Photoreactions of Biacetyl with Methionine and Related Compounds

Since WACKER, CHANDRA et al.[1,2] first discovered the ability of acetone to promote the photodimerization of pyrimidines, water-soluble ketones have been widely employed to induce the photodamage of nucleic acid constituents[3–5]. On the other hand, the photoreactions of such ketones with proteins are still unexplored, although a preliminary investigation in our laboratory[6] pointed out that acetone can efficiently sensitize enzymes to photoinactivation. In order to achieve a better understanding of the possibilities opened by the use of this new class of photosensitizers, we extended our studies to the photoeffects of a large number of ketones on amino acids. In this paper, we report our findings about the photoreactions of biacetyl with methionine, which disclose interesting prospects for the photobiology of proteins.

Materials and methods. Biacetyl was a product of Carlo Erba and was distilled prior to use. L-methionine (Met) and the other amino acids were purchased from Fluka. The peptide N-benzyloxycarbonyl-L-methionyl-L-aspartic acid (Z-Met-Asp) was synthesized in this Institute. Irradiations were performed by a 450-watt Xenon lamp[7]; narrow light bands were isolated by means of Balzers interference filters. The irradiated aqueous solutions, pH 5.9, were 1 m*M* in amino acid, 0.1 *M* in biacetyl and were contained in a Pyrex cuvette of 2-cm light path. Deaeration was achieved by flushing with ultrapure N_2 for 15 min prior to and during illumination. The temperature was kept at 18 ± 1°C. At the end, biacetyl was removed by distillation under reduced pressure and/or by lyophilization, and the recovered material was analyzed by thin layer chromatography on silica gel plates or by quantitative column chromatography on a Carlo Erba 3A27 analyzer, according to the procedures detailed elsewhere[7,8].

Results. Irradiation of biacetyl plus Met by unfiltered light, in deaerated solutions, caused the gradual conversion of the amino acid into 3 ninhydrin-positive products, which were identified as Met sulfoxide, Met sulfone and α-aminobutyric acid, respectively, by comparison of their chromatographic Rf values in different solvent systems with those of authentic samples. Under our conditions of irradiation, about 50 % of the original methionine was reacted after 90 min. Prolonged illumination caused a further conversion of Met sulfoxide to the sulfone. The former compound appeared to be an intermediate in the photosensitized oxidation of Met: actually, irradiation of Met sulfoxide plus biacetyl, under the same conditions as used for Met, resulted in a quantitative formation of Met sulfone. The yields of Met sulfone and of α-amino butyric acid after irradiation of Met under various conditions are reported in the Table. Apparently, only trace amounts of the desulfurated product were recovered if the illumination was run in the presence of 10 m*M* hydroquinone or in O_2-saturated solutions; moreover, the photoprocess could be specifically driven towards the formation of Met sulfone by using monochromatic 404 nm light. Conversely, α-aminobutyric acid was the main product upon irradiation at 313 nm.

Irradiation studies with Z-Met-Asp showed that the incorporation of Met into a peptide molecule has no appreciable effect on the relative yields of the two aforesaid products. In particular, the photoreaction appeared to occur also if anhydrous dioxane was used as the solvent. Finally, α-aminobutyric acid and the corresponding sulfones were obtained by biacetyl-sensitized photoreaction of aethionine and homocysteine, whereas cystine was recovered unchanged even after prolonged exposure to light. In all cases, colorimetric assay of the irradiated solutions with Ti^{4+} salts[9] gave no evidence of the presence of hydrogen peroxide.

Discussion. The photosensitization of Met by biacetyl appears to be noticeable both for the nature of the products obtained and for the possibility of controlling the reaction pathway by changing the exciting wavelength or by adding radical scavengers to the irradiated system. Indeed, all the previously used sensitizers, including acetone, quantitatively converted Met to the sulfoxide, even in deaerated solutions, with the exception of flavins which give rise also to methional[10,11]. Therefore, this photoreaction allows one to introduce new modifications into a protein molecule: in particular, the removal of the thioether function from the Met side chain may provide interesting information about the involvement of this amino acid in H-bonds and, more generally, about its importance in the secondary structure of proteins; on the other hand, the conversion of Met to the sulfone can be utilized to induce an irreversible oxidation of this amino acid, since Met sulfoxide has been shown to be reverted to Met by reducing agents[12]. The method appears to possess also a certain degree of selectivity, since our findings show that the photoreaction is of general occurrence for amino acids containing a thioether or a thiol function, but not a disulfide group.

