PYRIDINE CO-ENZYMES AS ELECTRON SCAVENGERS IN IRRADIATED DRY PROTEINS

B.B. Singh and M.G. Ormerod

Biology Division, Atomic Energy Establishment Trombay Bombay-INDIA

and

Physics Department, Royal Military College of Science
Shrivenham, Swindon,
England.

Received August 12, 1966

INTRODUCTION

In a series of investigations (Singh and Ormerod, 1965a, 1965b, 1966 and Ormerod and Singh, 1966) it has been shown that when proteins containing cystine groups are irradiated at 77°K, their electron spin resonance (ESR) spectra show the presence of cystine anions. These anions, which are formed by the trapping of thermalised electrons on the disulphide bonds, react on warming to room temperature to give $-CH_2-S^{\bullet}$ radicals (Ormerod and Singh, 1966). It has been found that metal ions, iodoacetamide and methacrylamide - all of which have a high reaction rate with the solvated electron (Baxendale et al, 1963 and Singh et al, 1966) - reduce the yield of the cystine anion in proteins irradiated at 77°K, and consequently reduce the yield of $-CH_2-S^{\bullet}$ radicals at room temperature (Singh and Ormerod, 1966 and Ormerod and Singh, 1966).

It has recently been reported (Sanner, 1965) that pyridine co-enzymes protect trypsin against high energy radiation both in the solid and in dilute solution. The reduced form of the

co-enzymes offered more protection than the oxidised form and this difference was ascribed to hydrogen donation to macromolecular radicals by the reduced co-enzyme. The protective effect of the oxidised co-enzyme has not been explained.

In the present communication it will be shown that the pyridine co-enzymes, NAD and $NADH_2$, are efficient electron scavengers and interfere with the reactions of thermalised electrons with disulphide bonds in trypsin and bovine serum albumin irradiated in the solid state at 77°K. They also reduce the yield of $-CH_2-S^{\bullet}$ radicals in trypsin at room temperature. The oxidised form was more effective than the reduced form and the difference is ascribed to their relative reactivities towards the thermalised electrons. The observed protection of trypsin by NAD is attributed partly to electron-scavenging by the additive.

EXPERIMENTAL

Trypsin and bovine serum albumin were obtained from Armour Pharmaceuticals Ltd. and Koch-Light & Co. Ltd. respectively. NAD and $NADH_2$ were also obtained from Koch-Light & Co. Ltd. and no further purification of these chemicals was made.

The proteins were freeze-dried with five percent by weight of either NAD or $NADH_2$ and the samples were then sealed under vacuum (10^{-5}mm Hg) for ESR observations. The samples were irradiated with Co^{60} gamma-rays at 77°K with doses varying from 0.5 Mrads to 5.0 Mrads and ESR observations were made first at 77°K then at room temperature. The technique of ESR measurements and the calculations of radical concentrations has been described elsewhere (Singh and Ormerod, 1965b).

RESULTS

As has been previously reported, both trypsin and BSA gave asymmetrical ESR spectra at 77°K. This asymmetry is caused by a single line on the low field side of the ESR spectrum which is attributed to the cystine anion (Ormerod and Singh, 1966). On warming to room temperature, a broad line on the low field side of the spectrum appeared. This line is due to the $-CH_2-S^\bullet$ radical (Kurita and Gordy, 1961). A second radical whose ESR spectrum is a doublet was also observed.

NAD and $NADH_2$ had the following effects:

(i) NAD completely removed the asymmetry in the ESR spectra observed at 77°K. That is, it removed the line due to the cystine anion. $NADH_2$ only partially reduced this line.

(ii) Both NAD and $NADH_2$ reduced the radical yield at 77°K by about 10%.

(iii) The yield of $-CH_2-S^\bullet$ radicals at room temperature was reduced by both compounds. NAD was comparatively more effective.

