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The Effect of V(III)–Adenine Complex on Yeast as a Model of Eukaryotic Cells

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The dinuclear μ-okso vanadium (III) complex compound $H_4V_2OCl_4(Ad)_2$ synthesized in our laboratory was investigated as a potential cytotoxic agent against yeast cells. The results of these studies could be helpful in the explanation of the mechanism governing the V (III) compound action on yeast as a simple model of eukaryotic cells. The important factors influencing the toxicity of this complex compound are: the stage of the yeast life cycle, the rate of growth and the pH of reaction mixture. The lethal effect was distinctly stronger when the reaction mixture was slightly acidic (pH = 4). In such solutions V(III) mononuclear species with adenine was relatively stable, and during the time of experiment possibly only a slow oxidation process to V(IV) occurred. In the solutions with pH = 7, several hydrolytic, perhaps mixed—valence, polynuclear species were present and their action on the yeast cells was rather weak. The increased lethal activity of this compound in acidic solutions may be useful in specific treatment against cancer cells whose cytoplasm and/or closest surrounding has lower pH value. The next important result was an observation that the killing activity of this compound was enhanced for yeast cells being in log phase. Also these which had a slower rate of growth (possessing some auxotrophic mutations) were more resistant than those growing faster. The extent of yeast mutagenesis caused by the complex compound is negligible, as the number of mutants found in experiments was within the limit of experimental error. These results are promising and the investigated complex can be considered as a potential anti cancer agent.

Key words: adenine, anti-cancer drugs, cancer treatment, vanadium complex compound, yeast.

Abbreviations: V(III), trivalent vanadium; IR, infra red; c.f.u., colony forming units; VA, adenine-vanadium complex.

Several vanadium compounds seem to have therapeutic effects. Of these, the best recognized is the so-called "insulin mimetic" effect of V (IV) and V (V) complexes with such ligands as maltol, pyridone, *N-N*-disalicilidine ethylene diamine, bis-α-furancarboxylate, etc (*1–5*). Recently such an effect was also observed for the V (III) complex with maltol (*6*).

The effect of V (V) and (IV) compounds on different animal cancer models and various types of malignant cell lines was also investigated (*7–9*). As a result, a distinct cytostatic effect was detected for mouse liver tumours, murine carcinogenesis, murine leukaemia and leiomiosarcoma (*9–13*). The V(III) complex with cysteine was found to exert a similar effect on a rat tumour chemically induced by benzo-α-pyrene (*14*, *15*).

Our studies on the interaction of the V(III)-cysteinate complex with hepatoma Morris 5123 cell lines prove that this compound reduces the viability of these cells by 70% at 100 μM of the vanadium species concentration in the culture medium. A statistically significant increase of the total and filamentous actin level (F/G ratio) was found in the cytoplasm of cells exposed to the vanadium(III)-L-cysteine complex (*16*).

Investigations of the vanadium complexes activity have a number of drawbacks when performed on real cancer cells. This is so because those cells exist always as part of a tight tissue. It is therefore practically impossible to estimate how many cells are killed by the lethal concentration of the drug. Besides, the access of chemical compounds to particular cancer cells is different under these conditions. The cells existing in the external part of a tissue are exposed to the action of a higher concentration of the drug than those located in the internal part. All this makes it difficult to obtain the answers to some interesting questions on the physical and physiological factors that can modify the anti-cancer effect of the tested compound.

In this study we attempted to throw light on the nature of the toxic action of the vanadium–adenine complex synthesized in our laboratory (*17*) against yeast as the model of eukaryotic cells. The mechanism governing the action of this compound on yeast apparently does not exactly reflect the subtle processes that occur in cancer cells. However, as it can be expected, investigations on yeast can help understand the general mode in which the vanadium–adenine complex acts and,

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what is especially important, enable to recognize the factors which influence the intensity of the toxic activity of this vanadium compound.

