Ion Pairing of Magnesium Sulfate in Seawater: Determined by Ultrasonic Absorption

Abstract. *Data from ultrasonic absorption in synthetic and natural seawater can be used to calculate pairing of magnesium and sulfate ions in seawater. Calculation under the assumption that data from ion pairing in single salt solution can be applied to seawater solution results in a value of 9.2 percent for the amount of magnesium paired with sulfate and 17.5 percent for sulfate paired with magnesium.*

Pytkowicz, Duedall, and Connors (*1*) discussed the extent to which the Mg^{++} ion is paired with the SO_4^{--} ion in seawater and concluded that between 39 and 53 percent of the total amount of the magnesium ions are paired with sulfate ions. This value is much higher than the figure of 11 percent calculated by Garrels and Thompson (*2*) and that of 3 percent calculated by Platford (*3*). Acoustic data show that 9.2 percent of the Mg^{++} is paired with SO_4^{--}.

Calculations of $MgSO_4$ ion pairing in seawater to date have not made use of ultrasonic-absorption measurements (*4*, *5*) in natural and synthetic seawater. In view of the fact that ultrasonic absorption in $MgSO_4$ solutions is proportional to the concentration of the associated or paired ions (*6*) (determined by conductivity measurements) and since neither $MgCl_2$, Na_2SO_4, nor NaCl demonstrates significant ultrasonic absorption in pure water or seawater (*4*, *5*), my calculations of the extent of $MgSO_4$ ion pairing in seawater were based on acoustic data.

Kurtze and Tamm (*4*) and Wilson and Leonard (*5*) found sound absorption in synthetic seawater equivalent to that observed in a pure-water solution of $MgSO_4$ at a concentration of 0.014 mole per liter. These two separate measurements agree within 10 percent. The measurements by Kurtze and Tamm (*4*) at 20°C were done with synthetic seawater containing (mole/liter): Na^+, 0.454; K^+, 0.01; Mg^{++}, 0.052; Ca^{++}, 0.010; Cl^-, 0.530; Br^-, 0.001; SO_4^{--}, 0.0275; and CO_3^{--}, 0.0025.

When we say that ultrasonic absorption in a solution of aqueous $MgSO_4$ (0.014 mole per liter) is equivalent to that observed in seawater, we mean that only 34 percent of this concentration (or 0.0048 mole per liter) is effective, because this is the extent to which $MgSO_4$ is paired in aqueous solution according to Fisher (*7*). Therefore, the extent of ion pairing of Mg^{++} to SO_4^{--} in seawater is 9.2 percent (0.0048/0.052), and that of SO_4^{--} to Mg^{++} is 17.5 percent (0.0048/0.0275). The values agree closely with 11 and 21.5 percent, respectively, obtained by Garrels and Thompson (*2*).

These calculations apply only to atmospheric-pressure measurements. Any attempt to make calculations at elevated pressures would have to take into consideration the large decrease in acoustic absorption (*8*) (about 60 percent) and the rather small decrease in the total amount of ion pairing of $MgSO_4$ (*4*) (about 10 percent) observed at 1000 atmospheres. Fisher (*8*) showed that these apparently contradictory effects are resolved within the Eigen and Tamm multistate dissociation model (*9*) in which three forms of ion pairs interact through two pressure-dependent chemical reactions.

Experiments with acoustic absorption are an independent source of data to be considered in defining one aspect of the chemistry of seawater, namely, the amount of Mg^{++} ions paired with SO_4^{--} ions. My initial calculation supports the results of Garrels and Thompson (*2*).

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Valyl-Transfer RNA: Role in Repression of the Isoleucine-Valine Enzymes in Escherichia coli

Abstract. *α-Aminobutyric acid is activated but not transferred to valine-specific transfer RNA and is unable to repress the isoleucine-valine enzymes in* Escherichia coli *strain W. α-Amino-β-chlorobutyric acid is activated and transferred to valine-specific transfer RNA and completely replaces valine in repression.*

There is evidence that certain amino acids must be activated in order to repress their own biosynthetic enzymes (*1*). The importance of activation for repression was shown by the effect of either the inhibition or the alteration (by mutation) of an aminoacyl-tRNA synthetase (*2*). Since these enzymes catalyze both the activation of the amino acid as well as the attachment of the amino acid to tRNA, the relevant reaction required for repression is not apparent. Thus, either the formation of the aminoacyl-synthetase complex, the amino acid-adenylate synthetase complex, or aminoacyl tRNA could be the key step for the participation of amino acids in repression. In one study, amino acid attachment to tRNA appears to be the important reaction in that strains of *Salmonella typhimurium* containing small amounts of histidine-specific tRNA were derepressed for the histidine biosynthetic enzymes (*3*).