TABLE 1

Proteins	G(77°K) ± 0.2	% decay of total radicals in 10^3 mins. at room temperature.	$CH_2S^\bullet$ radicals/gm in 10^3 mins at room temperature
Trypsin	2.4	22	1.5×10^{18}
Trypsin + $NADH_2$	2.1	48	0.7×10^{18}
Trypsin + NAD	2.1	42	0.2×10^{18}
Bovine Serum Albumin (BSA)	2.8	20	3.0×10^{18}
BSA + $NADH_2$	2.6	22	1.6×10^{18}
BSA + NAD	2.6	38	1.3×10^{18}

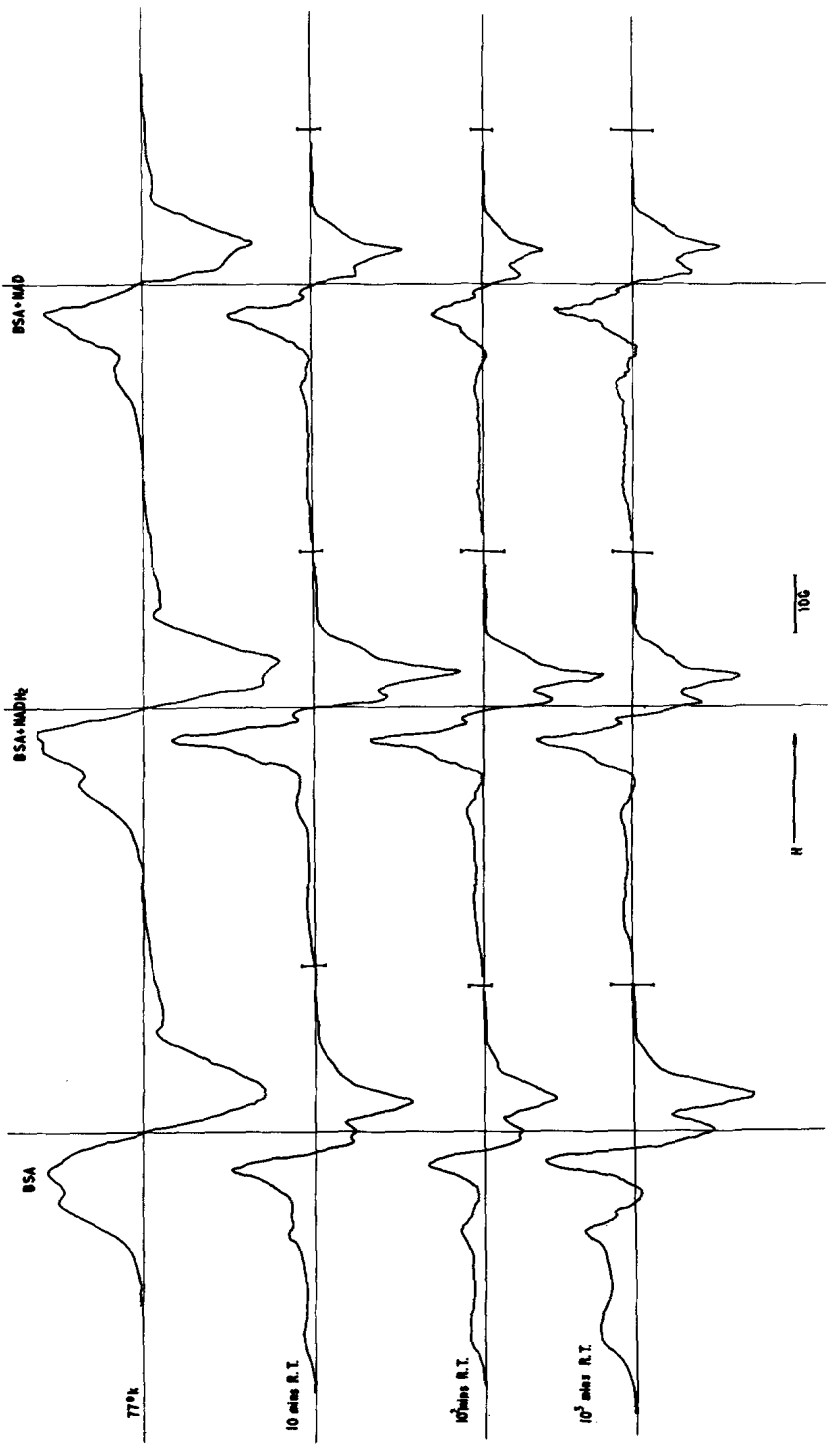

Fig 1. Effect of NAD and $NADH_2$ on the e.s.r. spectra of bovine serum albumin (BSA).