Yeasts are unicellular organisms, which multiply by budding (*18*). The optimal temperature for their cultivation is 28°C. Yeast cells multiply at this temperature every 2 h in complete medium with glucose as a source of carbon and energy. Furthermore, yeasts are easy to cultivate under laboratory conditions. Due to the evolutionary conservatism of cellular structures and functions, the knowledge about these organisms is helpful in understanding many processes that take place in higher eukaryotes. Approximately 30% of yeast proteins are homologous to those in mammalian cells (*19*). Many yeast genes exhibit significant similarity to human genes involved in the development of various diseases, including cancer (*20–23*).

Crans and others (*24*) investigated the effect of a new insulin-like compound (4-hydroxopyridine-2,6-dicarboxylato oxovanadate (V)) on a myoblast and yeast cell, and found significant inhibiting influence of the pH on their growth. For as many as four different Mo-hydroxylamido complexes studied the yeast growth inhibition was distinctly pH dependent. This effect was closely related to the speciation of particular Mo complex compounds (*25*).

The aim of our research was to characterize the vanadium complex compound by a simple system, consisting only of yeast cells, water and the compound. The advantage of this system is that many factors which might modify the action of the compound are eliminated under such conditions, so it is easier to draw conclusions as to the actual influence of a given factor. In this research the chosen factors were as follows: the stage of the life cycle, the pH and the rate of growth of the yeast cells.

MATERIALS AND METHODS

Vanadium(III)–Adenine Complex—A $H_4V_2OCl_4(Ad)_2$ compound was synthesized in our laboratory by the method described previously (*17*). The results of elemental analysis, IR and UV spectra measurements corresponded with the recently published data (*17*). IR spectra were measured in KBr pellets with a Bruker IFS 113v IR spectrophotometer in the 4000–200 cm^{-1} range. UV-Vis diffuse reflectance spectra were recorded on a Cary 5 spectrophotometer (with reflectance attachment). Absorption spectra in aqueous solution were recorded on a Cary 50 spectrophotometer.

Yeasts—The prototrophic *Saccharomyces cerevisiae* strain SM 002 was isolated from baker yeast. The auxotrophic SM 002/3 leu^- strain is a derivative of the prototrophic one. The auxotrophic diploid strain D7/144 a/α ade^- 2-40/ade^- 2-119, trp^- 5-112/trp^- 5-27 and the haploid XV-185-14c a trp^-, arg^-, lys^-, his^-, ade^- 2-1 were obtained from the Department of Biology, Brooklyn College of the City University of New York.

Prior to the experiments, the strains were pre-incubated in the full medium YPG (Yeast Extract, 1%; Bacto Pepton, 1%; Glucose, 2%) at 28°C for 16 h (log phase of growth) or 48 h (stationary phase of growth). Pre-incubated in such a way, the cells were washed twice by centrifugation in sterile, distilled water and then diluted to obtain a solute of cells at the concentration of 1×10^6 cells/ml. Thereafter, 100 μl samples of such a cell mixture were replaced to the cubes with 10 ml of water to obtain the reaction mixture variants required. The cells in the mixtures were incubated for 60 min, and after this time span 10 μl samples were taken and spread on the YPG solid medium (solidified with 2% Agar–Agar) in Petri dishes, to estimate the number of living cells in 1 ml of the reacting mixture.

A 100 μM concentration of the vanadium–adenine complex was chosen because at this concentration <100% of cells were killed in each variant of the reaction mixture. This appeared to be the most "sensitive" procedure which made it possible to compare particular variants of the reacting mixture, especially in terms of cell mortality due to the administration of the compound.

The diploid strain D7/144 was used to identify the mutants possibly induced by mitotic recombination, a process which may be triggered by a mutagenic factor. The mutant possesses an adenine mutation in the same gene in both homological chromosomes, but in each gene the mutations are differently located, so the mutations are termed heteroallelic. If there are two heteroallelic mutations and each of them occupies another homological chromosome, then the mutations complement, giving a normal, white colony of cells possessing such a system of mutations. However, if mitotical crossing-over occurs between the two loci of mutations in particular diploid cells, then the colony developing from the initial cell will have red and pink sector (*26*). The number of colonies with red/pink sectors reflects the number of the mitotic crossing-over events. Similarly, if gene conversion occurred in the mutation region of the initial cell, a red sector would appear in the colony which originated from the cell.

