

Radiation-Induced Changes on Beef Fat-Cephalin Mixtures by *gamma*-Radiation. Inhibition of *cis-trans* Isomerization

RUDOLF KOHN,¹ US Army Natick Laboratories, Natick, Massachusetts

Abstract

Ionizing radiation-induced *cis-trans* isomerization of unsaturated bonds in beef fat was inhibited by the addition of phospholipid. Infrared absorption at 10.35μ (966 cm^{-1}) of γ -irradiated beef fat-DL- α -cephalin dipalmitate mixtures was less than that of beef fat alone. These decreases were dose-dependent, but oxygen-independent. Reactions in the dose range 6 to 25 Mrad (see footnote 2) appear to differ from those in the dose range 25 to 100 Mrad. Some suggested explanations are offered for these phenomena.

Introduction

AN UNSOLVED PROBLEM in the analysis of irradiated foods is a simple and exact method for detecting a radiation treatment, especially for relatively low doses, e.g. 4 to 5 Mrad, applied to meat. A suitable solution to this problem would find wide application, since most countries have not approved the use of ionizing radiation for the preservation of food; but importation of irradiated food cannot be controlled for want of exact analytical procedures.

It has been observed that after irradiation by β - or γ -rays, fats show *cis-trans* isomerization of double bonds in addition to other chemical changes. A study, therefore, of irradiation-induced *cis-trans* isomerization in meat fat and of the effects of other meat components on the isomerization would be of interest.

Application of ionizing radiation to fats and oils causes polymerization, chain splitting and isomerizations. Oxidations occur only in the presence of oxygen, which reacts with the radicals formed by radiation. This paper deals only with radiation-induced *cis-trans* isomerization in beef fat and its observed inhibition by added DL- α -cephalin dipalmitate. Pan et al. (1) described *trans* isomerization during irradiation of oleic acid and potassium oleate. Similar effects upon beef tallow, horse fat, margarine, partially-hydrogenated margarine oil, olive oil and its methyl esters, peanut oil, pork fat and soybean oil were reported by Lück and co-workers (2,3). Both groups of investigators established that isomerization requires doses greater than 10 Mrad. In the present investigation it was seen that beef fat is quite resistant to radiation, even at doses up to 100 Mrad. This resistance made the assessment of the effect of the phospholipid additive in reducing *cis-trans* isomerization somewhat uncertain, especially since the effects were not appreciably different in concentrations ranging from 0.5 to 5.0%.

Little has been published on the high-dose radiation chemistry of natural phospholipids, perhaps because they are present in relatively small concentrations in animal foods. In beef muscle, for example, phospholipid content is between 0.8 and 1% (4,5). Radiation changes in lecithin were studied by Coleby (6), in cephalin after neutron bombardment by Hölzl (7), and in sphingomyelin and sphingosine sulfate by Landmann et al. (8). Neither Hölzl nor Landmann

investigated the easily demonstrable *cis-trans* isomerization.

Procedure

DL- α -cephalin dipalmitate (Mann Research Laboratories, Inc., New York, N. Y.) was added (0.5, 1.0, 2.0 and 5.0% w/w) to beef fat which had been extracted in a Soxhlet apparatus from beef adipose tissue by petroleum ether (b.r.: 30–60C); for composition see Table I. Samples of the mixture, and of controls, were melt-sealed in glass ampoules, part after evacuation (ca. 10^{-3} torr) for 3 hr, and part in air to insure adequate oxygen. Irradiation at 20C was in an underwater ^{60}Co - γ -radiation source (dose rate 0.75 Mrad/hour) to 6, 10, 25, 50 and 100 Mrad. The cylindrical arrangement of the 8 cobalt rods insured a homogeneous radiation field in the source. All samples were irradiated in duplicate.

The gas-chromatographic analyses were made with a Perkin-Elmer Vapor Fractometer Model 154B with a diethylene glycolsuccinate column (4 meters in length, 6, 4 mm I.D.: Gas-Chrom P 60/80 mesh). The temperature was 200C; the carrier gas was helium (20 psig). The preparation of the methyl esters was made by the BF_3 method.

Spectra of all samples were obtained in the region 9.5 to 11.0 μ (1053 – 909-cm^{-1}) with a Perkin-Elmer Model 21 IR Spectrophotometer (NaCl optics). For each sample, 10 sketched determinations were obtained. *Trans* isomer content (calculated as elaidic acid) was determined from a calibration curve established by known additions of elaidic acid methyl ester to beef fat. All peak areas were measured with a planimeter. Base lines for samples and calibration curve areas determinations were established between the maximum near 10.25 μ and the tangent near 10.7 μ .

