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REACTIONS OF LOW VALENT TRANSITION METAL COMPLEXES WITH HYDROGEN PEROXIDE. ARE THEY "FENTON-LIKE" OR NOT? 4. THE CASE OF FE(II)L, L = EDTA; HEDTA AND TCMA

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The question whether hydroxyl free radicals are formed in the reactions of divalent iron complexes Fe(II)L; L = edta; hedta; tcma (tcma = 1-acetato-1,4,7-triazacyclononane) with hydrogen peroxide in neutral and slightly acidic solutions was studied by using the β elimination reaction as an assay for the formation of hydroxyl free radicals, ·OH. The results show that at pH<5.5 the iron(II)peroxide intermediate complex decomposes rapidly to yield free hydroxyl radicals for L = edta and hedta. This is in contrast to the mechanism of the corresponding Fe(II)nta peroxide complex, which probably decomposes to form Fe(IV)nta which then reacts with organic substrates to yield aliphatic free radicals. Thus, the non-participating ligand L has an appreciable effect on the mechanism of reaction of the metal center with hydrogen peroxide. Blank experiments using ionizing radiation as the source of $\cdot CH_2CR(CH_3)OH$, R = H or CH_3 radicals indicate that when L = tcma intermediates of the type $LFe^{III}–CH_2CR(CH_3)OH_{aq}$ are formed, but their major mode of decomposition is not the β elimination reaction. Thus, the present assay for the formation of hydroxyl free radicals by the Fenton Reaction does not fit the latter system.

KEY WORDS: Fenton, hydroxyl free radicals, Fe(II)(edta), Fe(II)(hedta).

INTRODUCTION

Free radicals play an important role in many biological[1-5] and catalytic processes.[6-8] One of the major paths by which aliphatic free radicals are produced is via abstraction of a hydrogen atom from organic substrates by the hydroxyl free radical, ·OH, reaction (1):

$$RH + \cdot OH \rightarrow \cdot R + H_2O \tag{1}$$

The major sources of hydroxyl free radicals in biological systems are either ionizing

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radiation or the so-called "Fenton Like" (or "Modified Fenton") reactions,[9–12] reaction (2):

$$M^{n}L + H_2O_2 \rightarrow M^{(n+1)}L + OH^{-} + \cdot OH \tag{2}$$

There is no doubt that the reaction of low valent transition metal complexes with peroxides leads to a variety of biological deleterious processes. Therefore, the investigation of its detailed mechanism has been the subject of many studies.[13–21] The formation of the aliphatic free radical ·R, is not a proof for the formation of ·OH free radicals in reaction (2). The free radicals might be formed by a variety of alternative processes, *e.g.*[21]

$$M^{n}L{\cdot}H_2O_{2aq} + RH \rightarrow M^{(n+1)}L_{aq} + \cdot R + OH^{-} + H_2O \tag{3}$$

$$M^{(n+2)}L_{aq} + RH \rightarrow M^{(n+1)}L_{aq} + \cdot R + H_3O^{+} \tag{4}$$

Three general approaches are used to determine whether ·OH free radicals are formed in reaction (2):

1. The detection of ·OH adducts to spin labelling compounds, *e.g.*, DMPO. However, recent experiments point out that alternative mechanisms not involving ·OH free radicals might lead to the formation the same adducts.[22]
2. The use of ·OH scavengers to inhibit a deleterious process.[23–24] The difficulties with this approach are:
   a. The use of free radicals ·R formed in reactions (1), (3) and (4) might also induce the deleterious effect to some degree.[25–27] The reactivity of different free radicals in these follow-up reactions are different. Therefore, the relative effectivity of two compounds RH as scavengers is not directly related to their rates of reaction with the ·OH free radicals, even if the latter have been formed in the system.
   b. The "Fenton Like" reaction is a site specific and not a homogeneous process as it occurs where the transition metal complex is bound in the biological system.[28]

Therefore, the efficiency of different scavengers is not expected to be directly related to their rates of reaction with ·OH free radicals even if they have been formed in reaction (2).