The influence of the irradiation conditions on the pathway of the photoprocess provide also some mechanistic insights. It is likely that α-amino butyric acid is formed by

Percent recovery of Met sulfone and of α-aminobutyric acid after irradiation of Met in the presence of biacetyl

Conditions of irradiation	Met sulfone	α-aminobutyric acid
Unfiltered light, under N_2	56.7	33.5
Unfiltered light, under N_2, plus hydroquinone	67.3	5.0
Unfiltered light, in air	65.1	3.8
404 nm light, under N_2	92.4	0.0
313 nm light, under N_2	20.0	69.7

All irradiations were performed at 20 °C in aqueous solutions (pH 5.9), using a 450-watt xenon lamp as the light source. The solutions were contained in Pyrex cuvettes of 2-cm light path. The recovery was evaluated after 2 h of irradiation, by chromatographing known amounts of the irradiated solution on a Carlo Erba 3 A27 amino acid analyzer.

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[1] A. WACKER and P. CHANDRA, Studia biophys. *3*, 239 (1967).

[2] P. CHANDRA, P. MILDNER, H. FELLER and A. WACKER, Europ. photobiol. Symp. (Hvar, Yougoslavia, 1967), p. 145.

[3] A. A. LAMOLA and T. YAMANE, Proc. natn. Acad. Sci., USA *58*, 443 (1967).

[4] L. KITTLER and G. LOBER, Photochem. Photobiol. *10*, 35 (1969).

[5] P. CHANDRA, *Research Progress in Organic Biological and Medicinal Chemistry* (Eds. U. GALLO and L. SANTAMARIA; Academic Press, New York), Vol. 3, in press.

[6] G. GENNARI and G. JORI, FEBS letters *10*, 129 (1970).

[7] G. JORI and G. GAUZZO, Photochem. Photobiol. *12*, 231 (1970).

[8] G. JORI, G. GALIAZZO, A. MARZOTTO and E. SCOFFONE, Biochim. biophys. Acta *154*, 1 (1968).

[9] P. BONET-MAURY, C. r. Séance Soc. Biol., Paris *213*, 117 (1944).

[10] J. D. SPIKES and R. LIVINGSTON, Adv. Radiat. Biol. *3*, 29 (1969).

[11] J. D. SPIKES and M. L. MACKNIGHT, Ann. N.Y. Acad. Sci. *171*, 149 (1970).

[12] G. JORI, G. GALIAZZO, A. MARZOTTO and E. SCOFFONE, J. biol. Chem. *243*, 4272 (1968).

attack on Met by radical species deriving from the photolysis of photoexcited biacetyl: actually, the conversion of Met to this product is inhibited by O_2 or hydroquinone, which are known to be radical scavengers, and becomes appreciable only upon irradiation with wavelengths sufficiently low to promote photodissociation of biacetyl[13]. On the contrary, Met sulfone should be produced by direct attack of triplet biacetyl, which is known to be electrophilic[14], on the sulfur atom of Met. Actually, the irradiation conditions leading to the maximal yield of sulfone are coincident with those enhancing the quantum yield of the intersystem crossing to the first triplet state of biacetyl[13]. The possibility that photoexcited biacetyl primarily interacts with the solvent must be ruled out since the photoreaction proceeds also in anhydrous solutions; moreover, no H_2O_2, which could in turn oxidize Met, is formed during the photoprocess. Finally, since the oxidation of Met to the sulfone occurs also in anerobic conditions, our findings suggest that the triplet biacetyl acts also as an oxygen-donor for Met; a possible activation of molecular O_2 by the excited sensitizer should be only of minor importance.