Results and Discussion

The infrared (IR) spectra of beef fat and beef fat-cephalin mixtures, unirradiated with doses from 6 to 100 Mrad, in vacuum or in air, differ only slightly. The most prominent differences occur at 10.35μ (966 cm^{-1}), where absorption is attributed to the C-H deformation of isolated *trans* double bonds (Fig. 1). A distinct inhibition of *trans* isomerization results from the addition of cephalin. The observed inhibition was independent of the amount of added cephalin from 0.5 to 5.0% by weight of the beef fat.

Trans fatty acid contents (calculated as elaidic acid from the absorption at 10.35μ (966 cm^{-1})) are recorded in Table II. Differences between the pure fat

TABLE I
Composition of Used Beef Fat

Fatty acid	Wt. %
14:0	6.8
16:0	25.7
16:1	12.2
18:0	6.5
18:1	46.2
18:2	1.9
18:3	1.2
	100.0

¹From the Deutsche Forschungsanstalt für Lebensmittelchemie, Munich, Germany.

²10⁶ rads (current usage prefers "megarads").

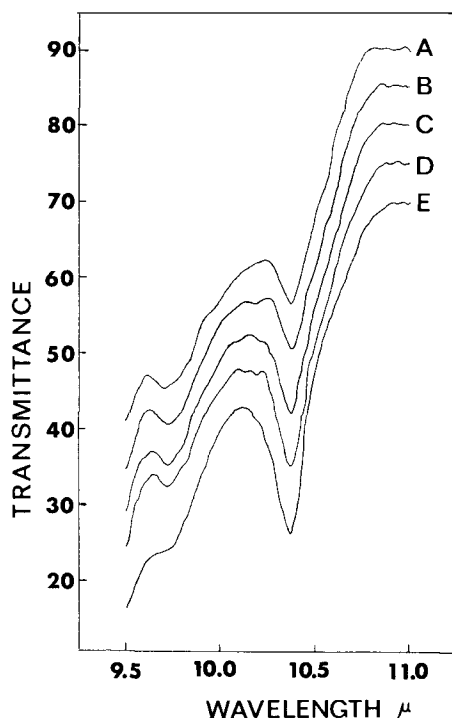


Fig. 1. IR spectrum of beef fat in the range from 9.5 to 11.0 μ ($1053\text{--}090\text{ cm}^{-1}$) A: nonirradiated control sample; B: 6 Mrad; C: 10 Mrad; D: 50 Mrad; E: 100 Mrad. Irradiated in vacuum. Concentration: 1.5 g fat and 4.5 ml CS_2 . Each curve is displaced by 5% transmittance units for clarity.

and the mixture, as well as differences due to dose applied are significant whereas the minor differences between samples irradiated in vacuo and in air are not significant. The effect of dose on the increase of *trans* isomer (elaidic acid) becomes most apparent by graphical presentation in which the so-called *trans* isomer content of the unirradiated sample is subtracted from that of the irradiated sample (Fig. 2).

Lück and Kohn (3) used a beef fat with a high initial content of *trans* isomers, and, therefore, did not observe an increase in *trans* isomer after irradiation. In the present study the *trans* isomer content of the original fat was relatively low, and the radiation-induced additional *trans* compounds and their dependence upon dose were readily observed.

Results recorded in Table II and Fig. 2 show that an addition of DL- α -cephalin dipalmitate inhibits radiation-induced production of *trans* isomer in beef fat and that the presence or absence of oxygen was of little importance. An explanation of these observations will be suggested after some fundamental considerations.

The bonding energy of an ethylenic bond is ca. 143

TABLE II
Effect of Ionizing Radiation on Beef Fat and Beef Fat-Cephalin Mixtures

Dose, Mrad	<i>trans</i> Fatty acid content (%) (calculated as elaidic acid) ^a			
	Beef fat		Beef fat and cephalin dipalmitate ^b 0.5 to 5.0%	
	+O ₂	Vacuum	+O ₂	Vacuum
Control samples	0.73		0.70	
6	0.95	0.95	0.70	0.73
10	1.37	1.57	0.83	0.87
25	1.52	1.65	0.87	0.93
50	3.00	3.03	2.70	2.87
100	4.13	4.40	2.73	3.83

^a Averages of 10 spectral determinations on each of 2 samples at each dose and treatment.

^b In the range shown, the effect of cephalin concentration was unmeasurable.