3. The measurement of the relative yields of the final organic products formed from the free radicals $\cdot R^1$ and $\cdot R^2$, formed in reaction (1), (the free radicals $\cdot R^1$ and $\cdot R^2$ might be formed by the reaction of ·OH radicals with a mixture of two organic substrates R^1H/R^2H, or by reacting with one organic compound RH at two different sites). The formation of the final products from the free radicals is often a complex, multiple-step process. Therefore, this assay is reliable only if both radicals $\cdot R^1$ and $\cdot R^2$ have very similar chemical properties, and if one can prove that indeed the yield of the final products equals the yield of the primary free radicals.

We have recently developed[29] a new and simple assay for the detection of ·OH free radicals, based upon the following reactions:

$$\cdot OH + CH_3CR^1R^2OH\ (R = H, CH_3) \rightarrow \cdot CH_2CR^1R^2OH + H_2O \tag{5}$$

$$\cdot CH_2CR^1R^2OH + LM^n_{aq} \rightarrow LM^{n+1}\text{–}CH_2CR^1R^2OH_{aq} \tag{6}$$

$$LM^{n+1}\text{–}CH_2CR^1R^2OH_{aq} \rightarrow LM^{n+1}_{aq} + H_2C{=}CR^1R^2 + OH^- \tag{7}$$

The relative yields of the alkenes produced when two alcohols are present are measured once in a N_2O saturated solution containing both the ferrous and the ferric complexes irradiated by ionizing radiation (where clearly $\cdot OH$ free radicals are formed) and once by adding hydrogen peroxide to a solution with an identical composition. As the chemical properties of the $\cdot CH_2CR^1R^2OH$ free radicals are very similar, this assay is usually not sensitive to solution composition. However, one has to demonstrate, prior to its application that the radicals $\cdot R^1$ and $\cdot R^2$ are transformed into the corresponding alkenes in a full yield via reactions (6) and (7).

In the present study we decided to apply this assay in order to check whether $\cdot OH$ free radicals are the major products of the reaction of Fe(II)edta with H_2O_2. Fe(II)edta was chosen as it is commonly used as a catalyst for the Haber-Weiss reaction, as a catalyst for reductively activated oxidations by dioxygen (the Udenfriend reactions)[30] and especially for determination of DNA sequence using the latter reaction.[31–35] In all these systems, it is assumed that $\cdot OH$ free radicals are being formed. However in a recent study we have shown that $\cdot OH$ free radicals are not the major product of the reaction Fe(II)nta + H_2O_2,[36] though nta is a ligand with very similar properties to those of edta.

Different assays were used to determine whether $\cdot OH$ free radicals are formed in the reaction of H_2O_2 with Fe(II)edta. Though conflicting reports were obtained, most studies indicate that indeed $\cdot OH$ free radicals are been formed in this reaction.[15–17,37–49] Recently, the group of Koppenol[13,16] reinvestigated the mechanism of this reaction. They concluded that $\cdot OH$ free radicals are not the major product of this reaction. However, in later studies,[50–52] using different assays, they concluded that indeed $\cdot OH$ free radicals are formed in this reaction. The different results concerning the detailed mechanism of the reaction of H_2O_2 with Fe(II)edta were summed up in a recent review.[21]

These contradicting conclusions are not surprising in view of the complicated mechanisms leading to the formation of the final products in the assays used.[24] It was noted in the literature that a simpler assay is required in order to solve this problem.[24] It was decided, therefore, to use the assay described above, which was previously employed for the analogous Fe(II)nta system,[36] for the study of the mechanism of the reaction of Fe(II)edta with H_2O_2.

A detailed thermodynamic analysis of the modified Fenton reaction[21,29] has shown that it cannot occur via a simple one-step outer sphere process, reaction (2). Based on the thermodynamic analysis, the mechanism suggested must involve the formation of a transient metal-peroxide complex, *e.g.*, $LM^n\cdot H_2O_2$. The transient metal-peroxide complex decomposes via one of the following pathways:

$$LM^n\cdot H_2O_2 \rightarrow LM^{(n+1)}_{aq} + \cdot OH + OH^- \text{ or} \tag{8}$$

$$LM^n\cdot H_2O_2 + RH \rightarrow LM^{(n+1)}_{aq} + \cdot R + OH^- + H_2O \text{ or} \tag{9}$$

$$LM^{n}\cdot H_2O_2 \rightarrow LM^{(n+2)}{}_{aq} + 2OH^{-} \qquad (10)$$

As the results pointed out that the mechanism of reaction of Fe(II)edta with H_2O_2 differs from that of the corresponding reaction of Fe(II)nta, it was decided to study also the reactions of Fe(II)L with H_2O_2, where L = hedta (N-hydroxyethyl-N,N′N′-triacetate-ethylenediamine), and tcma (1-acetato-1,4,7-triazcyclo-nonane). It was hoped that the additional results will shed light on the factors effecting the mechanism of the reaction of Fe(II)L with H_2O_2.

