

INTERACTION OF CLAY MINERALS WITH ADENOSINE-5-PHOSPHATES¹

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Abstract—Adenosine-5-phosphates (ATP, ADP, AMP) are adsorbed by clay minerals at very low concentrations (≤ 2 mg/liter). In contrast to quartz, the clay minerals exhibit a strong preference for ATP over AMP. The experimental data are expressed as recovery rates (adenosine-phosphate in solution to total nucleotide added). For example, the recovery rates of ATP, ADP, and AMP in the presence of sodium montmorillonite are 0, 17, and 100%; in the presence of quartz 95, 100, and 99%. The recovery rate of AMP on clays is markedly decreased by the presence of ATP, that is, ATP increases the adsorption of AMP by cooperative interactions.

A part of ATP not recovered in the equilibrium solution is dephosphorylated to ADP. For example, 45% of ATP not recovered in equilibrium solution with calcium montmorillonite is recovered as ADP; with sodium montmorillonite only ADP can be recovered in solution.

Key Words—Adenosine-5-phosphate, Adsorption, Anion exchange, Illite, Montmorillonite.

INTRODUCTION

The bonding of anions by clay minerals involves different mechanisms more complex than cation-exchange reactions. Besides exchange of anions in the double layers on the crystal edges, specific anion adsorption is very pronounced. A well-established example is the substitution of lattice OH^- ions by F^- anions on crystal edges and, in the case of kaolinite, on basal planes (Weiss *et al.*, 1956).

A particularly relevant problem encountered by soil scientists and biologists is that of the reaction of phosphate ions which are irreversibly bound at low coverages (Muljadi *et al.*, 1966a, 1966b). A study of adsorption and desorption of more complex anions, such as adenosine-5-phosphates (ATP, ADP, AMP) (Figure 1), is a prerequisite for biologists who wish to extract nucleotides from sediments during ecophysiological studies.

A large proportion of ATP (in comparison with ADP and AMP) is the basic requirement for cell growth (Chapman *et al.*, 1971). Ernst (1970) measured the ATP-content in marine sediments as an estimate of the microbial biomass. ATP was extracted from the sediment with solutions of "Tris" [= tris(hydroxymethyl)aminomethane], a buffer reported to be very effective. However, complete desorption of ATP was

not achieved. This was attributed to the presence of clay minerals (Conklin and MacGregor, 1972; Anderson and Davies, 1973), but a relationship between clay content and recovery rate could not be established (Ferguson and Murdoch, 1975).

A first insight into the interactions of adenosine-5-phosphates with clay minerals and silica may be obtained by determination of the recovery rates of ATP, ADP, and AMP in the presence of different clays and silica. The recovery rate is the ratio of the amount of a nucleotide in solution to the total amount of this nucleotide added to the clay or silica suspension. The recovery rate is representative for the system at low nucleotide concentrations. Representation of the experimental data by recovery rates was chosen because recovery rates can be directly deduced from the measurements. Representation by adsorption data requires the knowledge of dephosphorylation of the nucleotides at the clay surface (see below).

MATERIALS AND METHODS

Determination of recovery rates

The procedure used corresponds to ATP-determinations employed by biologists (Holmsen *et al.*, 1966; Karl and Larock, 1975). The adsorption of the nucleotides was studied at very low concentration, and the desorption was measured after dilution not only with water but also with buffer solutions, e.g., solutions of "Tris" at 100° and 0°C. (A boiling buffer is required for decomposition of the cells in biological experiments.)

¹ Contribution 255 of joint research program 95 "Interaction Sea–Seabottom."

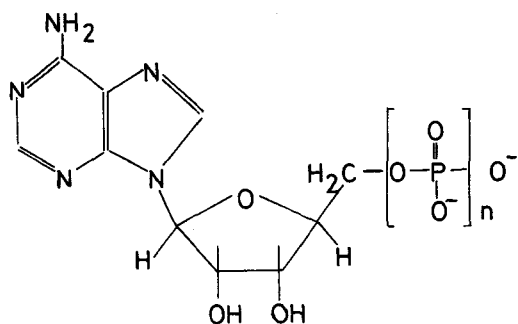


Figure 1. Adenosine-5-phosphates, $n = 1$: adenosine-5-monophosphate (AMP), $n = 2$: adenosine-5-diphosphate (ADP), $n = 3$: adenosine-5-triphosphate (ATP).

