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Negative Ames-test of *cis*-di(thiocyanato)-*N*, *N*'-bis(4,4'-dicarboxy-2,2'-bipyridine)Ru(II), the sensitizer dye of the nanocrystalline TiO_2 solar cell

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Abstract

Dye-sensitized nanocrystalline TiO_2 solar cells are currently under development. Since these cells contain an electrolyte solution we reviewed the health and safety aspects in view of indoor applications, where personal contact cannot be excluded. Only small amounts of chemicals are present in each cell and so there is no danger of acute toxicity. However, long-term effects often can be caused by incidental contact with minute amounts. For this reason we have tested *cis*-di(thiocyanato)-bis(4,4′-dicarboxy-2,2′-bipyridine)Ru(II), the sensitizer dye in the Ames test. The dye was not mutagenic in the *Salmonella typhimurium* reverse mutation assay and in the *Escherichia coli* reverse mutation assay.

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1. Introduction

The invention of the dye-sensititized nanocrystalline TiO_2 solar cell has opened new vistas with regard to the future of solar energy [1–3]. Although several prototype cells have been produced there is no large-scale production as yet. Market prospects for theses devices seem bright enough: application of smaller type cells for use in calculators, watches and electronic price tags seems within reach. A next step would be production for use in automotive or as part of architecture (window panes, roof tiles, etc.). The ultimate challenge will be production of larger sized modules for direct connection to the electrical grid.

We have chosen to work on the development of the small-scale cells, with particular emphasis on the indoor application in electronic price tags [4]. A major development item in this project is the long-term stability of the cells, particularly in relation to the sealing that is used. The next important target is simplification of the design to allow for a cheap production method. One of the major differences that sets these cells apart from commercial silica-based cells is the use of an electrolyte solution contained within the cell. This in turn raised the question of health and safety aspects of these devices. The fact that these cells will be used in price tags in supermarkets made this issue particularly acute. Due to the small size of the cells acute toxic effects of the chemicals involved is not expected to be of relevance. However, long-term adverse effects can be caused by minute amounts of material. The most suspect component in this respect is the dye, *cis*-di(thiocyanato)-bis(4,4′-dicarboxy-2,2′-bipyridine)Ru(II) (Fig. 1), which we will refer to as *N*-3. There is a history of mutagenicity surrounding Ru-complexes, in particular Ru(II)-phenanthroline complexes [5]. This is presumably related to their ability to bind to DNA [6]. More closely related Ru-bis(4,4′-diamino-2,2′-bipyridine) dichloride complexes were also found to be mutagenic [7]. In view of these facts we decided to investigate the mutagenicity of *N*-3, using the well-known Ames test.

2. The Ames test

The Ames test is based on a set of *Salmonella typhimurium* strains that revert to histidine independence upon exposure to mutagens [8,9]. This test is extended by

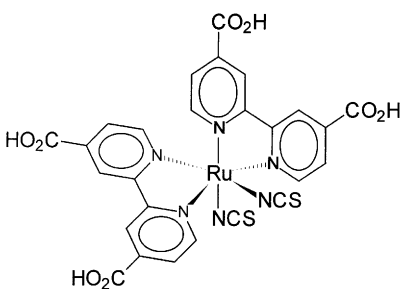

Fig. 1. The dye *N*-3.

adding a tryptophan-dependent *Escherichia coli* strain to make the assay more sensitive [10]. The bacterial strains contain specific types of mutations at well-defined locations in different genes of the histidine or tryptophan operon. These mutations prevent the performance of an essential biochemical function, i.e. the synthesis of one out of the 20 amino acids necessary for the synthesis of proteins. The strains of *Salmonella typhimurium* used in reverse mutation tests cannot synthesize the amino acid histidine and are therefore characterized as His^-. Similarly, the *Escherichia coli* strain used cannot synthesize the amino acid tryptophan and is referred to as Trp^-. Reverse mutations that restore the ability to synthesize histidine or tryptophan respectively are detected by colonies on histidine or tryptophan-deficient growth media.

In addition to the genetic markers directly concerned with the detection of mutations, the bacteria carry mutations, which enhance the sensitivity of the strains. The bacteria have been made more susceptible to mutagens by increased cell-wall permeability to large hydrophobic molecules and a deletion of the uvrB excision repair locus reduces their ability to repair DNA damages error-free. Furthermore, some strains contain the plasmid pKM101, which interferes further with DNA repair, making the host bacteria even more mutable.

A set of histidine-requiring strains and one tryptophan requiring strain are used for mutagenicity testing. Each tester strain contains a different type of mutation in order to be able to detect many different types of DNA damage.

It has been proved that many carcinogens have to be converted by enzymes in the liver or other tissue to an active form that is the true carcinogen. The bacteria are therefore treated both in the absence and presence of a post-mitochondrial supernatant (S9) prepared from the livers of mammals.

