A propos de l'extrémité N-terminale de la sérumalbumine

THOMPSON[1] a rapporté des observations intéressantes sur la nature du groupement α-aminé terminal libre des sérumalbumines bovine et équine. Contrairement aux résultats de TITANI, YOSHIKAWA ET SATAKE[2], cet auteur n'a pas retrouvé de cystine en position N-terminale. Or, au cours de recherches encore inédites sur la structure de la sérumalbumine, nous avions nous-mêmes été amené à étudier de nouveau la nature du groupement α-aminé terminal libre et nous avions obtenu des résultats qui correspondent exactement à ceux de THOMPSON.

Nous avons suivi strictement les conditions expérimentales de TITANI *et al.*[2] (dinitrophénylation, oxydation pendant 4 h à 0°, hydrolyse de la DNP-sérumalbumine oxydée pendant 4 h par HCl 6 *N* à 105°). L'extrait éthéré de l'hydrolysat contient uniquement de l'acide aspartique (identification en chromatographie sur papier bidimensionnelle dans les systèmes "toluène" de BISERTE ET OSTEUX[3] et "phosphate 1.5 *M*" de LEVY[4]). La phase aqueuse extraite par le mélange butanol secondaire-acétate d'éthyle (KOCH ET WEIDEL)[5] ne contient pas d'acide DNP-cystéique. En chromatographie unidimensionnelle dans le système butanol–acide acétique–eau (4:1:5), l'extrait contient un artefact jaune dont le R_F est plus élevé (0.64) que celui de l'acide cystéique (0.43). En électrophorèse sur papier à pH 3.9 (pyridine–acide acétique–eau), l'acide DNP-cystéique se détache facilement: aucune tache de même comportement ne peut être décelée quand on opère sur la phase aqueuse de l'hydrolysat de la DNP-sérumalbumine oxydée.

Ces constatations ont été faites aussi bien avec la sérumalbumine bovine qu'avec la sérumalbumine humaine. La sérumalbumine est donc bien constituée par une seule chaîne polypeptidique. Ce fait nous a semblé suffisamment important pour justifier notre intervention dans le débat.

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Abbréviation: DNP-, dinitrophényl-.

Reactions of haematins with peroxides and other oxidizing agents

The haemoprotein enzymes peroxidase and catalase each react with hydrogen peroxide to give three compounds which can be distinguished by their colour, absorption spectra, the conditions of their formation and their reactions with hydrogen donors. Methaemoglobin, however, gives only one compound with hydrogen peroxide[1,2]. Since all these haemoproteins have the same protohaematin prosthetic group which is primarily involved in the above reactions, it was of interest to study the effect of hydrogen peroxide on free haematins. However, different attempts to obtain a

protohaematin peroxide compound failed, as under the experimental conditions so far investigated, the protohaematin was either modified or destroyed by peroxide. Only urohaematin, which has eight carboxylic side chains on its porphyrin ring and which is more soluble in aqueous solvents and more reactive under certain conditions than protohaematin[3], was found to give a characteristic red compound[4] both with H_2O_2 and C_2H_5OOH. The absorption spectrum of this compound is of the same pattern as that of the methaemoglobin or metmyoglobin peroxide compound but its absorption bands in the visible region lie much nearer the blue end of the spectrum (at 547.5 and 521 mμ) as one would expect to find in haematin derivatives which are devoid of the unsaturated vinyl goups, while the Soret band lies at 413 mμ. The urohaematin peroxide compound is formed and is sufficiently stable for spectroscopic study only in a strongly alkaline solution such as 0.7–1.5 *N* NaOH. Even under these conditions it reverts within a few minutes to free alkaline urohaematin. In 0.1 *N* NaOH, 0.1–0.3 *M* Na_2CO_3 or 0.1 *M* phosphate buffer, pH 7.3, the incomplete formation of the urohaematin peroxide compound is almost immediately followed by the destruction of the urohaematin. Moreover, if urohaematin is added to a system composed of notatin (glucose oxidase), glucose and oxygen in phosphate buffer, pH 5–7.3, it is rapidly destroyed by the H_2O_2 generated during the oxidation of glucose without showing the formation of the urohaematin peroxide compound. This rapid destruction of urohaematin in neutral or slightly acid solution by a small concentration of generated H_2O_2 contrasts with the marked stability of this haematin in strongly alkaline solution towards a much higher concentration of H_2O_2.

