THE EFFECT OF PERMANGANATE ON THE ULTRAVIOLET ABSORPTION SPECTRA OF AROMATIC AMINO ACIDS AND PROTEINS*

by

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In a recent review on the effect of oxidizing agents on amino acids and proteins, OLCOTT AND FRAENKEL-CONRAT[9] point out that although the influence of oxidizing agents on the SH groups of proteins has received the most attention, the possibility that other oxidizable groups such as tyrosyl residues may play important roles in the biological activity of proteins should not be overlooked. Evidence for oxidation of the aromatic amino acids in proteins[1, 2, 5, 6, 8, 10, 11] has generally been indirect: the amount of the amino acid has been determined before and after oxidative treatment of the protein, using one or another of the accepted analytic procedures for the particular residue.

SIZER[10], however, treated bovine phosphatase solutions with various oxidants and found direct absorption spectrum changes that paralleled similar shifts in the spectrum of free tyrosine treated in a like manner. These changes were actual wavelength shifts of the peak of the ultraviolet absorption band, rather than the unselective diminution in total absorption that would be expected if tyrosine or other aromatic amino acid ring structures were simply destroyed by the oxidants. It seemed of interest, therefore, to reinvestigate the spectral changes that might take place in the oxidation of aromatic amino acids, both in solution and when combined in protein materials, in order to determine the details of the oxidative process. It also seemed probable that use in such an investigation of the low temperature absorption spectroscopy technique recently adapted for enzymatic systems[3] might yield results of interest.

EXPERIMENTAL METHOD

Potassium permanganate was chosen as the oxidizing agent because at any p_H below 3 it gives rise to a reduced form, the manganous ion, whose absorption in the ultraviolet is negligible at wavelengths longer than 2300 Å. Care was taken to measure absorption only after complete reduction, since permanganate ion absorbs strongly throughout the ultraviolet.

A sample of amino acid or crystalline protein was dissolved in a measured volume of p_H 2.0, $M/20$ KCl buffer in a 100 ml volumetric flask. A small amount of freshly made, concentrated (usually 10^{-2} or 10^{-3} M), $KMnO_4$ solution, also in p_H 2.0 buffer, was then added. The solution was quickly

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made up to volume with additional buffer and shaken. Original amounts of substrate and permanganate solutions were such as to give the desired final concentrations after dilution to volume. Substrate concentrations were chosen to give optical densities between 0.7 and 1.0 as measured with a Beckman Model DU Spectrophotometer. The final permanganate concentrations were usually between 10^{-4} and 10^{-5} *M*.

After complete discoloration of the permanganate had taken place, the absorption spectra of the oxidized solutions were measured directly with the Beckman Spectrophotometer at 293° K. The solutions were lyophilized to dryness and redissolved in approximately 1/10 the above volume of 60% glycerol, 40% water solvent for photographic determination of spectra at 77° K. by techniques discussed elsewhere[4]. Control solutions were made up and studied in the same manner, except that additional buffer solution was substituted for the concentrated permanganate.

RESULTS

Phenylalanine

As previously observed by BOWMAN[1], phenylalanine was extremely refractory to permanganate treatment. In very dilute permanganate solutions, 10^{-5} *M* and less, discoloration proceeded slowly; but no change in the absorption spectrum after oxidation could be noted. It therefore seems probable that reduction of the permanganate was due only to dissolved impurities in the solution. In more concentrated solutions of permanganate, reduction could seldom be forced beyond the stage of formation of a MnO_2 precipitate, even after boiling the solution. In the few cases where clear solutions were obtained by such drastic means, the spectra showed only continuous absorption, signifying the complete destruction of the phenyl ring.

Tyrosine and Tryptophane

Both tyrosine and tryptophane spectra showed changes with increasing oxidation by permanganate. The most pronounced over-all effect was a diminution in absorption at the peak and an increase in the minimum, associated with destruction of the selectively absorbing ring structures. The room temperature spectrum of tyrosine also showed an increase in absorption at the maximum at certain permanganate concentrations; and the 77° K spectra of both tyrosine and tryptophane (Figs. 1 and 2) showed pronounced changes in the relative intensities of the fine structure peaks.

The most probable explanation of the gradual decrease in the primary to secondary fine structure peak ratios (77° K spectra), leading to the actual reversal of their relative intensities during the permanganate treatment of tyrosine (Fig. 1), is the formation of a certain amount of dopa as an oxidative intermediate in the solution. Evidence for attributing this type of change to dopa formation has been given previously[3]. The early increase in total absorption of the tyrosine solutions mentioned above can be explained similarly since dopa ($\log \varepsilon_{282} = 3.43$, ref. 7) has a considerably greater absorption than tyrosine ($\log \varepsilon_{276} = 3.25$, ref. 12) in the 275–280 mμ region. Above a critical permanganate concentration, the total destruction of the tyrosine phenyl ring system by the permanganate is evidently sufficient to outweigh the increased absorption of the dopa molecules formed, and the absorption of the solution again decreases.