3. Materials and methods

3.1. Tester strains

All *Salmonella typhimurium* strains, TA1535, TA1537, TA98 and TA100 were kindly supplied by Dr. Bruce N. Ames (University of California at Berkeley, USA). The *Escherichia coli* strain WP_2uvrA was a gift of Prof. Dr. B.A. Bridges (University of Sussex, Brighton, UK).

The characteristics of the different *Salmonella typhimurium* strains were as follows: TA1537 mutated in the *his*C3076; TA98 mutated in the *his*D3052/R-factor; TA1535 mutated in the *his*G46: TA100 mutated in the *his*G46/R-factor. The strains TA1537 and TA98 are capable of detecting mutagens causing frame shifts and the strains TA1535, TA100 and WP_2uvrA are capable of detecting mutagens causing base-pair substitutions. *Cell cultures* were grown according to Refs. [8,9]; S-9 was prepared following Refs. [7,8].

3.2. Chemicals

The compounds used for control of the positive response were sodium azide (SA) (Fluka, Switzerland) for tester strain TA1535 (1 μg/plate); 9-aminoacridine (9AC)

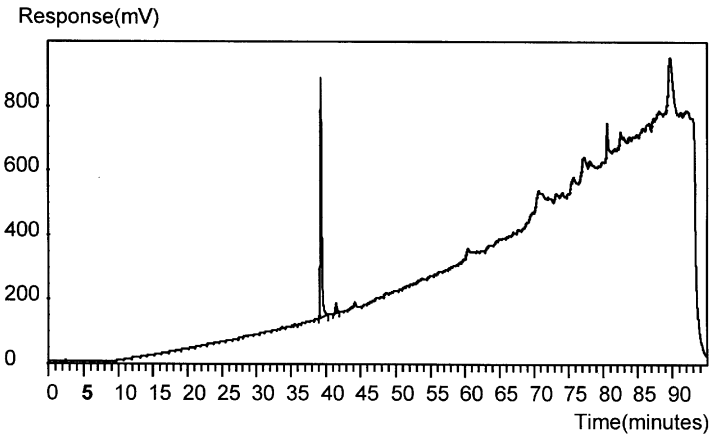

Fig. 2. HPLC-analysis of *N*-3.

(Janssen Chimica, Belgium) for tester strain TA1537 (60 μg/plate); Daunomycine (DM) (Sigma, USA) for tester strain TA98 (4 μg/plate); methyl methanesulfonate (MMS) (Merck, Germany) for tester strain TA100 (650 μg/plate); 4-nitroquinoline-*N*-oxide (4-NQO) (Sigma, USA) for tester strain WP_2uvrA (10 μg/plate); and 2-aminoanthracene (2AA) (Sigma, USA) for all strains in the presence of metabolic activation (TA1535, TA1537 and TA100: 1 μg/plate, TA1537: 2.5 μg/plate and WP_2uvrA: 5 μg/plate); SA, 9AC and DM were dissolved in physiological saline (Fresenius AG, Germany) and MMS, 4-NQO and 2AA were dissolved in dimethylsulfoxide (DMSO) of spectroscopic quality (Merck, Germany). The test substance *N*–3 was a gift from Johnson Matthey, UK. Its purity was established to be 96% (the impurity is described in footnote 2) using HPLC (25×0.4 cm I.D. Nucleosil 120-5 C_{18}, eluent A: 10 mM H_3PO_4; eluent B: CH_3OH; flow 1 ml/min; Gradient: 0–5 min: 100% A, 5–85 min 0–80% B, UV detection at 210 nm). See Fig. 2. *N*-3 was suspended in DMSO; the stock solution of 50 mg/ml was treated with ultra-sonication to obtain a homogeneous suspension. Stability of *N*-3 in DMSO was tested by making a solution of *N*-3 in *d6*-DMSO and recording the ^{1}H NMR spectrum immediately afterwards and after 4 h (see Fig. 3).[2] No changes were detected.

3.3. Experimental procedures

The study was performed according to the guidelines OECD 471 and 472 (adopted May 26, 1983) and EC B.13 and EC B.14 (adopted December, 1992).

[2] The minor set of peaks has been previously ascribed to the presence of an isomer in which one of the thiocyanato ligands has changed coordination from N to S [11]. The amount of this isomer (4–6%) corresponds to the amount detected by HPLC (Fig. 2). The isomer peak immediately follows the main peak; all other peaks are due to the solvent system.