I now report evidence for the participation of valyl-tRNA in the repression of the isoleucine-valine enzymes in *Escherichia coli* strain W. In these experiments two valine analogs, DL-α-aminobutyric acid (ABA) and DL-threo-α-amino-β-chlorobutyric acid (ACBA) were used (*4*). Both compounds have been shown, by the amino acid-dependent exchange of ATP and PPi^{32}, to be activated by *E. coli* valyl-tRNA synthetase (*5*).

The effect of ABA and of ACBA

Table 1. Supplements to the minimal medium. ABA was added at a concentration of 500 μg/ml and ACBA was added at a concentration of 75 μg/ml.

Supplement	Medium (μg/ml)		
	Complete	Valine limiting	Isoleucine limiting
L-Isoleucine	50	50	12
L-Valine	40	0	50
L-Leucine	50	50	50
Glycyl-L-valine	25	25	0
Pantothenic acid	10	10	10

on repression of the isoleucine-valine enzymes was determined with the use of *E. coli* W strain M4862H-5, which is an isoleucine, valine, and leucine auxotroph (*6*). In *E. coli* W the formation of the isoleucine-valine enzymes is controlled by multivalent repression; that is, an excess of isoleucine, valine, leucine, and pantothenic acid is required to repress these enzymes (*6*). In my experiments derepression was obtained by growth of the mutant in media containing growth-limiting amounts of one of the end products in the presence of an excess of the other three (Table 1). Both ABA and ACBA were added at the time of inoculation, and their effect on derepression was determined after harvesting the cultures at the early stationary phase of growth. The addition of ABA had no effect on derepression of the isoleucine-valine enzymes when the auxotroph was grown on limiting amounts of valine or isoleucine (Table 2). In contrast, the addition of ACBA to cells grown on limiting valine completely repressed the isoleucine-valine enzymes. Significantly, this compound had no effect on derepression due to isoleucine limitation. These data indicate that ACBA can specifically replace valine for repression and that ABA has no repressive effect.

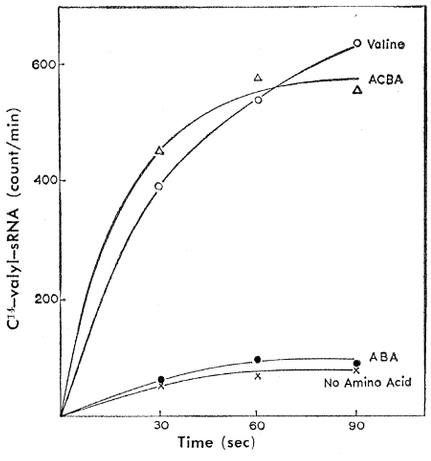

Fig. 1. Protection from periodate oxidation of valine-accepting capacity of tRNA by ABA and ACBA. Commercial preparations of *Escherichia coli* W tRNA were stripped of attached amino acids by incubation in 0.1*M* tris chloride buffer (*p*H 8.6) at 37°C for 2 hours and dialyzed for 3½ hours against 2000 volumes of distilled water at 4°C. Stripped tRNA (5 mg) was charged to completion by the method of Böck *et al.* (*11*) except that the concentration of all components in the assay system, except amino acids, was increased five times. The compounds to be charged were added at the following concentrations: L-valine, $2 \times 10^{-4}M$; ABA, $4 \times 10^{-3}M$; ACBA, $1.5 \times 10^{-3}M$. Samples were treated with periodate (*11*) and dialyzed for 3½ hours against 2000 volumes of distilled water at 4°C. They were then stripped of attached amino acid or analog as described. The valine-accepting capacity of the samples was measured by attachment of L-valine-C^{14} to *E. coli* W tRNA (*11*). The reaction was terminated by pipetting 0.1 ml of the reaction mixture onto a 25-mm filter-paper disc, and the disc was immediately placed into ice-cold 10 percent TCA. The discs were washed (*12*), and their radioactivity was determined in a Packard scintillation counter. Crude valyl-tRNA synthetase was prepared from *E. coli* W grown aerobically in glucose-minimal medium (*13*). All of the following procedures were carried out at 0° to 5°C. The cells were harvested during early stationary phase, washed once with 0.05*M* potassium phosphate buffer, *p*H 7.5, containing 0.01*M* 2-mercaptoethanol. The cells were broken in 12 ml of the wash buffer with a Branson model 110 sonic oscillator (6 amperes for 45 seconds). Debris was removed by centrifugation at 30,000*g* for 15 minutes, and the supernatant was centrifuged for 2 hours at 105,000*g* (Spinco model L-2 centrifuge; No. 40 rotor). The upper 8 ml of the supernatant was layered on a Sephadex G-25 column (24 cm^2 by 150 cm), eluted with 0.05*M* potassium phosphate *p*H 7.5, containing 0.01*M* 2-mercaptoethanol, and the unretarded fraction was collected.

