The Catalytic Effect of Molybdate on the Hydrolysis of Organic Phosphate Bonds

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(Received 29 November 1950)

The great instability of creatine phosphate (Fiske & Subbarow, 1929), acetyl phosphate (Lipmann & Tuttle, 1944) and 1:3-diphosphoglyceric acid (Negelein & Brömel, 1939) in solutions containing molybdate has been known almost as long as these compounds themselves. It could not fail to attract the attention of the analyst who used a method of phosphate estimation of the Briggs (1922) or Fiske & Subbarow (1925) type, because these methods entail a period of colour development during which the organic phosphate is in contact with ammonium molybdate concentrations of 0.2–0.7 %.

The acid-lability of a phosphate bond is apparently not invariably linked with the catalytic effect of molybdate. For ribose-1-phosphate, for example, which is intermediate in acid-stability between creatine phosphate and acetyl phosphate, Kalckar (1947) found, in dilute acids, only about 30 % increase of the hydrolysis rate in presence of molybdate, and Meyerhof & Lohmann (1928) even made the curious observation that the hydrolysis of arginine phosphate in acid solution was actually inhibited in presence of molybdate.

It is perhaps surprising that, apart from these scattered observations, no systematic study of the molybdate effect has yet been made. To some extent this may be due to the fact that with the older methods of phosphate estimation a tedious separation of organic and inorganic phosphate was necessary for its demonstration. In the extraction method of phosphate estimation introduced by Berenblum & Chain (1938) the time of contact between molybdate and phosphate before completion of the phosphomolybdate extraction can be reduced to about 15 sec. This time interval is sufficiently short to reduce the interaction between molybdate and all but the most labile phosphate bonds to insignificant proportions. The method of Berenblum & Chain is, however, somewhat laborious and unsuited to the handling of large series of analyses. The recent modification of Martin & Doty (1949) has greatly simplified it, and with the adaptations which we used in the present study the method is as easy and expeditious to carry out as the method of Fiske & Subbarow without sacrificing any of the advantages of Berenblum & Chain's method.

METHODS

Estimation of phosphate. For the purpose of this investigation the procedure of Martin & Doty (1949) has been modified as follows: (1) the addition of the silicotungstate reagent was omitted since no protein was present in the solutions to be analysed; (2) an economy in solvents and an increase in sensitivity was obtained by reducing the scale of the method; (3) pure isobutanol was used instead of the 1:1 mixture of isobutanol and benzene recommended by Martin & Doty. Excellent reproducibility of results was obtained and the method has given complete satisfaction.

Solutions

5 % Ammonium molybdate in 4N- H_2SO_4 (for inorganic P).

5 % Ammonium molybdate in water (for digests of total organic P, containing 1 ml. 60 % perchloric acid).

Stannous chloride. Stock solution: 10 g. SnCl_2 in 25 ml. conc. HCl.

Stannous chloride. Dilute: 0.5 ml. concentrated solution, diluted to 20 ml. with N- H_2SO_4 . Prepare fresh daily.

Acid ethanol. 10 ml. conc. H_2SO_4 + 490 ml. absolute ethanol.

Procedure

Add 6 ml. isobutanol and 1 ml. acid ammonium molybdate solution to 5 ml. of a test solution containing 2–25 μg . inorganic P and shake for 15 sec. in a glass-stoppered test tube. With a fine-tipped rubber-teat pipette draw off the isobutanol layer as completely as possible, carefully avoiding any admixture of the aqueous phase. Transfer the isobutanol extract to a second test tube containing a pinch of anhydrous Na_2SO_4 and shake until the extract is cleared of any emulsified droplets. Withdraw 5 ml. and transfer to a third test tube. Add 5 ml. acid ethanol, 0.2 ml. dilute SnCl_2 solution, mix and measure the absorption with a red filter (e.g. Ilford spectrum 608). Construct a calibration curve in the usual way.

Measurement of the molybdate effect. In the majority of experiments a final concentration of 1 % ammonium molybdate was used. The test solution was made up of 3 parts buffer, 1 part 5 % aqueous molybdate and 1 part solution of organic phosphate compound, the latter usually in a concentration of 2 or 4 mM (final concn. 0.4 or 0.8 mM). Buffer and phosphate solutions on the one hand and molybdate solution on the other were separately adjusted to the required temperature and mixed at time t_0 . Samples of 1–5 ml. were withdrawn at times t_1 , t_2 , etc., and, if necessary, cooled to room temperature. If the sample withdrawn for analysis amounted to y ml., the volume of 5 % molybdate in 4N- H_2SO_4 was reduced to 1.0–2 y ml. and 0.2 y ml. of 4N-

H_2SO_4 was added instead. In this way the molybdate and acid concentrations used for the phosphate analysis were kept constant.