Since small amounts of dispersed clay disturb the photometric ATP-determination, the clays were suspended in sodium chloride solution with a concentration high enough to ensure complete coagulation of the particles (cf. Frey and Lagaly, 1979). Thus, 20–30 mg of clay or 50–100 mg of quartz was suspended in 0.2 ml of 0.1 M solution of sodium chloride (pH 5–6) and stored for about 18 hr in a refrigerator. One tenth milliliter of nucleotide solution was added with cooling in an icebath, and the samples were shaken for a few seconds on a vortex mixer. After 5 min the solution was diluted with water or boiling buffer [0.02 M tris(hydroxymethyl)aminomethane, adjusted to pH = 7.8 with HCl] and stored again at 0°C. After centrifugation at 3400 rpm below 5°C the amount of nucleotide was determined in the supernatant. The contact time of clay with nucleotide was 25 min; longer times (1–2 hr) did not affect the results. The contact time could not markedly be increased because of the instability of the nucleotide solutions.

Determination of ATP

From 20 to 250 μ ml of the ATP solution was added to 1.5 ml of a solution of "Tris" and magnesium sulfate (20 mmoles "Tris," pH = 7.8, and 7.6 mmoles magnesium sulfate per liter) and made up with water to 2 ml in a scintillation vial. The vial was then inserted into a JRB ATP-photometer, and 0.5 ml of FLE-solution²) (diluted 2:5 with water) was injected with an automatic dispenser. A green-yellow emission (McElroy, 1947) whose intensity was proportional to the ATP concentration (Chase, 1960), was produced by reaction of the ATP with luciferine-luciferase. The signal was recorded on a millivolt recorder and the peak-height measured. The detection limit is about 0.5 picomoles (=0.2 ng) of ATP. All values reported are averages from at least 5 experiments.

² FLE = firefly-lantern extract, luciferine-luciferase extract from *Photinus pyralis*.

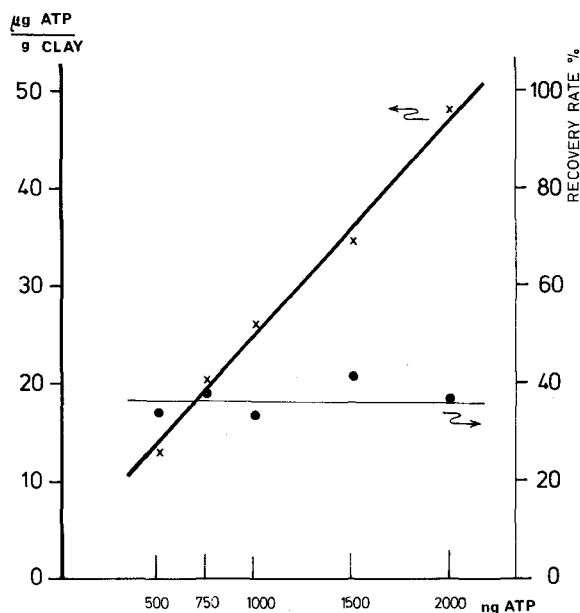


Figure 2. Adsorbed amounts of ATP (x) and recovery rate (●) (ATP in solution to total ATP added) vs. total ATP added (20 mg sodium montmorillonite, 500–2000 ng ATP in 0.3 ml of NaCl solution, diluted with 1.5 ml of boiling solution of "Tris."

