

to the unperturbed orbit and  $\Delta V_t$  and  $\Delta MV_t$  transverse to them. Let  $\Delta\theta$  be the angle by which the velocity is deflected. This will be

$$\Delta\theta = \Delta MV_t / MV_o \quad (2)$$

since  $\Delta V$  is small. But the change in momentum is proportional to the product of the force acting and the time during which it acts, so that

$$\Delta MV_t = D_t \Delta t \quad (3)$$

and

$$\Delta MV_o = D_o \Delta t, \quad (4)$$

where  $\Delta t$  is the time during which the forces act.

From Eqs. 1 and 3

$$\Delta MV_t = \frac{v_e}{V_o} D_o \Delta t, \quad (5)$$

or, by Eq. 4,

$$\Delta MV_t = \frac{v_e}{V_o} \Delta MV_o, \quad (6)$$

so that, substituting Eq. 6 in Eq. 2,

$$\Delta\theta = \frac{v_e}{V_o} \frac{\Delta MV_o}{MV_o} = \left( \frac{v_e}{V_o} \right) \left( \frac{\Delta V_o}{V_o} \right) \quad (7)$$

Since this deflection is assumed to occur near the equator, the orbit plane will be tilted by the same angle with respect to the equatorial plane, so that the orbit for the next revolution would not pass exactly over the poles. On each revolution, a similar effect occurs; but since the effect is small, we may assume that the polar orbit is approximately maintained and regard the equation as giving the total change in orbit inclination corresponding to any given change in orbital velocity, regardless of how many revolutions are involved.

Two further assumptions are to be made now, one with regard to the average orbital velocity, the other with regard to the fractional change of the velocity. We assume 18,000 mi/hr as a fair average and assume a change of 20 percent as a maximum value over the portion of the satellite's life during which elliptical orbit shrinks to a circular orbit. The transverse component of the relative wind velocity is just the earth's rotational velocity, taken as 1000 mi/hr at the equator. Then, over this phase of the satellite's lifetime:

$$\Delta\theta_{\max} = \frac{1000}{18000} = \frac{20}{100} = 0.01 \text{ radian or } 0.6^\circ \quad (8)$$

Somewhat more refined calculations, which take into account the inclination of the orbit and the progression of perigee, predict changes in orbit inclination of  $0.1^\circ$  to  $0.2^\circ$  from the original inclinations (of  $65^\circ$  and  $35^\circ$ ) for U.S.S.R. and U.S. satellites launched so far (over their lifetimes, but not includ-

ing the last revolution). Unfortunately, up to the time of writing, the observational data published on orbit inclinations have not been of sufficient accuracy to check these predictions on the first two U.S.S.R. satellites. Because of the smallness of this effect it is unlikely that it can be used to obtain any definite information on winds and tides in the upper atmosphere.

Although the transverse atmospheric drag effect is seen to be small throughout most of the satellite's lifetime, it becomes of major importance in the last few thousand miles of the final revolution, when the velocity decreases from around 17,000 mi/hr to some lower value at impact. For all satellites so far put in orbit, the mass-area ratios are such that the satellites may be expected to lose substantially all forward relative velocity in the lower atmosphere, and to fall nearly vertically before impact, if they withstand the frictional heating without burn-up.

As a simple case, consider impact at the equator. The "orbital" motion, just before impact, will be in the plane of the equator. Therefore, the change in orbit inclination will be equal to the initial inclination, whatever its original value.

Only large meteorites or satellites having mass-area ratios many times larger than present satellites would be expected to have any appreciable residue of forward velocity at sea-level impact. Even for these,  $\Delta\theta$  would be a major fraction of  $\theta$ , as may be calculated roughly by applying Eq. 7 in several steps over the velocity range.

Perhaps it should be pointed out explicitly that  $\Delta\theta$  is measured with respect to the initial orbit plane, fixed in space. From the moon, for instance, the curvature of the orbit path would be observable. To an observer stationed at the equator on the projected track of the orbiting satellite, no such curvature would be apparent. Neglecting the small change of the atmospheric cross wind with latitude near the equator, the apparent course of the satellite in polar orbit relative to the observer on the equator will be slightly westward, as the result of the orbital velocity and the rotational velocity of the earth's surface. The satellite will stay on this course, relative to the (rotating) observer, regardless of the time-velocity history along the course, since both the transverse and orbital components of velocity change proportionately with the decrease of the resultant. The observer will note only the slowing, and the change of motion from horizontal to nearly vertical. The satellite would arrive at the point of impact several minutes later than it would have passed over had it not been slowed or stopped by the atmosphere. During this delay the earth's revolution

would move the impact point many miles, but this motion is, of course, not apparent to the earth observer.

