after a 1 h incubation is recovered quantitatively. We have determined that normal human serum contains 200–300 pmol/ml of PQQ or of a related quininoid substance. The formazan measured could not have arisen from ascorbate or dehydroascorbate. Since human serum contains 20–80 nmol/ml of ascorbate, the 1–4 nmol of ascorbate present in the 50 μl serum aliquot give negligible color under the reaction conditions of the test.

Therefore, the redox-cycling assay has proven useful for the detection of PQQ (or other quininoid substance) in serum. Citrus juice may require the addition to the buffer of borate which significantly reduces the color contribution of ascorbate compounds to the test.

The method has been used for the determination of tissue levels of PQQ [5] and works well for the detection of quinoproteins after SDS-PAGE and protein immunoblotting to nitrocellulose. A manuscript documenting our experience with the redox-cycling assay with quinoid compounds is in preparation.

REFERENCES

[1] Flückiger, R., Woodtli, T. and Gallop, P.M. (1988) Biochem. Biophys. Res. Commun. 153, 353–358.
[2] Paz, M.A., Flückiger, R., Henson, E. and Gallop, P.M. (1989) in: PQQ and Quinoproteins (Jongejan, J.A., Duine, J.A. eds.) Kluwer pp. 131–143.
[3] Paz, M.A., Gallop, P.M., Torrelio, M. and Flückiger, R. (1988) Biochem. Biophys. Res. Commun. 154, 1330–1337.
[4] Van der Meer, R.A., Groen, B.W., Jongejan, J.A. and Duine, J.A. (1990) FEBS Lett. 261, 131–134.
[5] Myers, F., Smidt, C., Tran, D., Tinker, D. and Rucker, R. (1990) FASEB J. 4, A668.

FEBS 08434

Reply: The redox-cycling assay and PQQ

R.A. van der Meer, B.W. Groen, J.A. Jongejan and J.A. Duine

Department of Microbiology and Enzymology, Delft University of Technology, Delft, The Netherlands

Received 3 April 1990

The aim of the redox-cycling assay is to identify PQQ in a biological sample by measuring an activity, namely the reduction of a dye. As with any other identification method based on such a principle, one should be sure that the activity shown by a complex sample really derives from the presumed compound. Therefore, at least one independent check should demonstrate its presence and other compounds commonly present in the samples should not interfere. As reported [1], these basic requirements are not met: samples showing high activity in the redox-cycling assay do not show any trace of PQQ with the biological or other assays; several common biochemicals show activity too; the outcome is heavily biased by the conditions and the composition of the sample. The latter is of course related to the complicated dead-ended redox-chemistry of the assay. Thus, the presence of heavy metals, the O_2 concentration, the redox state of the compound, etc., will interfere with the chemistry and the termination of the process. This can be illustrated with ascorbic acid, giving different outcomes depending on the laboratory where the assay is performed ([1] and the comment), the higher activity of dehydroascorbic acid [1], the very high activities of ascorbic/dehydroascorbic acid mixtures [unpublished results], and the absence of an effect upon PQQ addition to citrus fruit samples [1]. Therefore, the point raised in the comment whether the activity of ascorbic acid is low or can be lowered, is trivial as it neglects the main objections against the redox-cycling assay.

Correspondence address: J.A. Duine, Department of Microbiology and Enzymology, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands

REFERENCES

[1] van der Meer, R.A., Groen, B.W., Jongejan, J.A. and Duine, J.A. (1990) FEBS Lett. 261, 131–134.