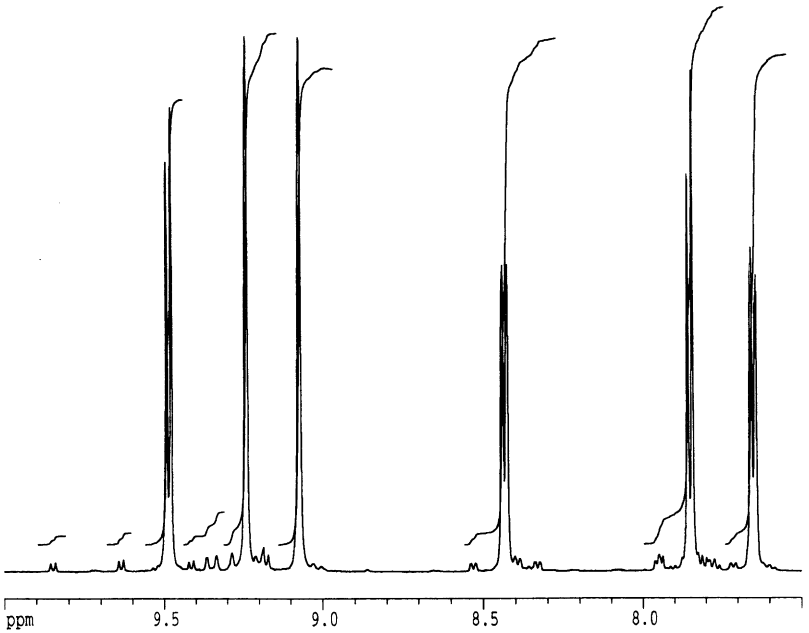

Fig. 3. ^{1}H NMR of *N*-3 in dmso-d_6.

Selection of an adequate range of doses of *N*-3 was based on a dose range finding test with strain TA100, with and without S9-mix. Seven concentrations were tested in triplicate up to concentrations of 1000 μg/plate. The highest concentration of *N*-3 used in the subsequent mutation assay was 5000 μg/plate.

In two independent experiments, five different doses (increasing with approximately half-log steps) of *N*-3 were tested in triplicate in each strain. *N*-3 was tested both in the absence and presence of S9-mix in each strain. Top agar in top agar tubes was heated to 45°C. The following solutions were successively added to 3 ml molten top agar: 0.1 ml of a fresh bacterial culture (10^9 cells/ml) of one of the tester strains, 0.1 ml of a dilution of *N*-3 in dimethylsulfoxide and either 0.5 ml S9-mix (in case of activation assays) or 0.5 ml 0.1 M phosphate buffer (in case of non-activation assays). The ingredients were mixed on a Vortex and the content of the top agar tube was poured onto a selective agar plate. After solidification of the top agar, the plates were turned and incubated in the dark at 37°C for 48 h. After this period revertant colonies (histidine independent for *Salmonella typhimurium* bacteria and tryptophan independent for *Escherichia coli* bacteria) were counted. The revertant colonies were counted automatically with a Protos model 50 000 colony counter or manually, if less than 40 colonies per plate were present.

4. Results and conclusion

Although *N*-3 was poorly soluble in the solvent dimethylsulfoxide, no precipitation of *N*-3 on the plates was observed at the start or at the end of the incubation period in

Table 1
Mutagenic response of *N*-3 in the *Salmonella typhimurium* reverse mutation assay and in the *Escherichia coli* reverse mutation assay

Dose (μg/plate)	Mean number of revertant colonies of three plates, in two experiments with different strains of *Salmonella typhimurium* and one *Escherichia coli* strain				
	TA1535 Exp.1/Exp.2	TA1537 Exp.1/Exp.2	TA98 Exp.1/Exp.2	TA100 Exp.1/Exp.2	WP_2uvrA Exp.1/Exp.2
Without S9-mix					
Positive control	296/255	473/369	547/519	715/810	1235/987
Solvent control	10/9	8/13	13/12	74/72	21/13
100	11/9	9/12	15/12	74/69	23/15
333	11/9	8/14	14/12	71/70	20/12
1000	7/8	11/10	13/9	72/70	16/14
3330	7/9	10/11	15/15	71/73	19/12
5000	11/7	9/12	13/11	73/70	19/14
With S9-mix					
Positive control	337/385	724/547	1916/1236	1998/1860	149/230
Solvent control	15/9	11/9	14/18	85/86	21/10
100	14/9	9/14	19/20	76/85	22/14
333	20/11	11/14	13/18	79/81	23/9
1000	18/11	14/14	12/16	75/85	20/13
3330	21/7	10/14	21/16	89/81	21/13
5000	17/10	10/13	11/13	78/80	19/9

Solvent control: 0.1 ml DMSO.

all tester strains. Therefore, *N*-3 was tested in the mutation assay up to the recommended maximum concentration of 5000 μg/plate in the absence and presence of S9-mix. No reduction of the bacterial background lawn and no decrease in the number of revertants were observed, therefore *N*-3 can be considered as not toxic for the bacterial strains. As shown in Table 1, all bacterial strains showed negative responses over the entire dose range, i.e. no dose-related, two-fold, increase in the number of revertants in two independently repeated experiments. The negative and strain-specific positive control values were within our laboratory background historical control data ranges indicating that the test conditions were adequate and that the metabolic activation system functioned properly.

Based on the results of this study it is concluded that *N*-3 is not mutagenic in the *Salmonella typhimurium* reverse mutation assay and in the *Escherichia coli* reverse mutation assay.

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