EXPERIMENTAL

Materials and Solutions

All chemicals employed were of analytical grade. Solutions were prepared using heat distilled water which was further purified by passing through a Millipore setup, the final resistance of the water being >10MΩ/cm. N_2O or He were purified from traces of oxygen by bubbling them through two washing bottles containing VSO_4 in diluted H_2SO_4 over Zn amalgam, followed by bubbling through a washing bottle containing pure water. The pH was adjusted with $HClO_4$ and NaOH.

As the Fe(II)L complexes react rapidly with dioxygen, the solutions have been prepared as follows: Solutions containing the required concentrations of the ligand and the alcohol were deaerated using the syringe technique by bubbling N_2O or He through them.[29,36] The required amount of solid $FeSO_4$ was added only after bubbling the gas for at least 15 minutes through the syringe. The pH was adjusted by gradual addition of deaerated dilute $HClO_4$ or NaOH while bubbling through the syringe into which the pH electrode was immersed. A known volume of the solution was injected through a rubber septum into small glass vials which were first washed with a stream of He or N_2O. These vials were then irradiated, or alternatively a deaerated solution of H_2O_2 was injected into them.

Tcma, 1-acetato-1,4,7-tri-azacyclononane, as the lithium salt was prepared as follows: to a solution of 5 g (36 mmole) of 1,4,7-triazatricyclo-[5.2.1.$0^{4.10}$]decane[53] in 40 ml toluene was added dropwise a solution of 2-bromoethanoic-acid-ethyl-ester (6 g, 36 mmole) in 50 ml toluene. The mixture was stirred for an hour at room temperature. The formed precipitate was collected by filtration. The white solid was then heated for ~ 5 hours in 4 M HCl (100 ml) under reflux. The solution was evaporated and the remaining oil was treated in ethanol (80 ml) and LiOH (6 g) to get the salt. The yield was 5.4 g (78%).

Irradiations

Two types of ionizing radiation have been employed:

1. A Varian 7715 linear accelerator which delivers 0.1–1.5 μ sec pulses (5 MeV electrons, 200 mA current) with a dose rate of approximately $1.2 \times 10^{26} eV\cdot sec^{-1} l^{-1}$.
2. A Co^{60} gamma source (Noratom) delivering 1.1 MeV γ ray photons with a dose rate of approximately 2.0×10^{18} $eV\cdot sec^{-1} l^{-1}$.

Dosimetry was carried out with the Fricke dosimeter[54] taking $G(Fe^{3+}) = 15.6$.

Analyses:

Concentrations of the stock H_2O_2 solutions were determined by titrations with $KMnO_4$. The quantitative determinations of propene and 2-methyl-propene were performed with a Varian 3700 gas chromatograph equipped with a TCD and FID connected in series. The gases were separated using a 9′ × ⅛″ stainless steel column packed with poropak Q mesh 60/80 particles at 160°C with He as carrier gas, the flow rate was 30 ml/min.

Assay for ·OH free radicals:

The assay used is based on the measurement of the ratio of the yields of two aliphatic radicals $\cdot R^1$ and $\cdot R^2$ in solutions containing a metal complex, M^nL, a mixture of two aliphatic alcohols, R^1H, R^2H and H_2O_2. The results are compared to the same solutions without H_2O_2 but irradiated by ionizing radiation.

Upon irradiation of N_2O saturated aqueous solutions containing two organic substrates R^1H and R^2H the following reactions take place:[54]

$$H_2O \xrightarrow{\gamma,\beta} e^-_{aq}(2.65),\ H\,(0.60),\ \cdot OH(2.65),\ H_2\,(0.45),\ H_2O_2(0.75). \qquad (11)$$

(Values in parentheses are G – values which represent the number of molecules of product formed per 100 eV energy absorbed by the solution.)

$$e^-_{aq} + N_2O \xrightarrow{H_2O} N_2 + \cdot OH + OH^- \qquad (12)$$

$$RH + \cdot OH \rightarrow \cdot R + H_2O \qquad (1)$$

These reactions are over within less than 1μsec from the time the radiation has been absorbed by the solutions.