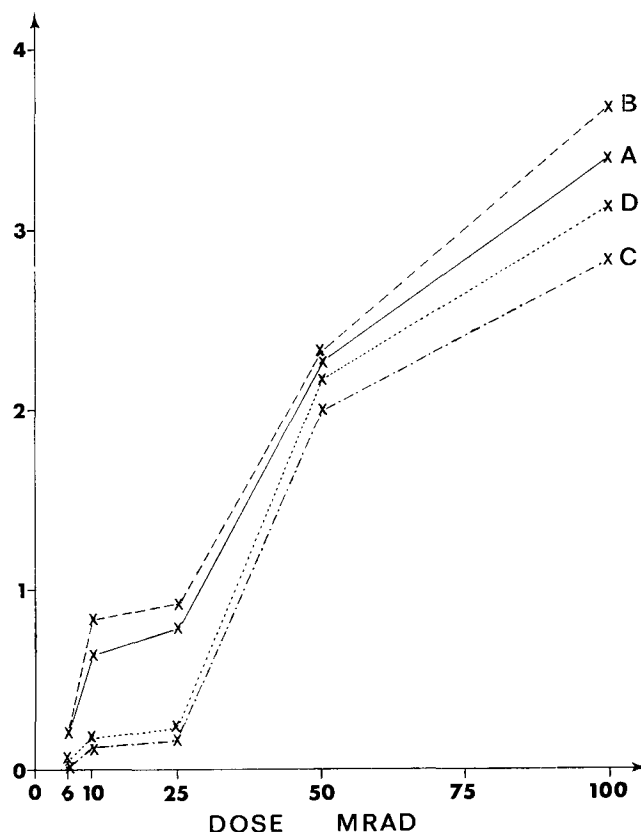


Fig. 2. Apparent increase of *trans* isomers in beef fat and beef fat cephalin dipalmitate mixtures. A, beef fat + O₂; B, beef fat + vacuum; C, beef fat + 1% cephaline dipalmitate + O₂; D, beef fat + 1% cephalin dipalmitate + vacuum.

kcal/mol; of a C-C single bond ca. 83 kcal/mol (9); of an activated state with decoupled π -electrons ca. 60 kcal/mol, corresponding to ca. 2.6 eV per bond, so that free rotation about the C-C bond is permitted. Thus, the energy required for uncatalyzed *cis-trans* isomerization is so great that heat alone cannot convert the more energy rich *cis*-configuration (oleic acid) to the more stable *trans* form (elaidic acid).

For ionizing radiation-induced chemical reactions the amount of product is independent of the concentration of the original substance, and dependent only on the radiation intensity or on the dose. For any given fatty acid concentration, however, this holds true only over a limited and small concentration range. Thermodynamic equilibrium is reached at a *trans* isomer content of 23% and iodine value of 38, as shown by Griffith and Hilditch (10) at ca. 66% elaidic acid.

Charlesby (11) established for the irradiation of unsaturated hydrocarbons (dose 4.5×10^8 r) that equilibrium occurs at 35% *trans* and 65% *cis* isomers. This is compatible with the results of Lück and Kohn (3) who found that the irradiation of beef fat with a high original *trans* fatty acid content resulted in a diminution of *trans* isomer even at 100 Mrad. The principle of Le Chatellier—in a radiation chemical reaction the increase in energy corresponds to an increase in temperature—explains the *trans* to *cis* conversion.

Ionizing radiation-induced chemical and photochemical processes are typical homolytic reactions; that is, activated molecules and free radicals are formed. For radiation-induced *cis-trans* conversions, two reaction schemes are possible (3). One (Fig. 3, eq. a) proceeds by a decoupling of electrons by a

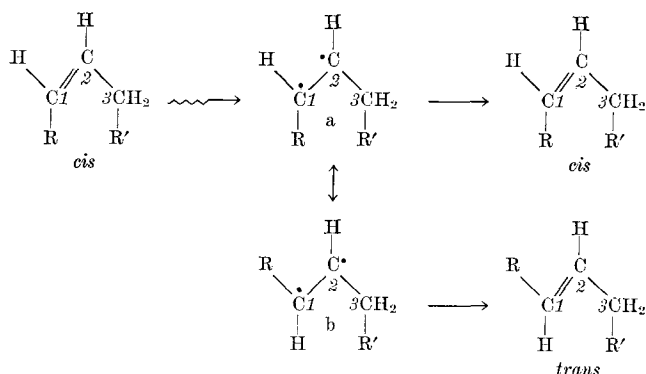


Fig. 3. Radical reaction mechanism of radiation induced *cis-trans* isomerization (equation a).

direct hit; the double bond is dissociated; a biradical is formed and free rotation around the $C_{(1)}-C_{(2)}$ bond is possible. After re-formation of the double bond, either the energy-rich *cis*- or the energy-poor *trans*-configuration may exist. Re-formation of the original steric form has a higher probability than does steric conversion. That is, more *cis* than *trans* isomers are formed.