The above explanation cannot be applied to the changes in tryptophane spectra upon permanganate treatment. The predominate effect of permanganate treatment of tryptophane appears to be an unselective diminution of the total absorption. It seems possible, however, that the formation of an oxidative intermediate with increased absorption in either or both of the 270 and 290 mμ regions might be responsible for the changes in the absorption band fine structure observed at 77° K. (Fig. 2).

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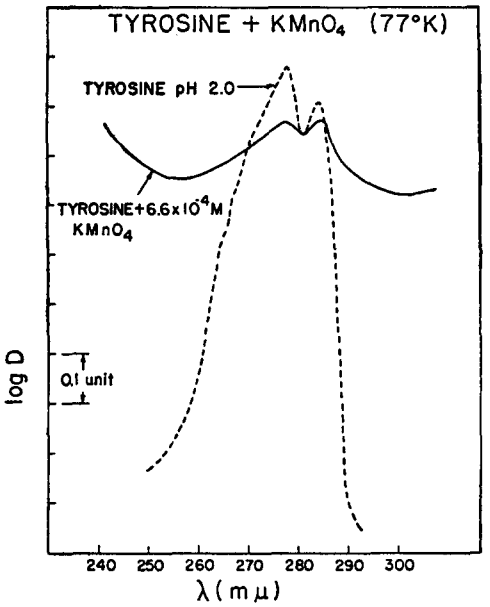

Fig. 1. Absorption spectrum of tyrosine (0.1 mg/ml in $M/20$ KCl buffer, p_H 2.0) before and after the addition originally of permanganate. Solutions were lyophilized and residues redissolved in 60% glycerol, 40% water at approximately 10 times the original concentration for determination of the absorption spectra at 77° K

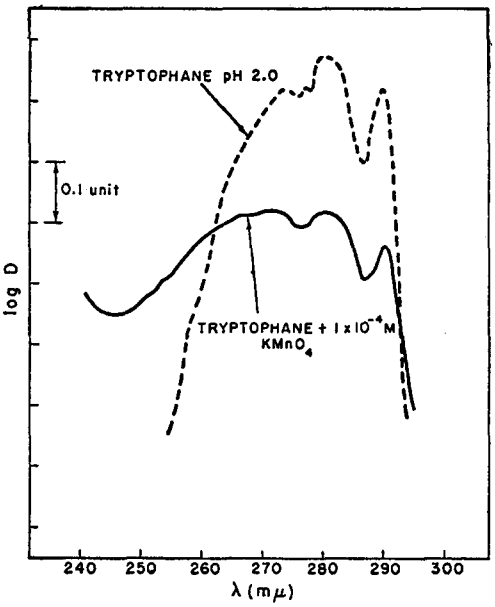

Fig. 2. Absorption spectrum of tryptophane (originally 0.04 mg/ml in $M/20$ KCl buffer, p_H 2.0) before and after the addition of permanganate. Solutions were lyophilized and residues redissolved in 60% glycerol, 40% water at approximately 10 times the original concentration for determination of the absorption spectra at 77° K

Insulin and Pepsin

As might have been expected, the absorption spectrum changes of permanganate treated insulin and pepsin paralleled those of tyrosine and tryptophane. As can be seen from the 77° K absorption spectra studies (Fig. 3 and 4), both insulin and pepsin showed the same changes in the relative intensities of the fine structure bands as did the free amino acids. Both proteins show a relative increase in the tyrosyl 282 mμ band. This change probably indicates the oxidative formation of dopa groups in the proteins (as in tyrosine solutions) and is comparable to the formation

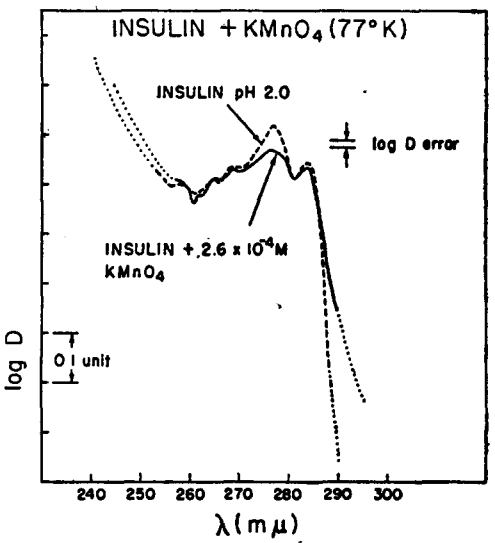

Fig. 3. Absorption spectrum of insulin (originally 1.0 mg/ml in $M/20$ KCl buffer, p_H 2.0) before and after the addition of permanganate. Solutions were lyophilized and residues redissolved in 60% glycerol, 40% water at approximately 10 times the original concentration for determination of the absorption spectra at 77° K

