 as estimated from counts in sections.

The first sample of DNA tested (a 4-year old preparation from herring sperm) was active, but fresh preparations from this source were inactive. However, on autoclaving water slurries of new DNA (*p*H 4.3) at 15 lb. for 30 min., they became extremely active. DNA from calf thymus behaved similarly. After extracting from aqueous solution (*p*H 6.8) with *n*-butanol and removing the alcohol, the active material in water was put on a cation exchange resin (Dowex 50) column and was eluted with 1.5 *N* HCl. The kinetin came off slowly and was detected by optical density readings at 271 mμ. The pooled active fractions were run through a similar column and after washing with water, the kinetin was readily eluted with 1 *N* NH_4OH. A band of crystalline material moving down the column was collected with as little dilution as possible. The precipitate from cooled solutions of this eluate was recrystallized from water, and then from absolute ethanol to give colorless platelets which sublimed at 220° (hot stage) m.p. 265–266° (sealed tube). The yield was about 30 mg. from 100 g. of DNA. The substance contained nitrogen, but no sulfur, phosphorus or halogens. Calcd. for $C_{10}H_9N_5O$: C, 55.81; H, 4.22; N, 32.55; mol. wt., 215.2. Found[4] (on separate preparations): C, 56.06, 56.13; H, 4.09, 4.16; N, 32.55, 32.58. Electrometric titration[5] in 1:1 ethanol:water showed pK_{a1} 2.7 ± 0.2, pK_{a2} 9.9 ± 0.2, n.e. (based on pK_{a2}) 223 ± 15. The ultraviolet spectrum[5] showed a single band, λ_{max}^{EtOH} 268 mμ, ϵ 18,650, λ_{min}^{EtOH} 233 mμ, ϵ 3200. The infrared spectrum[6] measured in a KBr pellet showed strong bands at 3.20, 3.30, 3.40, 3.59, 6.20, 6.30, 6.88, 7.12, 7.50, 7.64, 8.01, 8.72, 9.91, 10.7, 11.0, 11.2, and 13.3 μ. The biological activity was not destroyed by autoclaving aqueous solutions of kinetin at *p*H 0.5 or 12.0.

Kinetin is slightly soluble in cold water, ethanol, or methanol, freely soluble in dilute aqueous HCl or NaOH, and can be extracted from neutral aqueous solutions by shaking with ether. It is physiologically active at very great dilutions but only in the presence of auxin (added IAA). The lowest concentration tested, 0.01 p.p.m., was effective. The supply of kinetin, like that of auxin (IAA), must be renewed with each successive subculturing.

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(2) J. R. Jablonski and F. Skoog, *Physiol. Plantarum*, **7**, 16 (1954).

(3) In *l'Année biologique*, **30**, 412 (1954).

(4) Microanalyses by Micro-Tech Laboratories, Skokie, Illinois.

(5) Kindly carried out by N. S. Ling and R. M. Bock.

(6) Kindly determined by S. M. Aronovic.