Table 2. Effect of α-aminobutyrate and α-amino-β-chlorobutyrate on repression of the isoleucine-valine enzymes in *E. coli* W strain M4862H-5. The organisms were grown at 37°C in erlenmeyer flasks, with shaking. The harvesting of the cells, the preparation of the extracts, and the assays of enzymic activities were performed by described procedures (*6, 10*). The specific activity is expressed as the number of micromoles of product per milligram of protein per hour.

Addition to medium	Specific activity		
	Threonine deaminase	Dihydroxy-acid dehydrase	Acetohydroxyacid synthetase
	Complete		
None	8.2	9.8	1.8
	Valine limiting		
None	57.4	54.3	37.2
ABA	52.5	53.2	37.8
ACBA	7.4	8.7	1.9
	Isoleucine limiting		
None	36.7	32.6	7.7
ABA	39.4	37.2	9.2
ACBA	34.4	33.0	7.8

Since both ABA and ACBA can be activated by valyl-tRNA synthetase (*5*), it was of interest to see if these compounds could also be transferred to valine-specific tRNA. To investigate this, the analogs were tested for their ability to protect valine-specific tRNA from oxidation by periodate (*7*). Commercial preparations of *E. coli* W tRNA, stripped of attached amino acids, were subjected to charging conditions with valine, ABA, and ACBA. The charged tRNA was treated with periodate and chemically stripped of attached amino acids. The tRNA was then tested for its ability to accept valine C^{14}. Complete protection from periodate oxidation was obtained with either valine or ACBA. In contrast, ABA was unable to protect valyl-tRNA from oxidation by periodate. These results indicate that ACBA, but not ABA, has been transferred to valine-specific tRNA. Other experiments show that ABA C^{14} is not attached to tRNA (*8*).

Thus, while both ABA and ACBA are activated by the valyl-tRNA synthetase, only ACBA is attached to valine-specific tRNA. Parallel with this charging by ACBA is the fact that ACBA can specifically replace valine in repression of the isoleucine-valine enzymes while ABA is completely inactive in repression. Taken together, these observations support the hypothesis that charging of valine-specific tRNA by valine is an important (or required) step for repression mediated by the valyl-tRNA synthetase (*9*). However, the results do not exclude the possibility that a step in protein synthesis subsequent to aminoacyl-tRNA formation is the reaction directly involved in repression.

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Flow Characteristics of Human Erythrocytes through Polycarbonate Sieves

Abstract. *We used polycarbonate sieves with uniform cylindrical pores (2.4 to 6.8 microns in diameter) to filter suspensions of human erythrocytes (mean major diameter is 7.2 microns) in Eagle-albumin solution. With 6.8-micron sieves the pressure-flow curves are convexed to the pressure-axis at low pressures and become linear with high pressures. With 4.5-micron sieves, however, the pressure-flow relationship is linear throughout the range of study. In both types of sieves, flow rate is reduced progressively with increasing concentration of red blood cells (RBC) over a range of 0.5 to 95 percent. The resistance to flow of RBC suspensions is higher in 4.5-micron than in 6.8-micron pores. With filter pore diameters of 3.0 microns or more, the RBC concentration in the filtrate was 100 percent of that in the solution being filtered, but only 70 percent with 2.4-micron pores. The observed critical pore diameter for 100 percent cell transmission agrees with theoretical calculation based on the assumption that the RBC membrane is deformable but nonextensible. The importance of cell deformation in the passage of RBC's through small pores is shown by the inability of RBC hardened in acetaldehyde to pass pores with 6.8-micron diameter.*

Our data, presented here in three papers, show that the departure of blood from Newtonian behavior depends on two mechanisms: (i) erythrocyte deformation, which preserves the fluidity of blood at normal and high cell concentrations and also enables red cells to squeeze through the smallest capillaries, and (ii) erythrocyte aggregation (rouleaux formation), which accounts for the striking shear-rate dependence of viscosity at near-zero flow conditions. In this paper we examine RBC deformation by sieving tests; we have also compared the viscometric behavior of deformable and hardened (rigid) RBC (*1*) and have shown the influence of RBC aggregation (rouleaux formation) on blood viscosity (*2*).