The haploid strain XV–185–14-c forms a red colony because it is mutated in a gene which is necessary to metabolize a red colour compound arising on the pathway to adenine. If any other gene lying on the same pathway but before the one just described is mutated in a particular cell, the colour of the colony arising from the cell will be white. This enables us to identify the mutation altering a single gene.

Both strains were also used for the identification of respiratory mutations. Colonies arising from a cell which has a defective gene involved in respiration are smaller and do not grow on a medium with glycerol as a carbon source. We used for this purpose the medium N_3, which differs from the YPG only in that it includes 2% of glycerol instead of 2% of glucose.

The test procedure which was aimed at determining the viability of the yeast cells in the presence of the toxic agent consisted in keeping the cells for an hour in a mixture with the vanadium compound. After this period, the cells were spread onto a Petri dish with solid full medium so that the number of living cells could be detected. One-hour incubation time was chosen because in our previous tests it turned out that the compound did not kill yeast cells efficiently when the incubation time was 5 or 10 min. The yeast cells were killed in the range

useful for comparable investigations when they were treated with the vanadium compound for 60 min.

We must be aware of some disadvantages of the experimental stage when the cells were being spread. It is impossible to take samples of a liquid with exactly the same number of living cells and transfer them from the mixture to a Petri dish. This process is of a statistical nature. Furthermore, as it may be inferred from the tables, an important contributor to the extent of the toxic action of the vanadium compound is the physiological state of the cells. It is difficult to assure identical conditions for cell cultivation in every instance, so the cells may slightly vary in sensitivity in particular experiments. To overcome these limitations, we performed comparable tests using the same population of yeast pre-cultivated in the same liquid medium, so the organisms were in a similar physiological state. Such cells were, for example, used in the tests with different pH value of the medium. Also the same amount of vanadium compound was taken from the stock solution to both mixture variants. This made the results comparable, and such a procedure reduced to a great extent the random factors that might influence the results.

RESULTS

The polymeric $H_4V_2OCl_4(Ad)_2$ compound contains in coordination polyhedron two V^{3+} ions bounded by the oxygen bridge. V^{3+} ions can hydrolyze, and their hydrolysis products easily oxidize to mixed hydrolytic V(IV) and/or V(III)–V(IV) complex species. As it was proved earlier (*17*), the vanadium(III) adenine complex is stable in acidic aqueous solutions up to pH value 4.5. In the solid phase this compound is stable in the dry atmosphere. It was necessary, however, to check its behaviour in aqueous solutions during the biological experiments performed.

We measured the absorption spectra of this compound in aqueous solutions (pH = 3.81) during biological experiments performed. The detection of optical density data was possible, however, for the solutions of a vanadium compound concentration equalling 1 mM, which is ten times higher than in solutions administrated to the yeasts (100 μM). Results are presented in Figs. 1a and b.

As seen in Fig. 1, the concentration of complex species is decreasing during the first 25 min, then some equilibrium is reached, and no distinct changes are observed. It should be pointed out that a band ranging from 400 to 460 nm is characteristic of the dinuclear μ-oxo vanadium (III) complex species. No distinct absorption of the VO^{2+} ion is observed ($\lambda \sim 800$ nm) in these spectra. Aqueous solutions of this compound with higher pH values were opalescent as a result of the formation of polynuclear complex-hydrolytic species, thus quantitative measurement of optical density was impossible.