If $\cdot R^{1,2}$ have an alcohol functional group at the β position to the carbon-centered free radical, and the solution contains an appropriate transition metal complex, formation of a complex containing a metal-carbon σ bond, reaction (6), followed by a fast β elimination process of hydroxide, reaction (7), are expected for the two radicals.

Thus, if the precursor to the radicals $\cdot R^{1,2}$ is the free hydroxyl radicals the ratio between the two radicals should obey equation (I):

$$\frac{[\cdot R^1]}{[\cdot R^2]} = \frac{k_5^1[R^1H]}{k_5^2[R^2H]} \qquad (I)$$

For 2-methyl-2-propanol $k_5 = 4.2 \times 10^8\ M^{-1}s^{-1}$.[55] For 2-propanol, H abstraction by ·OH radicals occurs at the α and β carbons via reaction (5a).

$$(C^{\beta}H_3)_2C^{\alpha}HOH + \cdot OH \rightarrow \cdot C^{\beta}H_2CH(CH_3)OH / \cdot C^{\alpha}(CH_3)_2OH + H_2O \quad k = 2.0 \times 10^9 M^{-1}s^{-1}$$

$$13.3\%^{(30)} \qquad 85.5\%^{55} \qquad (5a)$$

Thus, if R^1H = 2-methyl-2-propanol and R^2H = 2-propanol, the calculated value of the radical ratio is:

$$\frac{[\cdot CH_2C(CH_3)_2OH]}{[\cdot CH_2CH(CH_3)OH]} = 1.58\,\frac{[(CH_3)_3COH]}{[(CH_3)_2CHOH]} \qquad \text{(II)}$$

The experimental value can be calculated by measuring the concentrations of 2-methylpropene and propene produced from the corresponding β hydroxyl radicals.

Production of Alkenes via the Reaction of the Metal Complex with Hydrogen Peroxide:

If the metal-peroxide complex produced undergoes decomposition to hydroxyl free radicals, reaction (8), then the measured ratio of the alkenes should obey equation (II). However, if the radicals are produced via reaction of the metal-peroxide complex with the organic substrates, reaction (9), or via reaction (10) followed by reaction (4) then the ratio $[\cdot R^1]/[\cdot R^2]$ will be:

$$\frac{[\cdot R^1]}{[\cdot R^2]} = \frac{k_{(9\text{ or }4)}{}^1}{k_{(9\text{ or }4)}{}^2} \times \frac{[(CH_3)_3COH]}{\alpha[(CH_3)_2CHOH]} \qquad \text{(III)}$$

(Where α is the fraction of the β-radicals formed.)

The chemical properties of the hydroxyl free radicals are expected to differ appreciably from those of the $LM^n\cdot H_2O_2$ or the $LM^{(n+2)}$ species. One expects therefore that usually this assay will differentiate between the pathway involving reaction (8) and reaction (10), followed by reaction (4) or reaction (9). A basic condition to fulfill the efficiency of the assay is that the β radicals formed react via reactions (6) and (7). Furthermore, the ratio of the alkenes formed has to be independent of the concentrations of LM^n, $LM^{(n+1)}$, RH, L and the H_2O_2 added.

The advantage of this approach over measurements of relative yields of different radicals is that the β hydroxyl radicals have very similar chemical properties, whereas other radicals usually differ considerably in their chemical properties.

RESULTS AND DISCUSSION

When N_2O-saturated solutions containing 2-methyl-2-propanol or 2-propanol or mixtures of these alcohols in the absence of iron complexes, in the pH range 4–7, are irradiated the yield of alkenes is negligible in accord with the known radiation chemistry of aqueous solutions of alcohols.

The yield of 2-methylpropene formed, when N_2O-saturated solutions containing $1\text{–}4 \times 10^{-3}$M Fe(II)L and 0.4 M 2-methyl-2-propanol are irradiated, was measured. For L = edta and hedta, the yield of 2-methylpropene was considerably lower than that of the ·OH free radicals, *i.e.* G = 6.0, at pH >5.5. On the other hand, at pH ≤5.5, a full yield of 2-methylpropene was observed. These results point out that the present assay for the formation of ·OH radicals is not useful for these ligands at pH >5.5.