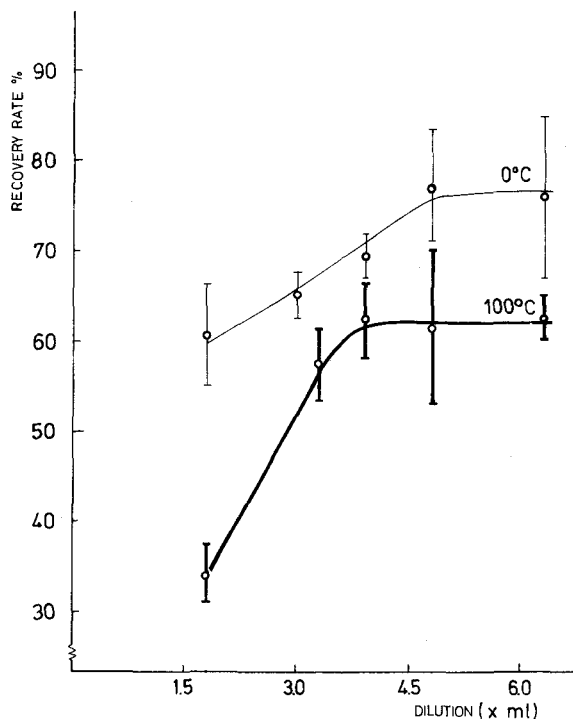


Figure 3. Recovery rates vs. dilution after adsorption (500 ng ATP added to 20 mg sodium montmorillonite in 0.3 ml of NaCl solution, then diluted with x ml of "Tris" at 0°C and 100°C. Recovery rate is the ratio of ATP now in solution to the total of ATP added).

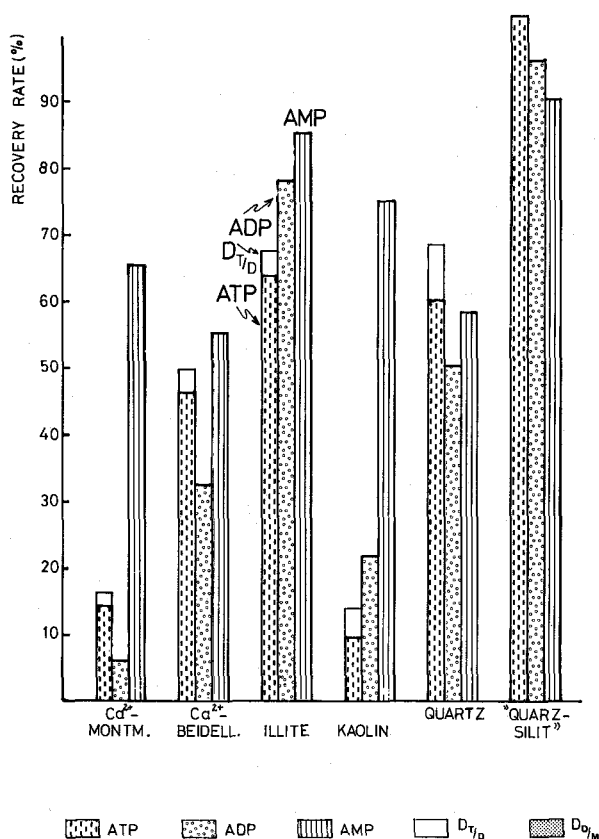


Figure 4. Recovery rates of adenosine-5-phosphates in the presence of clay minerals, quartz, and "quarzsilit" (2000 ng ATP, ADP, or AMP per 20 mg adsorbents in 0.3 ml of NaCl solution, diluted with 6 ml of boiling solution of "Tris"). $D_{T/D}$ refers to the dephosphorylation of ATP to ADP under influence of the solid surface. It is the recovery of ATP after conversion of the solution ADP into ATP. The same for ADP/AMP ($D_{D/M}$).

Enzymatic conversion

For determination of ADP and AMP, the nucleotides were converted to ATP by the enzymes pyruvatekinase and myokinase (Witzel, 1979). The conversion was initiated by the addition of 330 picomoles ATP.

Materials

Beidellite (Unterrupsroth, Rhön, Germany) and montmorillonite (bentonite from Geisenheim, Germany) were treated with dithionite solutions to remove the iron oxides and with H_2O_2 to destroy organic materials. The $<1\text{-}\mu\text{m}$ fraction was separated by sedimentation. Homoionic sodium and calcium forms were prepared by reaction with NaCl and $CaCl_2$ solutions. Kaolin (Georgia Hydrite, without industrial processing), illite (from Fuzeradvany, Hungary), quartz (1–3.16- μm fraction), and synthetic carborundum SiC ("Quarzsilit," Firma Kick, Schnaittenbach) were employed as received.