Many compounds kill eukaryotic cells quickly, even within seconds from the moment of administration. The duration of the killing action obviously depends on the target structure in the cell and on the mechanisms against the action of toxic drugs. A cell will die in a shorter time when the target site is located in the plasma membrane, but certainly in a longer time when the target is in the nucleus. So, it seemed interesting to use yeast cells in order to test how quickly the drug acted. The reaction mixture was composed only of yeast cells in the log phase and the investigated vanadium compound. The results illustrating the mortality of the cells during the spans of 5, 10, 20, 30 and 60 min are shown in Fig. 2.

The bars in Fig. 2 indicate that the toxic action of this compound is very slow. Only after 30 min did the mortality exceed 50%, and after 1 h time about 20% of the cells were still alive.

As has been described before, cancer cells differ from their initial cells in a variety of features involving

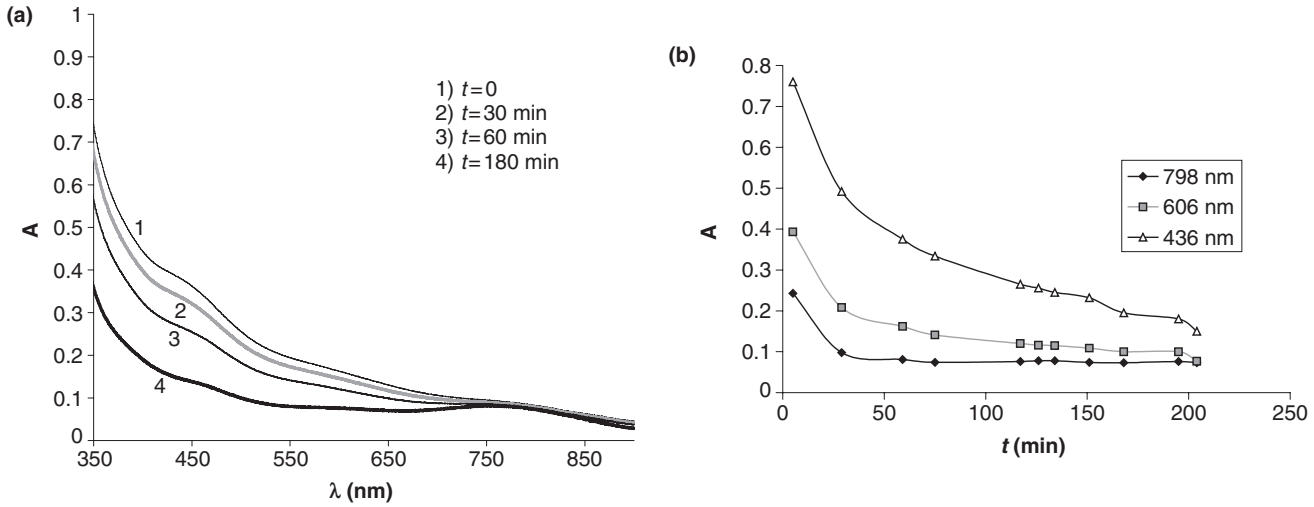

Fig. 1. **(a) Absorption spectra of the 1 mM $H_4V_2O_2Cl_4(Ad)_2$ compound diluted in water (d = 5 cm) measured during 180 min (pH = 3.81).** curve 1—absorption spectrum measured directly after dissolving of the V-Ad complex, curve 2—after 30 min, curve 3—after 60 min, curve 4—after 180 min. **(b) Dependence of the absorption of 1 mM $H_4V_2OCl_4(Ad)_2$ diluted in water as a function of time (d = 5 cm).** The concentration of complex species is lowering during the first 25 min, then some equilibrium is reached.

intracellular pH and intensity of metabolic processes. It was therefore essential that our research into the activity of the adenine–vanadium complex on the yeast included the contribution of the pH of cultured medium to the process of killing cells.

The reacting mixture consisted only of yeast cells, water and the compound studied. Slight amounts of Cl^- and Na^+ ions were present because pH adjustment was carried out with Na OH or HCl solutions. We decided that in this experiment one additional factor should be verified simultaneously, namely the stage of cell growth. The importance of this factor results from the fact that yeast cells in the log phase show a more intensive metabolism than in the stationary phase (*27*). It was intriguing to perform comparative experiments in which the influence of both the pH and the stage of growth could be verified. Thus a sample of the cells being in log phase and independently the cells in stationary phase were incubated in the mixture with pH = 4 and pH = 7 for 60 min. After that their viability was determined. The results are visualized in Table 1.