For L = tcma, the yield of 2-methylpropene was ≤2 in the whole pH range (4.0 ≤ pH ≤ 7.5) and thus, the present assay cannot be employed for this ligand.

When H_2O_2 was added to slightly acidic solutions, pH ≤ 5.5, containing Fe(II)edta

TABLE 1
Relative yields of 2-methyl-propene and propene formed in solutions containing Fe(II)edta by ionizing radiation and the Fenton Reagent.

$[Fe^{2+}] \times 10^3$ M	$[Fe^{3+}] \times 10^3$ M	$[edta] \times 10^3$ M	$[ROH]^a$ M	$[H_2O_2] \times 10^3$ M	pH	$C_4/C_3{}^b$ Radiation[c]	$C_4/C_3{}^b$ Fenton
5.0	–	8.0	0.675	1.0	5.0	1.25	1.36
"	–	"	"	"	5.5	1.48	1.85
1.0	–	"	"	"	5,5	1.70	1.85
1.6	–	"	"	–	5.5	1.30	–
5.0	–	"	"	1.0	"	–	1.39
7.5	–	"	"	–	"	1.69	–
5.0	–	"	"	1.0	"	1.39	1.67
2.5	–	"	"	–	"	2.01	–
5.0	–	4.0	"	–	5.5	1.48	–
"	–	5.0	"	–	"	1.63	–
"	–	6.0	"	1.0	"	1.49	1.48
"	–	8.0	"	"	"	1.51	1.58
"	–	"	"	"	"	1.65	1.77
"	–	"	0.337	"	"	1.46	1.33
"	–	"	2.25	"	"	1.53	1.82
"	–	"	1.0	"	"	1.47	1.51
"	0.5	"	0.675	"	"	1.72	1.57
"	1.0	"	"	"	"	1.68	1.39
"	2.0	"	"	"	"	1.49	1.76
"	2.5	"	"	"	"	1.71	1.61
"	5.0	"	"	"	"	1.56	1.72
"	–	"	"	1.0	"	–	1.77
"	–	"	"	3.9	"	–	1.81
"	–	"	"	0.4	"	–	1.67
"	–	"	"	0.2	"	–	1.54
					Average =	1.55	1.63

[a] Total alcohol concentration. The ratio of the alcohol concentrations was always 1.0.
[b] Relative yields of 2-methyl-propene and propene. Accuracy ± 0.25.
[c] In the radiation chemistry experiments no H_2O_2 was present even if the table indicates differently.

and Fe(II)hedta and 2-methyl-2-propanol or 2-propanol or a 1:1 mixture of these alcohols the yield of the corresponding alkenes was 100% (within experimental error). The ratios of the yields of the alkenes under different experimental conditions are summed up in Tables 1 and 2. These ratios are clearly independent, within experimental accuracy, of the composition of the solutions, within the concentration ranges used.

The results clearly point out that the assay used is adequate only for edta and hedta at pH <5.5. The question of whether ·OH free radicals are formed at higher pHs or when L = tcma cannot be answered using the present assay.

The results are in accord with the commonly accepted proposal that free ·OH radicals are formed via reactions (13) and (8) for L = edta and hedta. This finding is of importance, as Fe(II)edta is often used as a source of ·OH free radicals.

$$M^nL + H_2O_2 \rightarrow LM^n \cdot H_2O_2 \qquad (13)$$

It should be pointed out that the observation that under our experimental conditions ·OH free radicals are formed does not contradict results pointing out that another intermediate is formed when H_2O_2 is mixed with Fe(II)L. As stated above a peroxide complex, *e.g.*, Fe(II)L·H_2O_2 is always the primary product of the reaction.[29] The

TABLE 2

Relative yields of 2-methyl-propene and propene formed in solutions containing Fe(II)hedta by ionizing radiation and the Fenton Reagent.

