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Interaction of Glyoxal and Methylglyoxal with Biogenic Amines

(dopamine/charge transfer/free radicals/muscular dystrophy/photophosphorylation)

ALBERT SZENT-GYÖRGYI AND JANE A. McLAUGHLIN

Marine Biological Laboratory, Woods Hole, Massachusetts 02543

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ABSTRACT **Glyoxal and methylglyoxal interact with biogenic amines and form biologically active free radicals. Electron spin resonance absorption of the radical at room temperature is characterized by a signal at g = 2.004 with peak-to-peak width of 29 G. An optical absorption at 400 nm with molar absorptivity of 23,000 accompanies the formation of the radical. The dry powdered preparation of the same reaction, which is considered to be the secondary product, gives an electron spin resonance signal much narrower and $^1/_{200}$ in intensity compared with the one in solution. Similarly the 400 nm absorption intensity is $^1/_8$ that of the primary product.**

Possible biological significance of the primary and the secondary product, in relation to muscular dystrophy and photophosphorylation, is discussed.

Biogenic amines and dicarbonyls (ketone aldehydes or dialdehydes) play an important role in cellular regulations (1). Both are rather reactive and readily interact with one another. In the living cell they must be kept separated. The separation of substances within the narrow boundaries of the cell demands a high degree of order. If this order is disturbed, the interaction of the two groups of substances may lead to unwanted products and pathological conditions.

The possibility of such a cross reaction of the two regulatory systems was supported by the observations of Parker and Mendell (2), who found that after a derangement of the monoamine oxidase, the injection of serotonin into rats produced Duchenne-type muscular dystrophy. One of the characteristics of this degenerative disease is the appearance of fluorescent material in the sick muscles. The interaction of biogenic amines with glyoxal or methylglyoxal leads to the formations of fluorescent substances which, if excited by light of wavelength 400 nm (which corresponds to the maximum of their absorption), emit fluorescent light of wavelength 480 nm. If a 0.1% solution of dopamine hydrochloride is mixed with an equal volume of 0.25 M phosphate buffer of pH 7, and a 0.05 M solution of glyoxal, the appearance of a yellow color and an absorption at 400 nm wavelength indicates an interaction. The absorption rises with increasing speed, giving a curve characteristic for autocatalytic reactions (Fig. 1). The absorption is due to the development of a narrow structureless peak, suggesting electronic delocalization and a free radical chain reaction. The eventual extinction corresponds to a molar absorptivity of 23,000.

Methylglyoxal, used instead of glyoxal, behaves similarly, but the rise in absorption is about four times slower. The molar absorptivity of the product is also lower, 18,000.

Abbreviation: ESR, electron spin resonance.

If a mixture of dopamine and glyoxal or methylglyoxal is prepared that contains both substances in molar concentration at pH 7–7.4, a dark precipitate is formed. The reaction goes to completion in 60–90 min, and the precipitate can be isolated with a 75% yield. The precipitate formed by glyoxal and dopamine, in dried condition, is a blue-black powder which gives a strong electron spin resonance (ESR) signal, is thus a stable free radical. It can readily be dissolved in dimethylsulfoxide or dimethylformamide. In the spectroscope it gives a similar peak as the above solution but the molar absorbance is considerably lower, 3000, indicating that the isolated substance is not identical with the primary product. The dopamine complex of methylglyoxal formed under similar conditions is lighter in color and gives a weaker spin resonance signal. Glyoxal complexes contained 59.10% C, 5.21% H, 6.60% N, and 2% ash. Methylglyoxal complex: 59.29% C, 5.77% H, and 5.49% N (not analyzed for ash). Both complexes decomposed at higher temperature without melting.

Noradrenalin, adrenalin, and serotonin behaved similarly, forming highly colored complexes with glyoxal which gave high and narrow peaks around 400 nm, corresponding to a high molar absorbance, over 10,000.

The dried powdered complexes of dopamine–glyoxal, serotonin–glyoxal, and serotonin–methylglyoxal gave strong ESR signals at g = 2.005 with various peak-to-peak line widths and spin concentrations:

	Width (G)	No. of spins/mg ($\times 10^{-14}$)
Dopamine–glyoxal	8	2.5
Serotonin–glyoxal	9.8	24
Serotonin–methylglyoxal	12	5

The approximate number of spins was determined with the Varian weak pitch sample as a reference. The signals in the dry state were stable over the period of several weeks. In dimethylformamide solution (5–10 mg/ml) signals with little structure were obtained at the same g value and width; the intensity slowly decreased with time.