The results collected in Table 1 clearly indicate that pH, as well as the phase of growth, affects the killing activity of the adenine–vanadium compound against the yeast cells. Although in the case of the stationary phase the effect of the pH does not seem to be evident, its importance becomes obvious in the log phase. At pH = 4, only 8.6% of the cells survived, while at pH = 7 the survival was significantly higher—to 77%. As shown by these data, the stationary phase produces no toxic effect at pH = 7, and a rather weak effect at pH = 4.

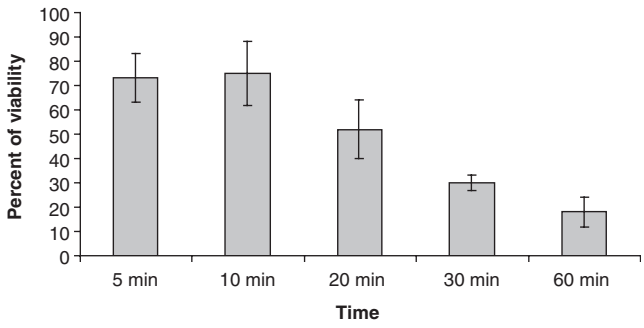

Fig. 2. **The killing activity of the 100 μM complex is not rapid.** The population of living yeast cells decreases but a significant number of cells are still not killed even after 1 h of incubation.

The higher sensitivity values obtained when the cells were in the log phase suggest that each cell which grew at a faster rate was more sensitive to the investigated compound. In order to verify this, comparable experiments were performed, in which—as a control—the prototrophic strain at the log phase was tested in the cultured medium at the same pH value as previously and, additionally, an auxotrophic strain, isogenic to the prototrophic one, was tested in the same manner. The auxotrophic strain (also in the log phase) was chosen because its growth was slower, which resulted from the fact that auxotrophs are genetically defective, unable to synthesize particular complex organic compounds (like amino acids), and therefore, forced to take up these compounds from the environment. Thus, the comparison of the sensitivities of the two strains might throw some light on the relationship between the toxic activity of the tested compound and the rate of cell growth. The auxotrophic strain used in the experiment was also, as the prototrophic one, in the log phase.

The results included in Table 2 indicate again that the strain growing at a faster rate is more sensitive than the one growing at a slower rate. Although the sensitivity of both strains is higher at pH = 4 than at pH = 7, the percentage of surviving cells, especially at acidic pH, is significantly greater in the auxotrophic than the prototrophic strain. It is worth noticing that the results obtained for the prototrophic strains (Table 2), in this experiment used as a control, are very similar to those obtained in the previous study (Table 1).

In comparative tests we used also two other strains which differ in that one is diploid and one is haploid. Besides, they have different mutations, as described earlier (*28*). We decided to test these two strains for the following reasons: firstly, it was an attempt to verify the vanadium–adenine complex as a mutagenic factor, which would be a proof supporting the thesis that this substance acts at the DNA level. Secondly, with these strains it was possible to examine the influence of the proliferation rate on the sensitivity to the tested compound. This could be achieved because the strains grow at different rates.

The shapes of the curves in Fig. 3 show that the diploid strain grows faster than the haploid one. After 10 h of incubation, the optical density of the haploid was lower by 30% than that of the diploid strain.

Both strains were taken from the culture in log phase and used in the experimental study of this compound. After keeping them for 1 h in water supplemented with

Table 1. **Influence of pH and stage of growth on the sensitivity of yeast cells to the adenine–vanadium complex compound.**

Log phase of growth				Stationary phase of growth			
pH = 4		pH = 7		pH = 4		pH = 7	
c.f.u.		c.f.u.		c.f.u.		c.f.u.	
Control	VA	Control	VA	Control	VA	Control	VA
348 ± 17	30 ± 3 (8.6%)	472 ± 26	365 ± 11 (77%)	564 ± 87	489 ± 61 (86%)	678 ± 84	707 ± 45 (100%)

c.f.u., colony forming units; VA, vanadium–adenine complex; %, percentage of living cells compared with the control.