Luciferine-luciferase ("FLE-250") was supplied by SIGMA Chemicals, Munich, Germany; pyruvatekinase, myokinase, phosphoenolpyruvate, and nucleotides by Boehringer, Mannheim, Germany; and tris(hydroxymethyl)aminomethane ($(HOCH_2)_3CNH_2$ ("Tris")) from Merck, Darmstadt, Germany.

RESULTS

Recovery rate

Part of the ATP added to the clay or quartz suspension was adsorbed (Figure 2). Dilution of the equilibrium solution with water or buffer increased the amount of ATP in solution to a limiting value (Figure 3), demonstrating irreversible adsorption of a certain amount of ATP. Since the adsorption isotherm is linear in the concentration range studied, the limiting recovery rate (total amount of ATP in solution to total amount of ATP added) remained independent of ATP-concentration (Figure 2). (Exception: with sodium beidellite the recovery rate decreased from 37% at 100 μg total ATP/g beidellite to 0% at 50 $\mu\text{g/g}$.) Extraction with boiling buffer resulted in a significant lower desorption (lower recovery) (Figure 3). Increased adsorption with rising temperature was observed for phosphate adsorption by kaolinite (Muljadi *et al.*, 1966a, 1966b).

ATP, ADP, and AMP

In suspensions of quartz and "quarzsilit" the recovery rates of ATP, ADP, and AMP were nearly identical—90% for "quarzsilit" and 50–60% for quartz. The clay minerals differentiate between ATP, ADP, and AMP (Figure 4). The recovery rate is especially high for AMP (55–85%) depending on the clay mineral. The specificity is most pronounced for kaolin and montmorillonite; only 14% of the ATP can be recovered in contrast to 75 and 65% of the AMP. The recovery of ATP increases from 14% for calcium montmorillonite, to 46% for calcium beidellite, to 64% for illite. ADP reacts differentially, i.e., smectites show a slight preference for ATP over ADP; for illite and kaolinite, this is not the case.

Adsorption at such low levels as used may be caused by impurities, especially quartz or amorphous silica which were not removed during the preparation of the homoionic forms of the clays. Figures 4 and 5, however, make evident that quartz and also amorphous silica do not reveal any specificity for nucleotide adsorption.

Influence of "Tris"

Although biologists start such extractions with boiling solutions of "Tris," the present results clearly indicate that this does not promote desorption but instead leads to an increase in the amount of irreversibly fixed nucleotides. This is especially true for AMP, ADP, and ATP on quartz and "quarzsilit" and for AMP on clay

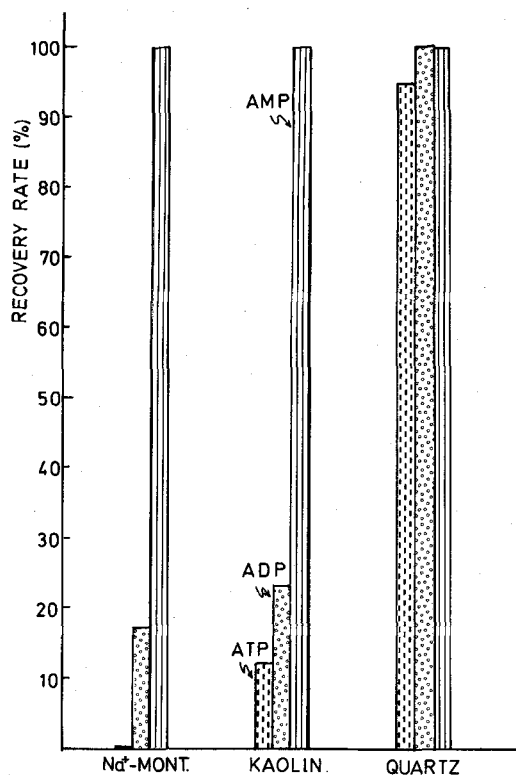


Figure 5. Recovery of ATP without dilution with "Tris" (experimental data as in Figure 3, but solutions not diluted with buffer).

minerals. The nucleotides are completely desorbed by dilution with water (recovery rate 100%, Figure 5), but not by dilution with "Tris." The recovery of ADP on clays is only slightly affected. ATP is completely fixed by sodium montmorillonite in aqueous solution and partially fixed in the presence of "Tris" (the recovery rate increased from 0 to 63%).