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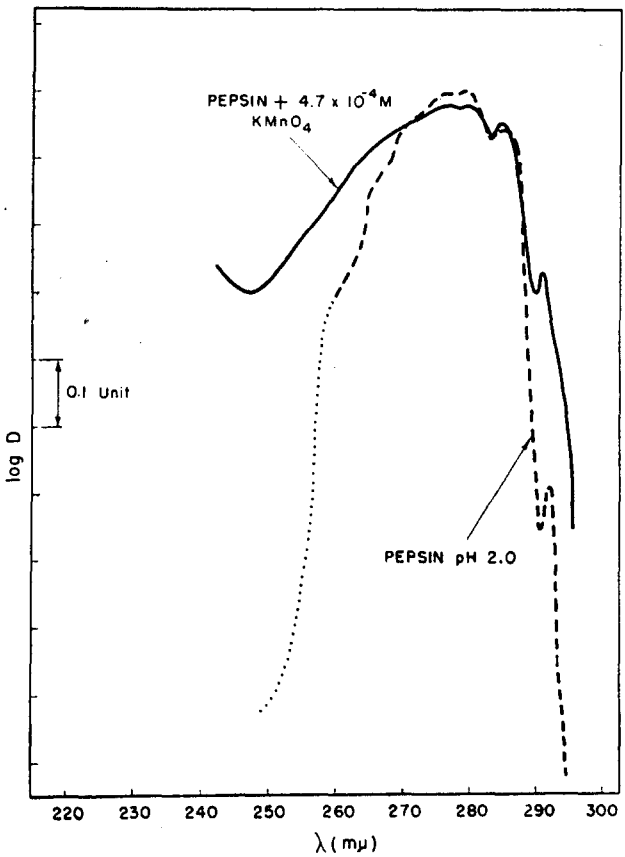

of dopa groups in proteins enzymatically oxidized with tyrosinase[3]. The relative changes in the tyrosyl fine structure bands are not as great in the proteins as in the free tyrosine solutions, and it is not surprising, therefore, that neither insulin nor pepsin showed any actual increase in absorption as a result of treatment. At the permanganate concentrations used, a smaller fraction of dopa groups are evidently formed in the proteins than are formed from free tyrosine. Fig. 4 also shows some relative increase in the 290 mμ absorption band of pepsin paralleling the change in the spectrum of tryptophane after treatment with permanganate (Fig. 2). No explanation is now available for this effect of permanganate on tryptophane.

Fig. 4. Absorption spectrum of pepsin (originally 0.8 mg/ml in *M*/20 KCl buffer, p_H 2.0) before and after the addition of permanganate. The solutions were lyophilized and the residues redissolved in 60% glycerol, 40% water at approximately 10 times the original concentration for determination of the absorption spectrum at 77° K

SUMMARY

1. The effects of permanganate oxidation on the 293° K and 77° K ultraviolet absorption spectra of the aromatic amino acids and crystalline insulin and pepsin have been studied.

2. The susceptibility of tyrosine and tryptophane, and the relative resistance of phenylalanine, to permanganate oxidation have been confirmed.

3. Spectroscopic evidence was obtained for the formation of dopa in solutions of tyrosine, and of dopa groups in solutions of insulin and pepsin, by permanganate treatment.

4. Permanganate induced changes in the fine structure of insulin and pepsin absorption spectra at 77° K are shown to parallel in several respects the spectrum changes in tyrosine and tryptophane similarly treated.

RÉSUMÉ

1. Les effects de l'oxydation par permanganate aux spectres d'absorption ultraviolets à 293° K et 77° K des acides aminés aromatiques, de l'insuline crystalline, et de la pepsine ont été etudiés.

2. La susceptibilité de la tyrosine et du tryptophane, et la résistence relative de la phénylalanine ont été prouvées à l'oxidation par permanganate.

3. L'évidence spectroscopique de la formation de dopa en solutions de la tyrosine et des groupes de dopa en solutions d'insuline et de pepsine traitées par permanganate, ont été prouvé.

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4. Le permanganate a induit des changes dans la structure fine des spectres d'absorption à 77° K de l'insuline et de la pepsine, lesquelles en plusieurs respects portent des changes spectroscopiques parallèles à celles de la tyrosine et du tryptophane traité similairement.

ZUSAMMENFASSUNG

1. Die Wirkung der Permanganatoxidation auf die bei 293° K und 77° K erhaltenen ultravioletten Absorptionsspektra der aromatischen Aminosäuren und des krystallinen Insulins und Pepsins wurden studiert.

2. Die Empfindlichkeit des Tyrosins und Tryptophans, und der relative Widerstandsfähigkeit des Phenylalanins gegen die Permanganatoxydation wurden bestätigt.

3. Spektroskopische Beweise der Formation von Dopa in Tyrosinlösungen, und von Dopagruppen in Insulin- und Pepsinlösungen wurden durch Permanganatbehandlung erlangt.

4. Das Permanganat ruft Änderungen in der Feinstruktur der Insulin und der Pepsin Absorptionsspektra bei 77° K hervor, die in mehreren Beziehungen parallel den Spektraländerungen des Tyrosins und Tryptophans nach ähnlicher Behandlung verlaufen.

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