Riassunto. L'irradiamento di soluzioni acquose di metionina e biacetile, con luce assorbita dall'α-dichetone, da' origine a metionin solfone e ad acido α-amminobutirrico. La fotoreazione puo' essere pilotata selettivamente verso l'uno o l'altro dei due prodotti mediante il controllo della pressione di O_2 e della lunghezza d'onda di eccitazione, oppure mediante introduzione di catturatori di radicali nel sistema irradiato.

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[13] W. Noyes, G. B. Porter and J. E. Jolley, Chem. Rev. *56*, 49 (1956).

[14] E. J. Baum, J. K. S. Wan and I. N. Pitts jr., J. Am. chem. Soc. *88*, 2652 (1966).

Rapid Quantitative Evaluation of Serum LDH Isoenzyme Patterns After Agar Gel Electrophoresis

Agar gel electrophoresis[1] has been widely used to separate serum LDH (lactic-dehydrogenase) isoenzymes[2,3], and consequently, different methods have been developed to evaluate the agar gel zymograms. Wieme[4,5], using enzymoelectrophoresis, proved that the spectrophotometric method can be directly applied to an agar gel and the modifications occurring at the level of NADH followed at 366 nm in a modified Eppendorf photometer. Variants of the spectrophotometric method have been described by Blanchaer[6], who used the disappearence of fluorescence in UV that accompanies the oxidation of NADH2, and by Laursen[7], who measured the bluish fluorescent light at 460 nm. Visual staining techniques followed by densitometric evaluation of isoenzymes are more common procedures, especially since the introduction of phenazine methosulphate as electron carrier and tetrazolium salts as suitable acceptors[8–10]. In these staining methods, the protons transferred to NAD to form NADH2 are taken up by phenazine methosulphate and thence transferred to a tetrazolium salt, which is thereby reduced to an insoluble formazan, appearing on the gel as a purple band. After staining, isoenzymes may be quantified by reflect-

Comparison of results from elution and densitometric procedures

		LDH Isoenzymes									
		1		2		3		4		5	
Diagnosis	No. of Cases	E	D	E	D	E	D	E	D	E	D
Myocardial infarction	10	45.6 ± 1.8	45.8 ± 2.2	41.0 ± 1.0	40.1 ± 1.2	13.4 ± 1.7	14.1 ± 1.6				
Infectious hepatitis	5	18.4 ± 2.0	18.0 ± 1.8	28.4 ± 2.0	28.4 ± 2.6	13.9 ± 1.7	13.5 ± 2.2	11.1 ± 3.8	10.6 ± 2.0	28.2 ± 4.0	29.5 ± 2.5
Neoplastic diseases	8	20.2 ± 0.9	21.2 ± 0.7	36.1 ± 1.6	35.6 ± 1.8	25.9 ± 0.7	25.8 ± 0.5	12.6 ± 1.2	12.7 ± 1.7	5.2 ± 0.6	4.7 ± 0.8
Normal subjects	7	28.6 ± 0.7	28.3 ± 0.5	39.6 ± 0.7	38.8 ± 0.6	19.0 ± 0.5	18.9 ± 0.7	10.4 ± 0.6	10.3 ± 0.6	2.4 ± 1.1	3.7 ± 1.1

E, elution technique; D, densitometric technique. The first figure in any row and column is the mean for all cases in each group. The second figure is the calculated standard error of the mean.

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[1] R. J. Wieme, Clin. chim. Acta *4*, 317 (1959).

[2] R. J. Wieme and Y. van Maercke, Ann. N.Y. Acad. Sci. *94*, 898 (1961).

[3] H. J. van der Helm, Clin. chim. Acta *7*, 124 (1962).

[4] R. J. Wieme, Behringwerk-Mitt. *34*, 27 (1958).

[5] R. J. Wieme, Clin. chim. Acta *4*, 46 (1959).

[6] M. C. Blanchaer, Clin. chim. Acta *6*, 272 (1961).

[7] T. Laursen, Scand. J. clin. Lab. Invest. *14*, 152 (1962).

[8] M. M. Nachlas, J. S. Margulies and A. M. Seligman, J. biol. Chem. *235*, 499 (1960).

[9] E. Kun and L. G. Abood, Science *109*, 144 (1949).

[10] N. M. Papadopoulos and J. A. Kintzios, Am. J. clin. Path. *47*, 96 (1967).