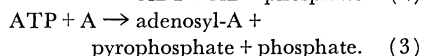
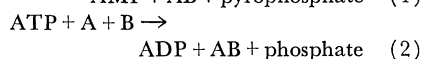
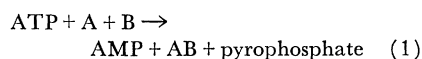
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16 June 1958

## Role of Magnesium in Enzyme-Catalyzed Syntheses Involving Adenosine Triphosphate

Adenosine triphosphate or one of the polyphosphates with which it is in equilibrium (guanosine, cytosine, and uridine triphosphates) is a reactant in each of a large number of enzymatic syntheses (1, 2), and these ATP- (3) or polyphosphate-dependent syntheses account for the preponderant proportion of all known biosynthetic reactions. These reactions fall into one of the following three categories (4):



Regardless of the category, all known ATP-catalyzed reactions show an absolute requirement for magnesium ions (2). Other divalent metals such as  $\text{Mn}^{++}$  or  $\text{Ca}^{++}$  may replace  $\text{Mg}^{++}$  in some cases, but the maximal activity which these ions induce may equal but never exceed the maximal activity which obtains in presence of  $\text{Mg}^{++}$ .

Since a very wide spectrum of synthetic reactions is encompassed by ATP-dependent enzymatic processes, the universal requirement for magnesium ions undoubtedly reflects some important underlying chemical principles. This report deals with several considerations which may throw light on these principles.

Magnesium ions chelate rapidly with ATP (5), polyphosphates (6), phosphoric esters, inorganic phosphate, hydroxy acids (7), amines (8), and amino acids (9) under physiological conditions. Thus all the reaction partners in known ATP-dependent reactions are capable of chelation with magnesium ions. The effect of magnesium chelation in such reactions is to lower the free energy of activation of the rate-determining step. This is accomplished in two ways—first by lowering the heat of activation of the reaction, and second by virtue of a stepwise mechanism that eliminates the unfavorable entropy of activation in the rate-determining step.

The manner in which chelation of the reactants with magnesium ions reduces the heat of activation has been well discussed by others (10). Essentially, mag-

nesium ion lowers the heat of activation by acting as a generalized acid catalyst.

However, chelation of the reactants by the magnesium in the enzyme is certainly also important in eliminating the unfavorable entropy of activation in the rate-determining step. For reasons given below, the entropy of activation term may be even more important in ATP-dependent reactions than in most non-enzymatic reactions.

The entropy of solvation is more negative for charged ions than for neutral molecules. Therefore, when neutralization of charge occurs during the formation of an activated complex, the entropy increases and the reaction is favored. The reaction is also favored if the charge is spread during the formation of an activated complex.

Solvation effects are little if any help to ATP-dependent reactions. Reactants in ATP-dependent reactions are commonly not charged at the reactive position. The charges present elsewhere in the molecules are usually of the same sign (negative) in both reactants. This causes the activated complex to be more highly charged than either reactant. In these reactions the entropy of solvation strongly hinders the reaction. For example, let us consider the displacement of AMP from AMP-OAc by an attack of the sulfhydryl group of coenzyme A on the carbon of the carbonyl group of the acetate group in AMP-OAc. This reaction probably does not involve any charges at the point of reaction. In addition, both reactants are negatively charged elsewhere in the molecule. The entropy of solvation does not favor and probably hinders this reaction. This is true for many reactions in which the reactant is ATP.

We now discuss the entropy terms other than solvation that are important. When two neutral molecules A and B are brought together to form an activated complex AB\*, the greater number of translational degrees of freedom of the reactants than those of the activated complex (II) causes the entropy of ac-

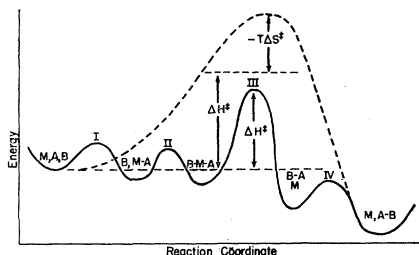


Fig. 1. The reaction path for the reaction of two molecules A and B with and without chelation by a metal M. The reaction path without chelation is represented by the dashed line, whereas the reaction path with chelation is represented by the solid line.

tivation to be unfavorable (a decrease) for the process. Therefore, these entropy terms hinder all reactions.