Control solutions contained 1 part of water instead of molybdate solution. Care was taken to reduce to a minimum the time of contact with molybdate during the extraction by rapid addition of the molybdate solution after all other components, including isobutanol, had been added.

Higher concentrations of molybdate were used in some experiments. Owing to the difficulty of buffering these solutions they were only used when strict pH control was not required and when the experiment could be carried out in the neighbourhood of pH 5. The addition of molybdate solution for phosphate analysis was again so adjusted as to reach the standard concentration.

Procedure in presence of acetyl phosphate. Acetyl phosphate is so labile in presence of molybdate that the separation method must be employed. At time t 1 ml. of the test solution was withdrawn and immediately delivered into a previously determined amount of alkali (or acid) sufficient to bring the pH of the mixture to 7.0. Inorganic phosphate was then precipitated with ethanolic CaCl_2 according to Lipmann & Tuttle (1944).

Buffering. With an ammonium molybdate concentration of 1% the following buffers were found suitable:

pH 1-6: 0.2M-sodium acetate-HCl and sodium acetate-acetic acid buffers (Walpole, 1914).

pH 6-9: 0.2M-sodium borate-HCl and sodium borate-NaOH buffers (Sørensen, 1912).

Values below pH 1-1.5 were obtained by the addition of HCl to a final concentration of 0.5-1.0N.

The final pH of the mixture was measured in every case with a glass electrode standardized against 0.05M-potassium biphthalate (pH 4.00; Marconi buffer solution tablets).

Preparations

Barium adenosinetriphosphate (ATP). Prepared from rabbit muscle by the method of Dounce, Rothstein, Beyer, Meier & Freer (1948). Total organic P, 11.65%; acid-labile P 7.82% (hydrolysed in N-HCl at 100° for 7 min.).

Creatine phosphate. Synthesized according to Zeile & Fawaz (1938). The Ca salt contained 7.68% acid-labile P (purity of 80%).

Acetyl phosphate. Synthesized according to Lipmann & Tuttle (1944, 1947). The crystalline Ag salt contained 8.15% acid-labile P.

Phospho-enol-pyruvic acid. Synthesized according to Baer & Fischer (1949). The Ba salt contained 7.64% iodine-labile P.

Glucose-1-phosphate. Synthesized according to Krahle & Cori (1949). The Ba salt contained 6.68% acid-labile P.

Glucose-6-phosphate. Synthesized according to Lardy & Fischer (1946). The Ca salt, dried *in vacuo* at 78°, contained 10.69% organic P.

Fructose-6-phosphate. Prepared from hexosediphosphate by the method of Neuberg, Lustig & Rothenberg (1943). The Ba salt contained 6.43% organic P.

Fructose-1:6-diphosphate. The acid Ba salt, prepared from a commercial sample by the method of Neuberg *et al.* (1943), contained 12.80% organic P.

Sodium pyrophosphate and sodium α -glycerophosphate were commercial samples.

RESULTS

Effect of molybdate concentration. The catalytic effect of molybdate increases with increasing concentration, although the increase is not strictly linear, especially at higher concentrations (Fig. 1).

Fig. 1. Effect of ammonium molybdate concentration on hydrolysis of ATP. ATP concn. 0.4 mM, pH 4.1, temp. 30°, time 30 min.

Fig. 2. Effect of temperature on the hydrolysis of ATP and sodium pyrophosphate in presence and in absence of ammonium molybdate. Concentration: substrates 0.4 mM, ammonium molybdate 2.5%; pH 5.2, 30 min. Hydrolysis in presence of ammonium molybdate, —; controls, ---; ATP, ○ and ●; pyrophosphate, △ and ▲.

On the other hand, a tenfold increase of substrate concentration did not change the percentage hydrolysis. The facts suggest that the catalytic effect depends on the formation of an unstable association complex. As long as molybdate is

present in great excess, an increase of substrate concentration will not affect the ratio of dissociated to undissociated complex. On the other hand, an

the data in Fig. 2 a temperature coefficient $d \ln k/dT$ of 0.075 has been calculated for the first hydrolysis constant of ATP (t in min.).

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

Fig. 9.

Fig. 10.

Fig. 11.