Fleischer *et al.* (*3*) recently described a new technical development of polycarbonate filters (sieves) with cylindrical pores of measurable diameter and known length. We performed experiments to see if these sieves could be used to obtain quantitative data on (i) the rheological behavior (pressure-flow relations) of erythrocyte (RBC) suspensions and (ii) RBC deformation in passing through cylindrical pores with diameters comparable to those of vessels in the microcirculation. We used only suspensions of washed RBC's to exclude the complicating effects of other formed elements (*4*) and of various cell-protein interactions (*5*) because our aim was to examine the properties of the sieves in relation to the flow characteristics of normal RBC's *per se*.

Human blood was washed three times with Eagle-albumin solution (*6*) and then suspended in it. Counts of RBC's were made with the Coulter electronic counter, and the cell percentage (H) was determined by the microhematocrit method (5 minutes centrifugation at 15,000*g*). From these results we calculated the mean corpuscular volume (MCV) of the washed RBC's, which agreed with that of the fresh blood. Microscopic observations showed normal biconcave RBC's after washing with Eagle-albumin solution. Suspensions of desired RBC concentrations were prepared immediately in the Eagle-albumin solution, and the sieving experiments were performed as soon as practicable.

The polycarbonate sieves used had various mean pore diameters (Fig. 1). The sieve and its metal supporting screen were clamped in a filter funnel with an effective filter area of 8.0 cm^2. The funnel stem was inserted through a rubber cork used to stopper a collecting cylinder. By setting the negative pressure in the cylinder, we could keep the filtration pressure (ranging from 0.5 to 20 cm of H_2O) constant.

All filtration experiments were done at 22° to 25°C. We wet each new filter with Ringer solution (*7*) before its installation and calibrated it immediately by timing the flow rate of the Ringer or Eagle-albumin solutions, both of which gave the same results. In filtering the RBC suspensions, we spread a measured volume (1 or 2 ml) of the mixed suspension uniformly over the filter and determined the passage time by observing the filter. The passage of successive samples of RBC suspensions through the sieves (especially those with pore diameters less than 5 μ) caused progressive occlusion of the pores and decrement in flow rate. Repeated washing of both sides of the filter under suction with Ringer solution usually cleared the occluded pores, as indicated by recalibration with Ringer solution. When this failed, the sieve was soaked in 6*N* nitric acid overnight, and new filter with comparable characteristics was used to continue the experiment.

We determined the rate of flow decrement of RBC suspensions caused by pore occlusion by timing the passage of discrete volumes in rapid succession at a constant filtration pressure. Figure 2 is a semilogarithmic plot of the flow rate and the cumulative volume passed (V) in one test. The data can be fitted by a simple exponential function:

$$\text{Flow} = \dot{V}_0 e^{-\lambda V}$$

where $\dot{V}_0$ (intercept on the flow axis) is the extrapolated initial flow rate in milliliters per second and λ is the

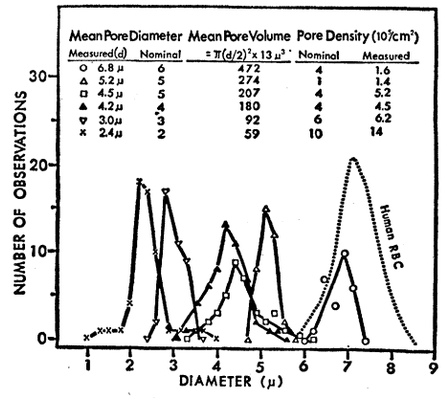

Fig. 1. Characteristics of polycarbonate sieves. The nominal values are data supplied by General Electric Atomic Products Division, Vallecitos Atomic Laboratories, Pleasanton, Calif. The filter thickness is 10 to 12 μ for all sieves. Because the pores are generally not perpendicular to the filter surface, we assume the mean pore length to be 13 μ in calculating the mean pore volume. The measured values for pore diameters and pore density were obtained by direct microscopic observations or from photomicrographs of the sieves. Solid lines represent the distribution curves for measured pore diamters of various sieves. Dotted line is the Price-Jones distribution curve for major diameter of normal human erythrocytes.