Influence of sodium diphosphate

Sodium diphosphate $\text{Na}_4\text{P}_2\text{O}_7$ is generally used as peptizing agent for smectites. Clay minerals treated with $\text{Na}_4\text{P}_2\text{O}_7$ prior to the adsorption of nucleotides had remarkably higher recovery rates, indicating a decrease in the amount of adsorption. For kaolinite, the recovery increased from 10 to 88%; for sodium montmorillonite (in the absence of "Tris"), from 0 to 38%.

Dephosphorylation of ATP

During adsorption of ATP some molecules were hydrolyzed to ADP by loss of the terminal phosphate group. Table 1 shows the extent of ATP-hydrolysis under the influence of different solid surfaces.

Thus, only a fraction of the unrecovered ATP was really adsorbed, the rest was transformed to ADP. The recovery of ATP with calcium montmorillonite, for example, was 14%; i.e., 86% of the ATP added could not

Table 1. Transformation of ATP to ADP.

Sample	ATP transformed into ADP ¹
Sodium montmorillonite	100%
Calcium montmorillonite	45%
Sodium beidellite	72%
Calcium beidellite	24%
Illite	14%
Kaolin	21%
Quartz	41%

2000 ng total ATP, 20 mg clay or quartz, extraction with "Tris" at 100°C.

¹ Percentage of ATP not recovered as ATP in solution.

be recovered as ATP in solution. About 45% of this amount was recovered in solution as ADP. The dephosphorylation of ATP was especially high on the surfaces of sodium smectites and of quartz in the presence of "Tris." Dephosphorylation of ADP into AMP was also observed with sodium montmorillonite and beidellite (Figure 6).

Cooperative reactions

In the presence of ATP the recovery of AMP in clay suspensions was drastically reduced. For illite, it was decreased to half of its value; for the other clay minerals it dropped to 5–10%: AMP was almost completely adsorbed. The recovery of ATP itself remained un-

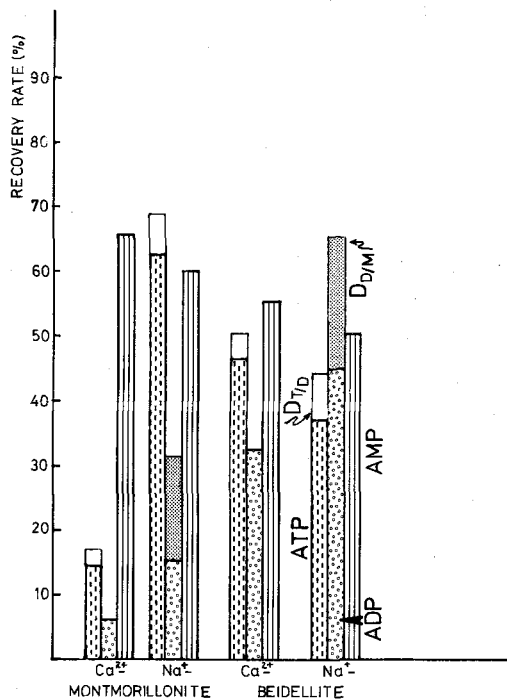


Figure 6. Recovery rates of adenosine-5-phosphates of calcium and sodium smectites after dilution with boiling buffer (experimental data as in Figure 3).

Table 2. Recovery of AMP in presence of ATP.

Adsorbents	Recovery rate (%) in absence of ATP	Recovery rate (%) in presence of ATP		Recovery rate (%) in absence of AMP
	AMP	AMP	ATP ¹	ATP
Sodium montmorillonite	60 ± 13	9 ± 4	60	63 ± 3
Calcium montmorillonite	66 ± 3	4 ± 3	10	14 ± 2.4
Sodium beidellite	50 ± 5	7 ± 2	0-2	37 ± 9.6
Calcium beidellite	56 ± 3	11 ± 7	26	46 ± 5.3
Illite	85 ± 4	43 ± 7	50	64 ± 8.3
Kaolin	75 ± 4	11 ± 7	8	9 ± 4
Quartz	59 ± 5	57 ± 15	60	60 ± 6

2000 ng AMP, 2000 ng ATP, 20 mg clay or 50 mg quartz, dilution with "Tris" at 100°C.