Table 2. **Influence of auxotrophy on the sensitivity of yeast cells to the adenine–vanadium compound.**

Auxotrophic strain				Prototrophic strain			
pH = 4		pH = 7		pH = 4		pH = 7	
c.f.u.		c.f.u.		c.f.u.		c.f.u.	
Control	VA	Control	VA	Control	VA	Control	VA
95 ± 10	33 ± 5 (35%)	105 ± 9	75 ± 8 (71%)	123 ± 38	13 ± 2 (11%)	106 ± 19	72 ± 26 (68%)

c.f.u., colony forming units; VA, vanadium–adenine complex; %, percentage of living cells compared with the control.

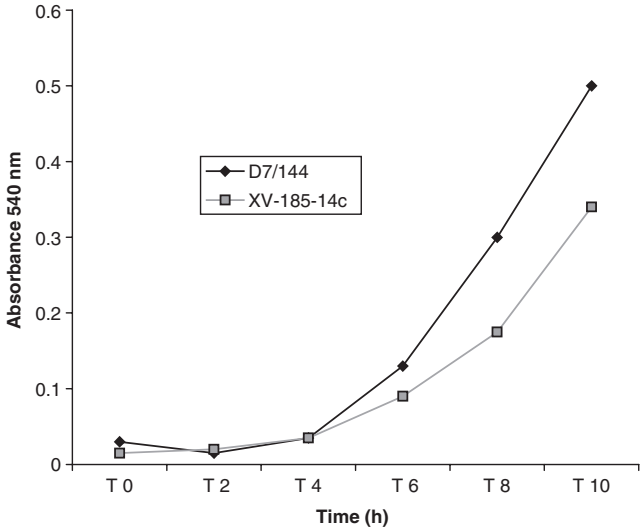

Fig. 3. **The diploid D7/144 yeast strain grows slower in full liquid medium than the haploid XV-185-14c.** After 10 h of incubation, optical density of the diploid strain culture was higher by 30% as compared with the culture of the haploid strain.

the 100 μM compound, the cells were spread onto Petri dishes with the solid full medium. The colonies were counted and especially their colour was detected in order to identify the mutants.

When we compare the data in Table 3, we can see that the haploid strain is more resistant under the conditions described before. No killing effect on the strain was noticed. A different effect was observed with the diploid strain whose cells (according to the data in Table 3) were evidently killed and only 37% of which survived. Hence, the response of these two strains to the investigated compound also indicates that yeast strain proliferating at a faster rate exhibit a higher sensitivity.

As for the number of new mutations, which possibly might arise, it is clear that the number is too small to allow the conclusion that the tested compound displays mutagenic activity. There was practically no new mutation event under these conditions, and this holds both for respiratory and adenine path mutations.

DISCUSSION

The vanadium complex with adenine $H_4V_2OCl_4$ $(Ad)_2$ is product of a partial hydrolysis of V^{3+} and its complexation reaction with adenine molecules. This compound was precipitated from hydrated methanolic solution and consists of two V^{3+} ions bounded each to an other by a μ-okso bridge (*17*). In biological experiments this compound was applied (administrated) as a diluted (100 μM) aqueous solution with two different (4 and 7) pH values. For each solution, obviously, different equilibria may be expected. A concentration of dinuclear species (both complex and hydrolytic only) should be rather low in such diluted solutions (*17*). On the other hand, in a recent paper we have estimated the stability constants of V(Ad) species, which are dominated in diluted solutions with pH ~ 3.8 (log K = 3.15).