Fig. 12.

Figs. 3-12. Effect of pH on the hydrolysis of phosphate bonds in presence and in absence of ammonium molybdate. ●—●—●, hydrolysis in presence of 1% ammonium molybdate. ○—○—○, control without ammonium molybdate. Fig. 3. ATP (0.4 mM), 35°, 30 min. Fig. 4. Creatine phosphate (0.8 mM), 0°, 30 min. Fig. 5. Sodium pyrophosphate (0.4 mM), 80°, 30 min. Fig. 6. Phospho-enol-pyruvate (0.8 mM), 80°, 30 min. Fig. 7. Fructose-6-phosphate (0.8 mM), 80°, 120 min. Fig. 8. Acetyl phosphate (0.8 mM), 0°, 15 min. Fig. 9. Glucose-1-phosphate (0.8 mM), 70°, 30 min. Fig. 10. Fructose-1:6-diphosphate (0.8 mM), 70°, 30 min. Fig. 11. α-Glycerophosphate (0.8 mM), 80°, 120 min. Fig. 12. Glucose-6-phosphate (0.8 mM), 80°, 120 min.

increase in the concentration of molybdate will favour the formation of the complex.

Effect of temperature. The temperature-activity curve has a typical exponential form (Fig. 2). From

Effect of pH. The influence of pH on the molybdate effect is illustrated in Figs. 3-12 for a number of organic phosphates. The temperatures and times used in the different experiments were so chosen for

convenience that a significant hydrolysis took place. In at least one case (pyrophosphate) where the effect was studied at 50, 70 and 80° the position of the pH optimum was unaffected by temperature. The effect has frequently a well pronounced pH optimum which varies from compound to compound. Some of the curves even show indications of two peaks, an 'alkaline' and an 'acid' pH optimum, as in the case of fructose-6-phosphate (Fig. 7), ATP (Fig. 3) and pyrophosphate (Fig. 5). With two of the compounds, pyrophosphate (Fig. 5) and glucose-1-phosphate (Fig. 9), the hydrolysis curves in presence and in absence of molybdate cross each other in the acid region of the pH scale. Here molybdate inhibits the hydrolysis, as has been found by Meyerhof & Lohmann (1928) for arginine phosphate.

Glucose-6-phosphate (Fig. 12) and α -glycerophosphate (Fig. 11) show a flat curve with a rise on the alkaline side of neutrality. The curve of glucose-1-phosphate is similar; the rise in the acid range is closely followed, and eventually overtaken, by the curve of the uncatalysed hydrolysis.

Table 1. Hydrolysis of glucose-1-phosphate in presence of 1 % ammonium molybdate

(9 ml. 0.2M-acetate-HCl buffer, pH 2.6, 3 ml. 5 % ammonium molybdate, 3 ml. 0.0033M-glucose-1-phosphate. pH of mixture 3.32. Temp. 70°. Total acid-labile P: 295 μ g./10 ml.)

Time (min.)	P (μ g./10 ml.)	Hydrolysis (%)	$k_{Mo} \times 10^3$
60	29.5	10.0	2.03
90	42	14.2	1.99
120	55.5	18.8	2.04
190	80	27.1	1.98
300	116	39.3	2.01
		Mean	2.01

Hydrolysis constants. As is to be expected the time course of the hydrolysis, in the case of compounds containing one phosphate bond, is that of a first-order reaction, in presence as well as in absence of molybdate. An example is given in Table 1.

Table 2. Effect of 1 % ammonium molybdate on the hydrolysis of some phosphate bonds

(k_{Mo} : hydrolysis constant in presence of molybdate; k_c : hydrolysis constant in absence of molybdate (control).)

Substrate	pH	Temp. (°)	$k_{Mo} \times 10^3$	$k_c \times 10^3$	k_{Mo}/k_c
Acetyl phosphate	4.0	0	226	1.12	202
ATP (terminal group)	4.1	70	78	0.50*	156
Creatine phosphate	2.5	0	54.3	0.40	135
Glucose-1-phosphate	3.3	70	2.01	0.312	6.4
Fructose-6-phosphate	5.8	80	4.17	0.82	5.1
Pyrophosphate	2.2	80	24.5	5.3	4.6
Phospho-enol-pyruvate	4.0	80	55	12.5	4.4
α -Glycerophosphate	6.3	80	0.364	0.150	2.4
Glucose-6-phosphate	6.3	80	0.306	0.155	2.0

* In absence of molybdate both acid-labile phosphate groups of ATP are assumed to be hydrolysed equally (cf. text p. 290).