¹ Limits of error not given because of three parallel measurements only.

changed by the addition of AMP; only in the presence of beidellite was it markedly decreased (Table 2). No mutual influence was observed on quartz.

DISCUSSION

Oxides and clay minerals adsorb phosphate anions by exchange of OH-groups, as described extensively for alumina and kaolinites by Muljadi *et al.* (1966) and for TiO₂ surfaces by Flaig-Baumann *et al.* (1970) and Cornejo *et al.* (1978). In general, anions of medium-strong or weak oxo-acids are tightly bound on oxide surfaces. Phosphate anions are especially strongly adsorbed, and are only slowly removed by extensive washing with water and incompletely displaced by other anions. Anion exchange also governs the sorption of polyphosphate anions (Lyons, 1964; Joyce and Worall, 1970; Balzer and Lange, 1979). Lyons (1964) estimated the number of adsorption sites of kaolinite for triphosphate anions to be 0.1 mmole/100 g. A comparable value ($V_1^M = 0.7$ mmole/100 g) was deduced from phosphate adsorption on kaolinite (Muljadi *et al.*, 1966). It is likely that sorption of adenosine-5-phosphates proceeds by similar exchange mechanisms. The highest amounts of ATP adsorbed in the concentration range studied were about 0.02 mmole/100 g.

Muljadi *et al.* (1966) suggested that the exchangeable OH-groups of clay minerals are OH⁻ ions linked to aluminum ions at the crystal edges. Surface OH-groups bound to silicon ions are more difficult to exchange which explains the high recovery of nucleotides in the presence of silica and "quarzsilit" reported in the present work.

Specific interactions were observed with clay minerals: AMP was not adsorbed or was adsorbed only to a small extent in contrast to the strong adsorption of ADP and ATP. The bulky adenosine group in AMP apparently affected the anion exchange by sterically hindering close contacts between exchangeable sites and the phosphate group. If the terminal phosphate group is farther from the adenosine group, as in ADP and ATP, the probability of displacing OH⁻ ions from aluminum ions would be larger.

In all experiments the clays were used in a coagulated state. The structure of the coagulate may influence the number of bonding sites which are accessible to the nucleotides, and, therefore, may affect the recovery. Edge-edge and edge-face contacts in the "Kartenhaus" structure can blockade sites for the bonding of phosphate anions. In sodium smectite suspensions, the "Kartenhaus" is built up by single silicate layers or by a few (up to 6) almost parallel silicate layers. In the presence of calcium ions,³ larger aggregates are formed by face-to-face contacts, and new adsorption sites for anions are created by equimolar bound calcium ions (Weiss, 1958)



Figure 6 shows that the effect of calcium ions was most pronounced for montmorillonite and less for beidellite. The different behavior of both clays requires further studies.

Adsorption of diphosphate anions prior to adsorption of nucleotides increased the negative charge at the clay edges and hindered the adsorption of adenosine phosphate; the recovery rates were increased.

AMP was not adsorbed by clays. The marked adsorption which occurred in the presence of ATP hints at cooperative phenomena. It may be that ATP molecules enlarge the edge-edge and edge-face distances and create new sites for AMP adsorption or that AMP molecules surrounding the adsorbed ATP adopt a conformation which favors exchange of lattice OH⁻ ions.

The effect of "Tris" requires further studies. Since "Tris" increases the pH from 5 to 7.8, a decreased adsorption would be expected analogous to phosphate adsorption. The increased adsorption of AMP may have resulted from changes of the ionic charge and/or conformation. A more probable explanation is that

³ In the experiments with calcium smectites the ratio mval Ca²⁺/mval Na⁺ was about 1.

AMP is bounded via tri(hydroxymethyl)aminomethane molecules on the surface.