We were unable to determine possible concentration of mono and dinuclear complex species directly in such diluted solutions. We measured then UV-Vis absorption spectra of solutions of 1 mM concentration with pH = 3.81 (Fig. 1a). A freshly dissolved complex compound indicated the presence of a V^{3+} ion d–d band at 606 nm which corresponds to $^3T_{2g} \leftarrow {}^3T_{1g}$ transition. This band is shifted to the higher energy values which is typical of V^{3+} bounded with a nitrogen atom. On the other hand a very intense (ε ~ 2800–3000) CT band is observed in all μ-okso dinuclear species. In this spectrum such a band is observed at 436 nm, but its intensity indicates that the concentration is ~4% of total vanadium concentration. So, it seems reasonable to assume mononuclear complex species with adenine predominate under the biological experiment conditions.

The next problem is oxidation of V^{3+} to VO^{2+} (Fig. 1a and b). Distinct lowering of the band at 436 nm is observed during the experiments. On the other hand the band at 798 nm, characteristic of a VO^{2+} ion, does not change significantly. It means that in an administrated solution at pH = 4 mononuclear complex species with chloride ions and adenine molecules play most significant role in the interaction with the yeast cells. It should be pointed out that our previous studies prove that the VO^{2+} ion does not form any complex species with adenine in acidic solutions (*17*).

In a freshly prepared 100 μM aqueous solution of $H_4V_2OCl_4(Ad)_2$ at pH = 7 some polymerization of mixed hydrolytic adenine species occurs very quickly and it is difficult to determine the vanadium oxidation state. In such solutions presumably hydrolytic polymers of V(IV) are predominating.

Using a 100 μM concentration of this compound we found, generally, that its toxic action on the yeast cells is rather slow. At pH = 4 after 1 h of exposure to the compound the survival of yeast cells approached 20%. Such results can suggest that the target of the

Table 3. **Activity of the vanadium–adenine complex against diploid and haploid adenine mutants of *S. cerevisiae* differing in the number of mutations and rate of growth.**

	Diploid D7/144		Haploid XV-185-14c	
	Control	VA	Control	VA
Number of living cells	549 ± 116	203 ± 53	266 ± 39	261 ± 36
Percentage of living cells compared with the control		37%		98%
Number of mutation events in adenine path	1	0	2	1
Percentage of mutation events in adenine path	0.18%	0%	0.75%	0.38%
Number of respiratory mutation events	4	2	4	3
Percentage of respiratory mutation events	0.72%	0.98%	1.5%	1.1%

VA, vanadium–adenine complex.

vanadium–adenine complex is not situated in the plasma membrane. However, the prolonged cell survival may have been caused by a slow uptake of the drug through the plasma membrane. Another possibility is that the extent of toxic activity depends on the DNA synthesis in the course of the replication process. This would explain the observation that the log phase cells were more sensitive to the drug than the cells at the stationary phase.

As it can be inferred from the data of Table 1, the investigated compound is more toxic to the yeast when the pH of the cells environment is acidic, not neutral. The results of our experiments have revealed that both vanadium speciation and the oxidation state of the vanadium ion differ with the acidity of the solution changing. As discussed earlier, in solutions at pH = 4 mononuclear V^{3+} species are responsible for biological activity of these solutions, whereas polymeric hydrolytic species, existing in solutions with pH = 7, react distinctly weaker.

It is very interesting to compare our data with those of G.R. Willsky and others (*29*), who found that 5 mM aqueous solutions of vanadyl sulphate stimulated growth of *Sacharomyces cerevisiae*. Our solutions were more diluted (100 μM) and these which were distinctly active had significantly lower pH values. It suggests that these are the V^{3+}/VO^{2+}-complex with adenine species that are responsible for the lethal effect.

These results confirm well the results of our recent paper where the cytotoxic activity of the vanadium (III) complex with L-cysteine was proved (*16*).

The difference in the stability of the vanadium–adenine compound depending on the solutions acidity allow to explain the different activity of this compound against yeast in reaction mixtures having different pH values.