The unfavorable entropy of activation may be eliminated by chelation. Chelation allows a stepwise reaction mechanism which eliminates the entropy terms in the rate-determining step, as is shown in Fig. 1. The dotted line refers to the reaction path without chelation, the solid line to the reaction path with chelation. The entropy of activation and the heat of activation would both occur during the rate-determining step in the absence of chelation.

When chelation obtains, the entropy of activation is taken care of in steps I and II (12) because this is when the reactants are gathered together. Thus, the heat of activation, which is now less, is the only terms which is important in the rate-determining step. The total free energy of activation of the reaction is thus less, so that the reaction is faster. We then see that reactants will react with a lower free energy of activation when they are both chelated to a common metal ion.

Looking at the problem in a different manner, we might consider the great stability of ethylenediamine chelates as compared with methylamine chelates. This difference is due primarily to the dissociation of the ethylenediamine chelates into fewer particles, giving therefore a smaller increase in translational entropy than that in the corresponding methylamine complexes (13).

The greater stability of the ethylenediamine complex over that of the methylamine complexes means that thermodynamics would favor the formation of a bond between the methyl groups of the methylamine complex. Although the reactions between methyl groups of methylamine complexes to give ethylenediamine chelates are impossible, were similar bond-forming reactions to occur in other systems they would tend to occur in the chelated rather than in the unchelated form.

The considerations discussed above are germane to reactions involving ATP. The usual reactants (coenzyme A, thiamine pyrophosphate, diphosphopyridine nucleotide, triphosphopyridine nucleotide, and ATP) are all molecules with pyrophosphate groups which provide handles for chelation. The reactions in which these molecules participate would require a metal chelator with a pyrophosphate specificity and a chelating strength which is intermediate between high and low. The intermediate chelating strength is necessary so that the products may dissociate from the enzyme. Magnesium ion fulfills all these requirements and is the ion universally associated with ATP reactions.

Considering the ideas presented above,

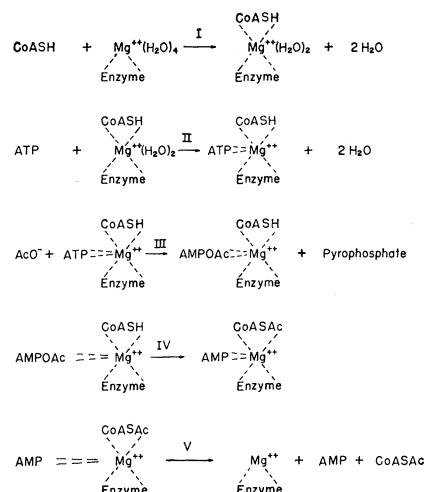
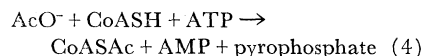


Fig. 2. A possible mechanism involving chelation by magnesium ions for the formation of acetyl coenzyme A. There is no significance in the order of occurrence of reactions I and II.

one may write a possible mechanism for reaction 4 which involves all six available coordination positions of magnesium, as is shown in Fig. 2.



The loss of entropy of translation of CoASH and ATP occurs in steps I and II, so that the reaction between these two groups (step IV) probably occurs with very little entropy of activation.

Step III, in which acetate ion displaces pyrophosphate ion, may not require chelation by the acetate ion. Here we have a small particle charged at the reactive position approaching to give an activated complex. The entropy of solvation may favor this reaction.

It is quite probable that one or another of the reactants is chelated much more strongly than another reactant. This situation could lead to inactive dichelates of ATP or perhaps CoASH. One of the important functions which the unique configuration of the enzyme fulfills is that of imposing such steric restrictions that only mixed chelates of ATP and CoASH are formed with the magnesium ion in preference to dichelates of ATP or CoASH.

Acetyl AMP has been shown by Berg (14) to give rise to acetate and ATP when it is incubated with acetic thio-kinase in presence of pyrophosphate and to give rise to CoASAc and AMP when it is incubated with the enzyme in presence of coenzyme A. Despite this powerful evidence that acetyl AMP is the intermediary in the acetate activation reaction, Berg and others have been unable to isolate or demonstrate acetyl AMP as an intermediary in the over-all reaction.

The acetate activation reaction serves as a model of ATP- and Mg-dependent reactions in general. The principles which have been applied to the model reaction are equally applicable to the large number of enzyme systems which catalyze the reactions described by Eqs. 1 to 3.

