

## Correspondence

## Comment: Redox-cycling is a property of PQQ but not of ascorbate

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We have found that pyrroloquinoline quinone (PQQ) in the presence of glycine at pH 10, efficiently reduces nitroblue tetrazolium (NBT) to its formazan [1]. In this reaction, 1 mol of PQQ reduces approximately 1000 mol of NBT in 1 h at room temperature. Since no other biological substance tested to date showed a comparable redox-cycling, we used this property for the detection of PQQ in proteins and biological fluids [2,3].

A recent publication in this Journal [4] states that ascorbic and dehydroascorbic acid promote color formation in the redox-cycling assay to the extent of PQQ on a molar basis. This reactivity and possible in-

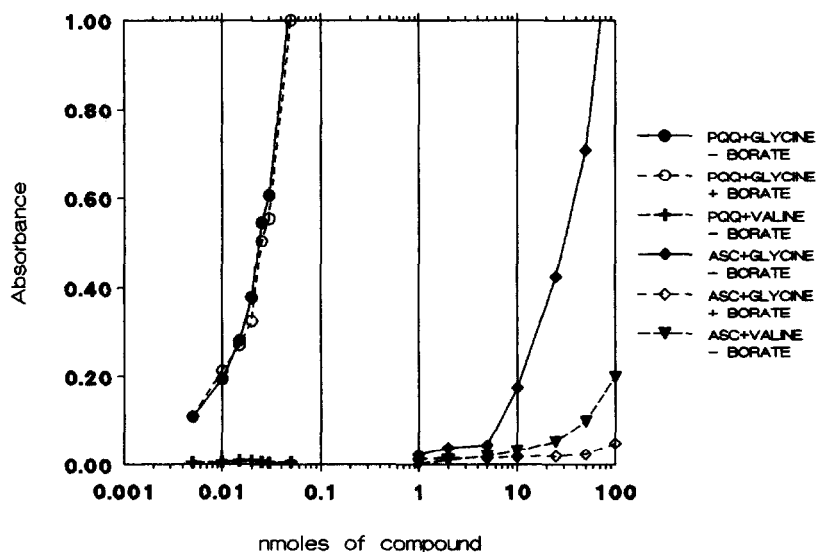
terference by a number of other commonly occurring biochemicals led the authors to conclude that claims based on the results of the redox-cycling assay should be disregarded.

Our experience with the redox-cycling reaction for the measurement of PQQ and ascorbate is clearly at variance with this report, as shown in Fig. 1.

On a molar basis, in a 1 h reaction using glycine, PQQ yields 1000 times more color than ascorbate. While PQQ does not redox-cycle with valine and gives no color, ascorbate reduces NBT to a significant extent in the presence of valine. Moreover, we recently found that borate (0.02 M) greatly reduces the reactivity of ascorbate in glycinate-NBT, and has little effect on the redox-cycling of PQQ. Ascorbate, therefore, does not limit the use of the redox-cycling assay for PQQ.

PQQ added to human plasma (50  $\mu$ l) and measured

FORMAZAN PRODUCTION BY PQQ and ASCORBATE



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 quinoid 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  $\mu$ l 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 quinoid 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 detec-

tion 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

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## Reply: The redox-cycling assay and PQQ

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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<sub>2</sub> 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.

## REFERENCES

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