The detection of an increased activity of the investigated compound at a lower pH seems to support its potential application for cancer therapy, where (due to a more intense metabolism) the acidity of the intracellular space should be lower than in normal cells (*30*, *31*).

Other evidence for increased toxicity against tumour cells under conditions of enhanced metabolism is the effect of higher mortality of the yeast cells, when they are in the log phase of growth. Various factors may participate in this phenomenon of increased sensitivity. It is possible that the uptake of the drug by the cells becomes enhanced in the log phase of growth as compared with the stationary stage. Besides, in the log phase the cells multiply, their DNA is replicated and synthesized which would enable the vanadium–adenine compound to act at the level of the DNA structure.

The importance of the yeast culture growth rate seems to be confirmed by the test with the auxotrophic strain. The strain with the mutation which blocked leucine synthesis was more resistant to the investigated compound than the prototrophic isogenic initial strain, as shown by the data in Table 2. In this case, both strains were in the log phase, so the intensity of metabolism can be regarded as comparable. The observed decreased sensitivity of the auxotrophic strain suggests that the growth rate is an important factor affecting the toxic action of the drug. It is a well-established fact that each auxotrophic strain grows slower than its prototrophic parent.

An additional attempt to verify the significance of the growth rate factor was made in the experiment with two strains differing in the type and number of mutations. The growth rate was then exactly determined (Table 3). As expected, the strain possessing more defective genes grew slower than the one with fewer mutations. And again, the "slower" one appeared to be less sensitive to the toxic action of the drug than the one growing faster.

Another aim of using these mutants was to detect the increasing amount of new mutations possibly arising via the mechanisms described earlier. The results gathered in Table 3 show that there was no mutation in the adenine metabolic path induced by the drug (*32*, *33*) under conditions when about 63% of the cells had been killed. We also revealed no significant difference in the respiratory mutations numbers, which would suggest that such mutations were not induced by the vanadium–adenine complex. This finding seems to suggest that if the drug acts at the DNA level, it does not induce the kind of mutations which are recognized by the two strains. However, it is also possible that it does not induce any mutation at all. The latter conclusion is very promising for the potential therapeutic application of the investigated compound in cancer diseases.

Investigations into yeast are highly valuable for the determination of the basic features of the tested vanadium–adenine complex. Yeasts are model eukaryotic organisms living in water solution as a population of independent cells, which can be used in a variety of experiments. The results obtained in our study show that even a small change in the metabolic activity of those organisms alters the toxic action of the administrated compound. It is more active when the metabolism of the cells is more intense and if the cells are in the phase of multiplication.

All factors considered here deserve particular attention as they notably enhance the sensitivity of cancer cells to the vanadium–adenine compound as compared with normal cells.

On the basis of the results obtained in this study we can formulate the following general conclusions:

(i) The activity of the investigated compound, administered to the yeast cells, depends significantly on its concentration in the reaction medium and accounts for significant killing of the yeast cells.

(ii) The toxicity of the compound studied is much stronger for yeast cells being in the log phase of growth.

(iii) The pH of the cultured medium is an important parameter influencing the activity of the investigated compound. This should be attributed to the hydrolysis of this compound in an aqueous solution at pH = 7. The formation of several hydrolytic polymeric species may be responsible for the weaker interaction with the yeast cells.

(iv) The number of the yeast mutants obtained through treatment with the vanadium complex is within the limit of experimental error. It suggests that this compound does not affect the nuclear DNA.

(v) An inspection of the complex equilibria in diluted aqueous solutions of $H_4V_2OCl_4$ $(Ad)_2$ proved that throughout the biological experiment, the V^{3+} to VO^{2+} equilibrium among complex species is preserved. One can conclude that cytotoxic activity is characteristic of V(III)–adenine complex species.

All these observations enable us to draw the conclusion that the $H_4V_2OCl_4$ $(Ad)_2$ compound should significantly strongly interact with the cells having a fast metabolism in the solutions with a lower pH, and this suggests that they should act selectively on cancer cells.

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