The second reaction (Fig. 4) proceeds by a primary loss of hydrogen, leaving a radical in a position *a* to the double bond. A resonance hybrid can be postulated such that structures I, I' and I'' are equivalent to free rotation about $C_{(1)}-C_{(2)}$. Radical reaction through structure I'' by way of II leads to the *trans* isomer III; whereas reaction of I leads to retention of the *cis*-form IV. Actually, structures I' and I'' have no free rotation; they merely have a lower activation energy for rotation of the thermodynamically-favored *trans* form. Conversion of the radical to the *trans* form is favored if a substance (e.g. oxygen) is present which is able to totally decouple the π -electrons which are distributed over 3 carbon atoms of radical I. In this case, *trans* hydroperoxy radicals are formed, which after reaction with hydrogen, produce *trans* fatty acid peroxides. In this reaction scheme, if either structure I' or I'' enters the reaction, displacement of the double bond is possible.

Trans isomer content was easily determined by (IR) spectroscopy. Isolated *trans* double bonds absorb at 10.35μ (966 cm^{-1}), conjugated *cis-trans* octadecadienoates at 10.18 and 10.54μ ($982-940 \text{ cm}^{-1}$) and *trans-trans* isomers at 10.12μ (988 cm^{-1}). Neither isolated *cis*, nor conjugated *cis-cis* bonds absorb in this region of the electromagnetic spectrum. Because of the relatively small content of higher unsaturated fatty acids in beef fat (ca. 2-3% linoleic acid) no *cis-trans* or *trans-trans* isomers were expected, even at 100 Mrad, and none were observed (Fig. 1).

Almost all natural fats and some partially-hydro-

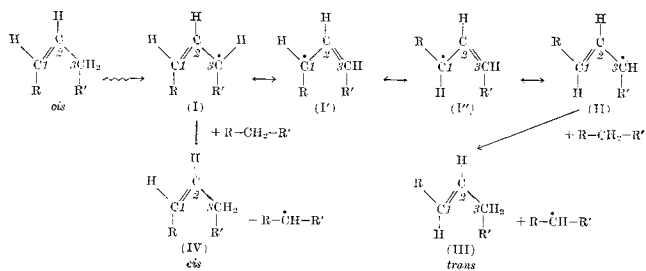


Fig. 4. Radical reaction mechanism of radiation induced *cis-trans* isomerization (equation b).

genated fats and oils exhibit a vibration at 10.35μ (966 cm^{-1}). Reduction in intensity of this IR band in beef fat by dilution with cephalin dipalmitate (beef fat: 0.73% elaidic acid, mixture 0.70%) is very much smaller than the difference seen after irradiation. This inhibition of isomerization has not hitherto been described. This phenomenon can be explained only by the already observed high resistance to radiation of the pure DL- α -cephalin dipalmitate and its protective effect upon the fat. Some saturated triglycerides are also relatively stable against radiation (12). It may be, therefore, that the presence of a phosphatidyl ethanolamine group in the saturated glyceride leads to additional increase in resistance to radiation and, by the so-called radical capture effect, to a protective effect on the surrounding area. It was well known that other saturated organic acid esters are stable against radiation.

An acceptable description of the reactions of the influence of cephalin on the observed *cis-trans* isomerization of the fatty acids of beef fat is very difficult, because unequivocal information about the chemical structure of the shared radical types, as well as the reaction end products, is still missing.

Some reactions between the cephalin and the unsaturated fatty acids of beef fat are possible. By γ -radiation radicals of the following type from the ethanol amine residue can be formed: $\dots O-CH_2-$

$\dot{C}H-NH_2$. Such a radical is able to inhibit the radical reaction which transforms the *cis* biradical into the *trans* biradical (Fig. 3). The reaction end product is a dimer molecule of the triglyceride and the cephalin. By such a reaction the creation of the *trans* biradical and then of the *trans* fatty acid containing triglyceride can be blocked.

On the other hand, a recombination reaction between the *cis* radicals and hydrogen can inhibit the formation of *trans* forms as also can the combination of two *cis* fatty acid radicals to form a dimer triglyceride.

IR, NMR and ESR and mass spectrometry are the most suitable methods to resolve these problems, but it is still necessary to isolate and prepare the reaction end products.

A look at the other reaction mechanism, also possible and described in Fig. 4 shows that the similar possibilities exist for ethanolamine and fatty acid radicals together or with hydrogen in the reaction between the radicals I' and I''.

Another observation requiring an explanation is the difference in the slope of the curves for radiation-induced increase in *trans* isomer in the pure fat and in the mixture (Fig. 2). Although, at the present state of this investigation, no completely satisfactory explanation is possible, some comments can be made. The small increases in *trans* isomer between 6 and 25 Mrad are regarded as an initial phase in which the protective effect is most apparent. This may be similar to the protection against radiation shown by systems with conjugated C-C bonds (e.g. β -carotene), or by antioxidants. At higher doses above 25 Mrad, the protective effect disappears. Isomerization becomes dependent only on the double bond content. At doses of more than 50 Mrad, the curves show that the formation of *trans* isomers levels off slightly. This effect is caused by increasing polymerization and destruction of fatty acids and the reduction in number of double bonds (diminution of the iodine value) (13).

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