It has been shown previously[1] that the reaction of methaemoglobin with peroxides is reversible only with regard to the methaemoglobin, the H_2O_2 being used up in some peroxidatic reaction. In this respect H_2O_2 was found to differ from substances such as cyanide, azide or fluoride which form perfectly reversible complexes with haemoproteins. A more recent study[5–7] of this problem revealed that the metmyoglobin peroxide compound is only one oxidizing equivalent higher than metmyoglobin itself and that a compound indistinguishable from metmyoglobin peroxide could be obtained by treating metmyoglobin with oxidizing agents such as sodium chlorite, potassium chloriridate and others. The product of the reaction between metmyoglobin and H_2O_2 cannot, therefore, be described as a metmyoglobin-H_2O_2 equilibrium complex but as a derivative of metmyoglobin with an effective oxidizing number of + 4. Although the true nature of such a compound has not yet been properly elucidated, it behaves in its reactions as a metmyoglobin with a quadrivalent iron atom. It can, therefore, be referred to as a higher oxidation state of metmyoglobin or simply as a metmyoglobin peroxide compound but not as a metmyoglobin peroxide complex, the term complex being reserved for perfectly reversible products of the reactions of haemoproteins with ligands such as cyanide, azide and fluoride.

Since urohaematin has already been shown[3] to give with H_2O_2 and C_2H_5OOH derivatives analogous to the methaemoglobin or metmyoglobin peroxide compounds, to complete the analogy it was important to determine whether similar compounds could be obtained using other oxidizing agents such as sodium chlorite ($NaClO_2$) and sodium chloriridate ($NaIrCl_6$) instead of peroxides. When $NaClO_2$ is added to urohaematin in 1 *N* NaOH, the colour of the solution turns from brown to red and its absorption band at 596 mμ is replaced by two bands at 547.5 and 521 mμ indistin-

guishable from those of the urohaematinperoxide compound[3]. However, these absorption bands soon disappear and the solution turns from red to green owing to the destruction of the haematin whereas the urohaematin peroxide compound obtained with peroxides (H_2O_2 and C_2H_5OOH) tends to revert to free urohaematin.

The effect of $NaIrCl_6$ could only be studied in Na_2CO_3 solutions as it is rapidly destroyed in strong alkali. In the presence of 0.3 M Na_2CO_3, urohaematin reacts immediately with $NaIrCl_6$ giving the same absorption spectrum as with H_2O_2 or $NaClO_2$ but the compound formed is very unstable reverting to free urohaematin. Urohaematin ($6.6 \cdot 10^{-4}$ M) treated with $NaIrCl_6$ or with $NaClO_2$ in a 1:1 molecular ratio forms about 60% and 75% respectively of the urohaematin peroxide compound. These results are of the same order as those obtained in the reaction between $KIrCl_6$ and metmyoglobin[6].

The effects of $NaClO_2$ and $NaIrCl_6$ on other haematins (proto-, haemato-, deutero- and coprohaematin) were also investigated. Of these haematins coprohaematin (prepared from coproporphyrin obtained from the urine of a case of bovine congenital porphyria) is of special interest since it has 4 carboxylic side chains; it therefore occupies an intermediate position with regard to its solubility and several other properties between urohaematin, which has 8 carboxylic side chains, and proto-, haemato-, deutero- and mesohaematin which have only 2 carboxylic side chains in the porphyrin ring. Yet coproporphyrin, unlike uroporphyrin, gives only with $NaClO_2$ an incomplete and evanescent absorption spectrum corresponding to the urohaematin peroxide compound. In this respect haemato- and deuterohaematin behave like coprohaematin.

These results show that the action of oxidizing agents on free haematins leads in most cases to their destruction rather than to the formation of haematin peroxide compounds. Only urohaematin, in strongly alkaline solution, gives with peroxides and other oxidizing agents ($NaClO_2$ or $NaIrCl_6$) a characteristic haematin peroxide compound. However when a haematin (protohaematin) is combined with specific native proteins to form methaemoglobin, metmyoglobin, peroxidase and catalase, it acquires the property of entering far more easily into higher oxidation states and shows greater resistance to oxidative destruction.

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