The hydrolysis of ATP, a compound with two acid-labile phosphate bonds, is more complicated. The time curve (Fig. 13) of the molybdate-catalysed hydrolysis shows a break after the hydrolysis of only 50 % of the acid-labile P. The hydrolysis constants for the two phosphate groups, k_1 and k_2 , were calculated from the data by making an estimate of several possible values for k_2 from that part of the curve lying between 50 and 100 % hydrolysis, and selecting that value which gave the best straight line when the hydrolysis of the terminal phosphate group, corrected for the simultaneous hydrolysis of the intermediate phosphate group at the assumed rate, was plotted on a logarithmic scale. For the experiment of Fig. 13 the hydrolysis constants of the terminal and intermediate phosphate groups in presence of molybdate, k_{Mo_1} and k_{Mo_2} , respectively, were found to be 0.078 and 0.0080. In Fig. 13 the

Fig. 13. Time curve of hydrolysis of the labile phosphate groups of ATP in presence and absence of ammonium molybdate (1 %). ATP concn. 0.4 mM. Temp. 70°.

curve is the calculated one, formed by the superimposition of the two first-order reactions, whereas the crosses represent the actual observations. The close agreement between observation and calculation shows that the estimates for k_{Mo_1} and k_{Mo_2} were substantially correct. The catalytic effect of molybdate on the terminal phosphate group is thus ten times greater than that on the intermediate phosphate group. In contrast to this, as has been shown

by Lohmann (1932), the two phosphate groups are hydrolysed at the same rate in absence of molybdate. The hydrolysis constant of the control has therefore been calculated on the assumption that the hydrolysed phosphate groups were derived equally from the terminal and the intermediate phosphate groups of ATP.

In Table 2 a number of hydrolysis constants in presence (k_{mo}) and in absence (k_c) of molybdate are listed. The experiments were usually performed at the pH optimum of the molybdate effect and at a temperature convenient for the measurement of the reaction rate within a period of several hours.

The catalytic effect of molybdate has been expressed numerically by the ratio of k_{mo}/k_c . No compound has yet been found where the molybdate effect was completely absent, but there is a large variation of the ratios with different compounds, ranging from about 2 to about 200.

Effects of tungstate and vanadate. Since the catalytic effect of molybdate is presumably connected with its tendency to form a heteropoly acid complex with phosphoric acid or its derivatives the question arose whether tungstate and vanadate, which form similar complexes, have also similar catalytic properties. Both tungstate and vanadate interfered with the direct colorimetric estimation of phosphate and it was therefore necessary to carry out a preliminary precipitation and separation of inorganic phosphate as magnesium ammonium phosphate ($MgNH_4PO_4$). The technique was tested on standard solutions of phosphate in presence of tungstate or vanadate and a satisfactory recovery was obtained.

Table 3. *Hydrolysis of ATP in presence of molybdate, tungstate and vanadate*

(Concn. of ATP 1686 μ g. acid-labile P/10 ml. Incubated at 70° for 30 min.)

Addition	pH	Hydrolysis	
		P (μ g./10 ml.)	Acid-labile P (%)
Nil	3.98	46	2.7
Sodium tungstate (1%)	4.32	46	2.7
Ammonium vanadate (0.9%)	4.42	76	4.5
Ammonium molybdate (1%)	4.21	650	38.6

In the experiment in which the catalytic activity was examined a third sample was set up with molybdate in which inorganic phosphate was precipitated with magnesia mixture in the same way. The result (Table 3), while showing the catalytic effect of molybdate, gave no significant indication of a similar effect of either tungstate or vanadate.

DISCUSSION

The relation of the catalytic effect to the properties of the phosphate bond. The k_{mo}/k_c ratios of the substances investigated fall into two sharply separated groups.