Dopamine was dissolved in a pH 7.0 phosphate buffer, then glyoxal was added and the mixture was stored for one hour at room temperature, during which time the yellow color developed. Then the solution was frozen at 77°K. In the ESR a structureless symmetrical signal with peak-to-peak width of 27 G was obtained at g = 2.005. Another similar solution was frozen 5 min after mixing. The signal obtained was of the same shape as before but its intensity was 3.5 times larger, which indicates that the radicals declined rapidly.

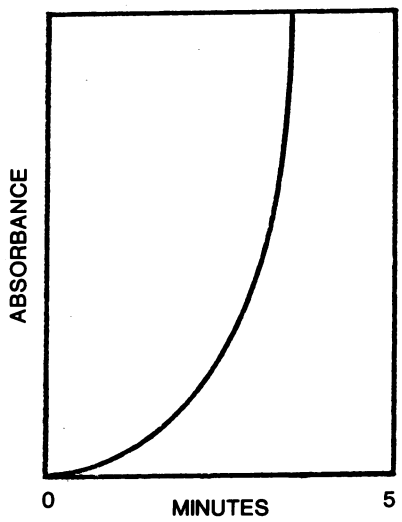

FIG. 1. Absorbance of a mixture of glyoxal and dopamine.

Similar measurements were made at room temperature, starting 5 min after mixing. A large symmetrical signal with peak-to-peak width of 29 G and at least 10 poorly resolved hyperfine structures was obtained at 2.004 g. During the first 20 min the signal rapidly decreased, to decrease more slowly later. In 2 hr the intensity decayed to about $^1/_4$ with no apparent change in shape.

The solid dry and black powder of the dopamine–glyoxal complex was suspended in water. The quantity of the powder was comparable to the quantity of the methylglyoxal–dopamine complex formed in the above experiments. At 77°K an asymmetrical signal was obtained at g = 2.005, but the peak-to-peak height was only $^1/_{16}$ of the earlier one, and the peak-to-peak width was only 7.5 G. The number of spins per gram was $^1/_{200}$ of the former.

All this brings out the fact that biogenic amines and dicarbonyls form free radicals. The primary product of the reaction is a hitherto unidentified compound. Charge transfer then takes place within the complex. Cyclization may occur later.

Dr. Daniel I. Arnon kindly tested the solid glyoxal and methylglyoxal complex of dopamine for their ability to catalyze photophosphorylation. He found them inactive in cyclic photophosphorylation, but active in pseudocyclic photophosphorylation in which ATP formation is coupled to a noncyclic unidirectional type of electron flow in which electrons from water photoreduce a cofactor which is reoxidized by oxygen. The dopamine complexes functioned similarly to methyl viologen, which is reduced by water and reoxidized by oxygen, transferring electrons. The reaction was inhibited by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), which specifically inhibits electron flow from water to oxygen.

Anderson (3) called attention to the fact that nutritional muscular dystrophy and human myocardial infarction can be caused in the absence of antioxidants by the auto-oxidation of highly unsaturated fatty acids. Schauenstein and his associates (4) showed that the auto-oxidation of highly unsaturated fatty acids leads to the formation of unsaturated hydroxyaldehydes, the various chemical and biological reactions of which are similar to those of glyoxal and its derivatives.

That the described reactions may have a biological meaning is indicated by the fact that only amines which had a regulatory function showed them. The reactions of dopamine were not shared by tyramine, nor were the reactions of serotonin shared by tryptamine, though the primary reaction has to be between the amino group and the carbonyl. Somehow the second OH in dopamine and the 5-hydroxy group in serotonin, which lend the biological activity to the molecule, must be involved.

Cotzias *et al.* (5, 6) have shown dopa to have a beneficial effect in Parkinson's disease, which lends a clinical importance to the described reactions.

How a cross reaction between the two regulatory systems, that of biological amines and that of ketone aldehydes, affects cellular reactions remains to be shown. It seems possible that pathological conditions may be produced by the mutual inactivation of the two groups of regulatory substances or by the toxic products of their interaction. Free radicals are known to be biologically active. In mice the products were found to be toxic.

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