A fraction of the ATP anions were hydrolyzed to ADP under the influence of the mineral surface. Evidently, the terminal phosphate group was anchored on the surface by anion exchange and the activation energy for breaking the P–O–P bond between the terminal and second phosphate group was decreased. This corresponds to Lyons' (1964) experimental results from studies of hydrolysis of tripolyphosphate on kaolinite. Lyons estimated an activation energy under the influence of the kaolinite surface of about 63 kJ mole compared with 117 kJ mole in free solution.

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Резюме—Аденоцин-5-фосфат (АТФ, АДП, АМП) адсорбируются глинистыми минералами при очень низких концентрациях (≤ 2 мг/литр). В противоположность кварцу, глинистые минералы проявляют сильное предпочтение к АТФ по сравнению с АМП. Экспериментальные данные выражались в виде степени регенерации (аденоцин-фосфаты в растворе к общему добавленному их количеству). Например, степенями регенерации АТФ, АДП, и АМП в присутствии натриевого монтмориллонита являются 0, 17, и 100%; в присутствии кварца 95, 100, и 99%. Степень регенерации АМП на глинах заметно уменьшается в присутствии АТФ, то есть, АТФ увеличивает адсорбцию АМП в результате кооперативного взаимодействия.

Часть АТФ, не регенерированная в равновесном растворе, дефосфорируется в АДП. Например, 45% АТФ нерегенерированные в равновесном растворе с калиевым монтмориллонитом регенерируются как АДП; в присутствии натриевого монтмориллонита только АДП может быть регенерирована в растворе. [N. R.]

Resümee—Adenosin-5-phosphationen (ATP, ADP, AMP) werden von Tonmineralen bereits bei sehr kleinen Konzentrationen (≤ 2 mg/litre) adsorbiert. Im Gegensatz zu Quarz binden die Tonminerale ATP bevorzugt vor AMP. Die experimentellen Daten werden durch die Wiederfundraten ausgedrückt, die angeben, welcher Anteil des einer Suspension zugesetzten Nucleotids in der Gleichgewichtslösung zurückbleibt. Zum Beispiel werden in Natriummontmorillonit-Suspensionen 0, 17, und 100% des zugesetzten ATP, ADP, und AMP wiedergefunden, während bei Quarz keine Adsorption erfolgt (Wiederfundraten 95, 100, und 99%). Die Wiederfundrate von AMP an Tonmineralen sinkt stark ab, wenn gleichzeitig ATP anwesend ist: die Adsorption des AMP wird durch kooperative Wechselwirkungen mit ATP begünstigt.

Die Abnahme des ATP-Gehaltes der Suspension geht nicht nur auf die Adsorption des ATP zurück; ein Teil des ATP wird an der Festkörperoberfläche in ADP hydrolysiert. So finden sich z.B. in Calciummontmorillonit-Suspensionen 45% des ATP als ADP wieder, bezogen auf die Differenz: zugesetztes ATP–ATP in der Gleichgewichtslösung. Bei Natriummontmorillonit wird sogar nur ADP in der Lösung gefunden.

Résumé—Les phosphates-adenosine-5 (ATP, ADP, AMP) sont adsorbés par des minéraux argileux à de très basses concentrations (≥ 2 mg/litre). Contrairement au quartz, les minéraux argileux montrent une forte préférence pour l'ATP par rapport à l'AMP. Les données expérimentales sont exprimées en taux de récupération (l'adénosine phosphate en solution par rapport au nucléotide total ajouté). Les taux de récupération d'ATP, ADP, et AMP en présence de montmorillonite sodium par exemple, sont 0, 17, et 100%; en présence de quartz, 95, 100, et 99%. Le taux de récupération d'AMP sur les argiles est remarquablement amoindri en présence d'ATP, c'est à dire, l'ATP accroît l'adsorption d'AMP par interaction cooperative.

Une partie d'ATP non récupéré dans la solution d'équilibre est déphosphorilaté en ADP. Par exemple, 45% d'ATP, non récupéré dans la solution d'équilibre avec la montmorillonite calcium sont récupérés comme ADP, avec la montmorillonite sodium, seul l'ADP peut être récupéré en solution. [D. J.]