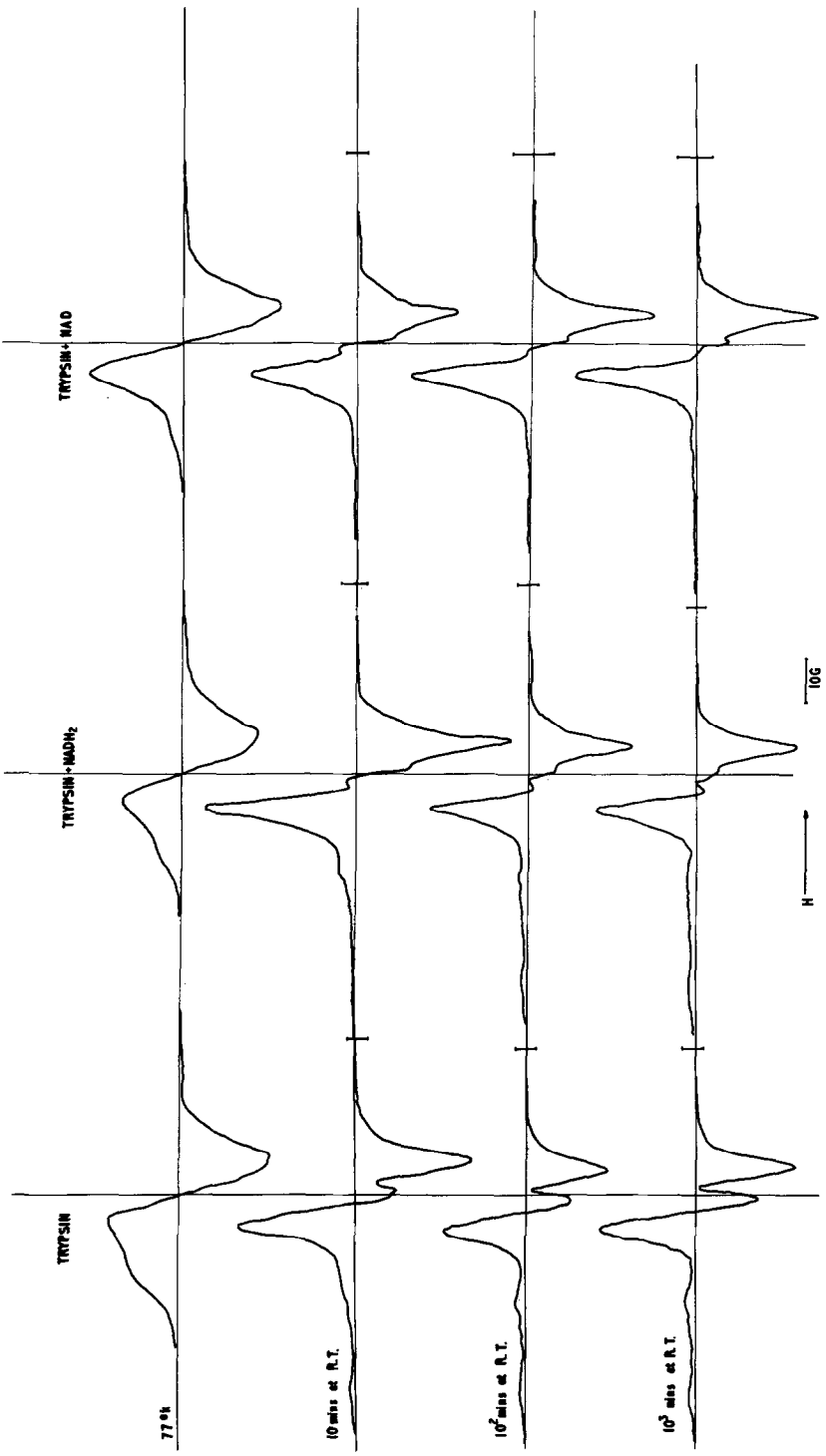

Fig 2. Effect of NAD and $NADH_2$ on the e.s.r. spectra of trypsin.