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3. The following abbreviations are used: ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; A and B, various reactants; AMP-OAc, mixed anhydride of adenosine monophosphoric acid and acetic acid; AcO<sup>-</sup>, acetate ion; CoASH, coenzyme A; CoASAc, acetyl coenzyme A.
4. For an example of reaction 1, see H. Beinert, D. E. Green, H. Hift, R. W. Von Korff, C. V. Ramakrishnan, *J. Biol. Chem.* 203, 35 (1953); for reaction 2, see J. F. Speck, *J. Biol. Chem.* 179, 1405 (1949); for reaction 3, see G. L. Cantoni and J. Durell, *J. Biol. Chem.* 225, 1033 (1957).
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11. The entropy of translation is approximately proportional to the logarithm of the molecular weight, so that the translational entropy of AB\* is greater than either A or B but less than the sum of the entropies of translation of A and B.
12. Actually the free energy of activation of the chelation process may be quite largely composed of favorable entropy terms [R. G. Charles, *J. Am. Chem. Soc.* 76, 5855 (1954)]. The release of water molecules from the magnesium during the chelation more than makes up for the unfavorable entropy of bringing the reactants together.
13. See, for example, F. H. Westheimer and L. L. Ingraham, *J. Phys. Chem.* 60, 1168 (1956); C. G. Spike and R. W. Parry, *J. Am. Chem. Soc.* 75, 2726 (1953).
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23 May 1958

## Pigments in the Flower of "Fu-Yong" (*Hibiscus mutabilis* L.)

The pigments found in *Hibiscus* flowers are almost exclusively of the flavone type—that is, cannabiscitrin (*H. cannabinus* (1)), gossypin [*H. esculentus* (2)] and *H. vitifolium* (3)], gossypitrin, hibiscitrin, quercetin, sabdaritrin (*H. sabdariffa*) (4), and saponarin (*H. syriacus*) (5). As far as we are aware, the only *Hibiscus* flower known to contain anthocyanin pigment is *H. rosa-sinensis*. The deep red flower of this plant was reported to contain a delphinidin glycoside (6). Recent work, however, has revealed that the coloring matter of the flower is cyanidin diglucoside (7). We were much interested to find that no chemical work has been reported on the pigment in the beautiful flower of "Fu-Yong" (*H. mutabilis* L.), one of the most widely cultivated flowers in Chinese and Japanese gardens.

Since the outer petals of the flower are faintly pink colored, it was necessary to use a large number of flowers to get a sufficient amount of the pigment solution. The pigments of the flowers were extracted with ethanol hydrochloric acid, concentrated in a vacuum and hydrolyzed. Both paper chromatography and the color reactions of the isolated anthocyanidin fraction showed that its chemical constituent is cyanidin.

The outer petals (500 g) of the flower of *H. mutabilis* L. were repeatedly extracted with 0.01 percent ethanol hydrochloric acid. The combined extracts (500 ml) were concentrated in a vacuum at room temperature until no more solvent could be distilled out. After the concentrate had stood at room temperature for 4 days, the yellow-brown precipitate formed was removed by filtration. The filtrate (5 ml) was further hydrolyzed by adding 1.7 ml of 10 percent hydrochloric acid and refluxing for 30 minutes. (When the filtrate was hydrolyzed by adding the same volume of concentrated hydrochloric acid and boiling for 2 minutes, a large amount of black tarlike substance was formed.) The resulting brown precipitate was filtered, and the orange-red filtrate was extracted with isoamyl alcohol. The anthocyanidin in the isoamyl alcohol phase was transferred, by extraction with a mixture of 1 percent aqueous hydrochloric acid and benzene (1:1 by volume), into aqueous phase, and then again transferred into isoamyl alcohol phase.

Paper chromatography of the anthocyanidin solution was carried out by

using a mixture of glacial acetic acid, 36 percent hydrochloric acid, and water (5:1:5); the chromatograms were developed by the descending method (8). From the  $R_f$  value of the spot (0.38) and the comparison of this value with the values of cyanidin [0.38, from red rose (9)], of pelargonidin [0.51, from *Pelargonium zonale* (10)], and of malvidin [0.44, from *Iris kaempferii* (11)], the anthocyanidin was found to be cyanidin. The extract of this spot ( $R_f$  0.38) also showed color reactions of cyanidin—that is, it turned red-purple on the addition of sodium acetate and blue on the subsequent addition of ferric chloride.

The brown precipitate obtained was extracted with ether. After the ether was expelled, the yellow residue was dissolved in ethanol. The solution turned to orange-red when it was treated with magnesium powder and hydrochloric acid, showing the existence of flavone (12, 13).

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13. We wish to thank K. Hayashi (Tokyo Kyoiku University) for his advice, and C. S. Hu (head, department of horticulture), Y. F. Shen (head, department of botany), and T. T. Wang (head, department of forestry of our university for permitting us to collect the flowers at their gardens).

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3 January 1958