Acetyl phosphate, ATP-terminal phosphate and creatine phosphate show ratios 20 to 100 times higher than the rest, while the differences of ratios are comparatively small within the two groups. It is clear that there is no strict correlation between the stability of a phosphate bond towards acid and the catalytic effect of molybdate. The best example for this lack of correlation is the difference of the molybdate effect on the two acid-labile phosphate groups of ATP. Both these groups have the same heat of hydrolysis (Meyerhof & Lohmann, 1932), and are hydrolysed by hydrogen ions at the same rate (Lohmann, 1932). Yet biologically they are not equivalent since, according to current views, only the terminal phosphate group of ATP is directly utilized in transphosphorylation or hydrolysed by the specific adenosinetriphosphatases of muscle and other tissues. This differential behaviour towards enzymes is reflected in the difference in the effect of molybdate. The high-energy phosphate bonds of acetyl phosphate and creatine phosphate have a molybdate-lability similar in magnitude to that of the terminal group of ATP. On the other hand, the phosphate bond in pyrophosphate, whose energy content is high but biologically unavailable, has a molybdate lability of the same order as the intermediate phosphate group of ATP, and as most of the low-energy phosphoric esters investigated. Phosphoenol-pyruvic acid seems to be exceptional, since it combines a low molybdate lability with a phosphate bond which is both rich in energy and biologically available. But, as has recently been pointed out by Oesper (1950), the phosphate bond of phosphoenol-pyruvic acid belongs to the family of low-energy esters, and the energy released on hydrolysis is probably largely derived from the conversion of the unstable enol to the stable keto configuration.

The difference between the effect of molybdate on acid-resistant phosphate bonds, as in glucose-6-phosphate, and on acid-labile bonds of the low-energy type, as in glucose-1-phosphate, is small compared with the difference between groups of similar stability towards acid, but a different content of biologically available energy.

Mechanism of the catalytic effect and its biological significance. It has been suggested (Lipmann & Tuttle, 1944) that the catalytic effect of molybdate is due to the removal of inorganic phosphate in complex form. This view cannot be correct, for the following reasons: (1) The theory presupposes the existence of an equilibrium in which the removal of one reaction partner simply reveals, but does not accelerate, the rate of the forward reaction. The normal dynamic equilibrium would be maintained by the reverse (synthetic) reaction and molybdate would act by suppressing the latter. The theory thus leads to the assumption of the highest rate of spontaneous synthesis in exactly those phosphate

compounds, e.g. creatine phosphate and acetyl phosphate, which have the highest degree of instability. In fact, the thermodynamic equilibrium lies so far in favour of the split products that the rate of the reverse reaction must be quite insignificant. (2) The complex between molybdate and inorganic phosphate is very stable, and a small excess of molybdate is sufficient to convert inorganic phosphate quantitatively into the complex. The sustained increase of the catalytic effect with increasing molybdate concentrations, however, points to the existence of an unstable complex, the concentration of which determines the rate of the reaction. (3) The existence of a sharp pH optimum in some cases, and the fact that it varies from compound to compound, are incompatible with a mechanism which would be generally identical. It rather suggests that the rate of reaction depends on the formation of specific complexes with either a stability maximum or a decomposition maximum in a narrow range of the pH scale, depending on whether the formation or the decomposition of the complex is the rate-determining step.

It may therefore be suggested that the effect of molybdate is due to the formation of a complex with the substrate as a first step in the breaking of the phosphate bond. Complexes of molybdate with organic compounds, phosphorylated or not, are well known; those with α -hydroxy acids have been particularly studied owing to the analytical interest attached to the large increase in optical rotation which they frequently show (see, for example, Auerbach & Krüger, 1923; Meyerhof & Schulz, 1938; Krebs & Eggleston, 1943).

An evaluation of the thermal data (Fig. 2) is consistent with the assumption of an intermediary complex between catalyst and substrate. The following figures have been calculated for the overall reaction from the equation $k = Ae^{-\Delta E/RT}$, where k is the velocity constant, A the temperature-independent factor, ΔE the activation energy, R the gas constant and T the absolute temperature. A temperature-independent factor (A) of 10^{13} is obtained for the uncatalysed hydrolysis of sodium pyrophosphate, which is of the order found for 'normal' reactions. In presence of molybdate this factor is decreased to 10^7 in the case of sodium pyrophosphate and to 10^6 in the case of the terminal phosphate group of ATP, indicating a higher degree of restraint in the transition state, such as would be expected if an intermediary complex were formed. For the overall activation energy of the uncatalysed hydrolysis of sodium pyrophosphate a value of about 29,000 cal. has been calculated, which is also of the order commonly found for reactions of this type. In presence of molybdate the activation energy is lowered to about 17,200 cal. in the case of sodium pyrophosphate and to about 13,000 cal. in the case

of the terminal phosphate group of ATP. If it is assumed that the catalysed hydrolysis of pyrophosphate takes place side by side with the uncatalysed reaction, the activation energy for the catalysed reaction would be about 14,000 cal. These values are of the same order as those frequently found for enzymic catalysis, which is generally assumed to depend on the formation of intermediary complexes.