(iv) The total radical decay at room temperature was accelerated by NAD and $NADH_2$.

(v) In the presence of NAD and $NADH_2$ new lines were observed in the ESR spectra at room temperature. These were probably due to radicals formed on the additive.

These data are shown qualitatively in Figures 1 and 2 and are summarised quantitatively in Table 1.

DISCUSSION

The reduction in the yield of cystine anion at 77°K and the reduction in the $-CH_2-S^{\bullet}$ radical concentration at room temperature by NAD in irradiated trypsin and BSA demonstrate that the NAD is competing with the disulphide group of the proteins for thermalised electrons. That is, NAD is an efficient electron scavenger in the solid state. Likewise, the results show that $NADH_2$ also scavenges electrons, but less effectively.

Using the pulse radiolysis techniques Land (1966) has shown that both NAD and $NADH_2$ give a high value for their reaction constant with the e^-_{aq} in water and that NAD reacts five times faster than $NADH_2$. The rate constants are $2.6 \times 10^{10} M^{-1} sec^{-1}$ and $5.3\ 10^{9} M^{-1} sec^{-1}$ respectively.

The protective effect of NAD on trypsin irradiated in the dry state and in solution observed by Sanner (1965) can be attributed partly to its high electron scavenging property in competition with that of the disulphide bonds. In the solid state, however the cleavage of the disulphide bond does not play an important role in the radiation inactivation of trypsin (Ormerod, 1966). The protection by NAD would probably be due to the reduction in concentration of the other protein radicals. From a comparison of the reduction in radical G-value at 77°K

reported here (~ 10%) and the dose-reduction factor reported by Sanner (1.9), it seems that the reduction in radical yield is too low to account for radiation protection. However, Sanner (1965) used an additive concentration four times higher than that used in this study, and a direct comparison of the two results is not possible unless the effects of higher concentration of the additive is studied systematically.

Viewed as an electron scavenger, $NADH_2$ should be considerably less efficient as a protective agent than NAD. The observation that it protects more efficiently suggests that at least in the solid state $NADH_2$ protects by a repair mechanism involving hydrogen donation as suggested by Sanner (1965). Unfortunately it was not possible to show this effect directly from the ESR observations due to the close overlapping of the signals from the protein radical and the spectra from the radicals on $NADH_2$.

ACKNOWLEDGEMENTS

We thank Professor A. Charlesby and Dr. P. Alexander for their helpful discussions and encouragement. B.B. Singh received financial assistance from the Colombo Plan.

REFERENCES

Baxendale J.H., Fielden E.M. and Keene J.P., 1963 Proc.Chem.Soc. 242.
Kurita Y. and Gordy W., 1961, J.Chem.Phys. 34, 282.
Land E.J., 1966. Unpublished work, personally communicated.
Ormerod M.G. and Singh B.B., 1966, Biochim.Biophys. Acta 120, 413.
Ormerod M.G., 1966. Radiation Res. (In press.)
Sanner T., 1965, Radiation Res. 26, 95.
Singh B.B. and Ormerod M.G., 1965a, Nature 206, 1314.
Singh B.B. and Ormerod M.G., 1965b, Biochim.Biophys. Acta 109, 204.
Singh B.B. and Ormerod M.G., 1966, Int.J.Rad.Biol 10, 369.
Singh B.B., Charlesby A., Keene J.P. and Swallow A.J., 1966., 3rd International Congress of Radiation Research, Cortina.