$[Fe^{2+}] \times 10^3$ M	$[Fe^{3+}] \times 10^3$ M	[hedta] $\times 10^3$ M	[ROH][a] M	$[H_2O_2] \times 10^3$ M	pH	C_4/C_3[b] Radiation[c]	C_4/C_3[b] Fenton
5.0	–	6.0	0.675	1.0	4.0	1.26	1.87
"	–	"	"	–	4.5	1.48	–
"	–	"	"	1.0	5.0	1.57	1.77
"	–	"	"	–	5.5	1.52	–
"	–	"	"	1.0	5.7	–	1.58
1.0	–	"	"	"	5.5	1.77	2.05
1.6	–	"	"	–	"	1.42	–
7.5	"	"	"	–	"	1.60	–
5.0	–	"	"	1.0	"	1.52	1.58
"	2.5	"	"	–	"	1.77	–
0.8	–	"	"	1.0	"	–	2.09
					Average =	1.55	1.82

[a] Total alcohol concentration. The ratio of the alcohol concentrations was always 1.0.

[b] Relative yields of 2-methyl-propene and propene. Accuracy ± 0.25.

[c] In the radiation chemistry experiments no H_2O_2 was present even if the table indicates differently.

mechanism of its decomposition, *i.e.* reactions (8), (9) and (10) depends on the nature of L and RH, the concentration of RH and the pH. Thus, different assays are expected to yield different results.

It is interesting to note that, using the same assay, we concluded that the main product of the reaction of H_2O_2 with Fe(II)nta at pH >3 are not ·OH free radicals![36] Rush and Koppenol[56] came to the same conclusion, though their interpretation was contested.[57] In acidic solutions,[36] the results suggest that also in this system, ·OH free radicals are been formed. This result seems surprising, since edta, hedta and nta are ligands with similar chemical properties. A plausible explanation to this observation is that reaction (14)

$$\mathrm{Fe(II)L{\cdot}H_2O_2 \rightarrow Fe(III)L(OH) + {\cdot}OH} \qquad (14)$$

is acid catalyzed, *i.e.*:

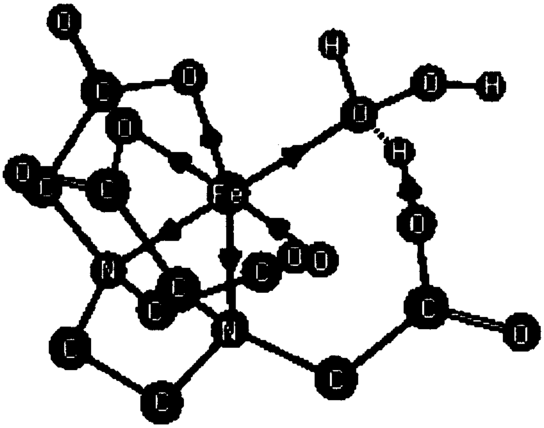
$$\mathrm{Fe(II)L{\cdot}H_2O_2 + H_3O^+ \rightarrow Fe(III)L(H_2O) + {\cdot}OH} \qquad (15)$$

For L = amincarboxylic acids the detailed mechanism of the latter reaction involves probably the following steps:

$$\mathrm{Fe(II)L{\cdot}H_2O_2 + H_3O^+ \rightleftharpoons Fe(II)LH^+{\cdot}H_2O_2} \qquad (16)$$

$$\mathrm{Fe(II)LH^+{\cdot}H_2O_2 \rightarrow Fe(III)L(H_2O) + {\cdot}OH} \qquad (17)$$

i.e., the acid catalysis of the redox step is an intramolecular step with an intermediate of the type:



Fe(II)edtaH^-·H_2O_2

It is reasonable to suggest that such a catalysis will be significant at higher pHs for L = edta and hedta than for nta. Such a mechanism could explain the observation that ·OH free radicals are the major product of reaction of Fe(II)L, L = edta and hedta, at pH ≤5.5 (or even at higher pHs), whereas for L = nta they are the main product only at pH <3.

It is of interest to note that a similar intramolecular catalysis was observed for reaction (18):[58]

$$(\text{edta})\text{Fe}^{\text{III}}\text{–}\ CH_2C(CH_3)_2OH \rightarrow \text{Fe(III)edta} + H_2C = C(CH_3)_2 + H_2O \qquad (18)$$

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58. L. Luzzatto, H. Cohen and D. Meyerstein Results to be published.

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