The formation of a molybdate-substrate complex may perhaps explain the specific inhibition of 'acid' phosphatases by molybdate (Munemura, 1933; Courtois & Bossard, 1944; Bossard, 1947). A direct reaction of molybdate with the enzyme can of course not be excluded.

There are numerous examples of inorganic catalysts whose action is the prototype of an enzyme action, and frequently the enzyme concerned contains the same element as part of its active centre. But there is at present no reason to attach to molybdate any such significance as a phosphatase model. It is true that molybdenum is an essential trace element, at least for plants (Arnon & Stout, 1939; Arnon, 1940), but its function seems to be connected with processes of nitrogen reduction (Mulder, 1948) and nitrogen fixation (Burk & Burris, 1941; Burris & Wilson, 1945) rather than with phosphatase activity. The toxicity of molybdate, on the other hand, may be connected with its inhibitory effect on phosphatases, since Comar, Singer & Davis (1949) found disturbances of bone development in growing rats subjected to molybdate poisoning.

Passing reference should be made to some related phenomena. Lohmann (1931) was the first to point out the enhanced instability of calcium pyrophosphate and of calcium ATP compared with the sodium salts, especially in weakly alkaline solution. Lipmann & Tuttle (1944) found the same to be true for acetyl phosphate, and Bielschowsky (1950) has recently reported a special instability of the terminal group of magnesium-ATP. Bamann & Meisenheimer (1938) and Bamann & Nowotny (1948), in a series of papers, showed that the salts of polyphosphates and glycerophosphates with lanthanum and other rare earths have an increased tendency towards hydrolysis. The lanthanum effect has a well marked pH optimum of about 8, while in acid medium the hydrolysis, at least of pyrophosphate, is inhibited. It may be recalled that similar observations were made in the case of molybdate.

Analytical implications. The catalytic effect of molybdate has been used for the estimation of creatine phosphate since the work of Fiske & Subbarow (1929). The present results suggest that the same principle could be extended with advantage to distinguish between compounds of similar acid lability, but differing molybdate lability, e.g. between acetyl phosphate and ribose-1-phosphate or between ATP and glucose-1-phosphate.

In recent years the method of Lowry & Lopez (1946) has been widely used in biological research for phosphate estimations. Though the authors compared the hydrolysis of creatine phosphate, acetyl phosphate and ribose-1-phosphate under the conditions of their method and of the method of Fiske & Subbarow (1925) they did not investigate the activity-pH curve of the molybdate effect systematically, and one is left with the impression that the effect increases with increasing acidity. In the particular case of creatine phosphate it may be an advantage to carry out the analysis at pH 4, but it must not be assumed that this is true generally. For example, the pH optimum of the molybdate effect with ATP is 4, precisely the pH chosen by Lowry & Lopez; although the hydrolysis of ATP may not be significant at room temperature and at the low concentration of ammonium molybdate used in their method, it should be borne in mind that there is no particular region of the pH scale which could be judged universally safe as far as the catalytic effect of molybdate is concerned.

SUMMARY

1. The catalytic effect of molybdate on the hydrolysis of a number of phosphate compounds has been investigated.

2. The effect increases with increasing concentration of molybdate, though not in a strictly linear fashion, and also exponentially with temperature. Evaluation of the thermal data indicates a lowering

of the activation energy and of the temperature-independent factor by molybdate.

3. The molybdate effect may have a well pronounced pH optimum which varies from compound to compound. In two compounds hydrolysis was inhibited by molybdate at the acid end of the pH scale.

4. For the substances investigated, the ratios of the hydrolysis constants in presence and in absence of molybdate fall into two groups. The first group consists of acetyl phosphate, creatine phosphate and the terminal phosphate group of adenosinetriphosphate, which show ratios 20 to 100 times higher than those of the second group, which comprises the other substances studied. The differences of the ratios within these two groups are relatively small. A catalytic effect of molybdate was demonstrated in all the compounds tested.

5. Although the terminal and intermediate phosphate groups of adenosinetriphosphate are hydrolysed at the same rate by acid, their hydrolysis constants in the presence of molybdate differ by a factor of 10.

6. No catalytic activity was found when molybdate was replaced by tungstate or vanadate.

7. It is suggested that the catalytic effect of molybdate involves the formation of an unstable transition complex between catalyst and substrate. The biological and analytical implications of the effect are discussed.

We are greatly indebted to Dr J. Stern for the thermodynamic calculations and discussion.

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