Biochemical Studies on Inositol

VI. MECHANISM OF CLEAVAGE OF \(^{14}C\) INOSITOL TO D-GLUCURONIC ACID*

Frixos C. Charalampous

From the Department of Biochemistry, School of Medicine, University of Pennsylvania, Philadelphia 4, Pennsylvania

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In a previous publication (1, Paper V of this series) the purification and properties of the inositol-cleaving enzyme were described. It was shown that a highly purified enzyme was obtained from rat kidneys which cleaved inositol to D-glucuronic acid. The purified enzyme was shown to possess SH groups and was found to be in carbon atom 2 to the extent of 98% or more. Uniformly labeled n-glucuronate-C\(^{14}\) and more. Uniformly labeled glucose-C\(^{14}\) was a product of the Tracerlab Inc. Uniformly labeled n-glucuronate-C\(^{14}\) were prepared enzymatically from uniformly labeled D-glucuronate-C\(^{14}\) as described later in this paper. myo-Inosose-2 and \(\alpha\)-epi-inosose-2 were prepared enzymatically from uniformly labeled inositol-C\(^{14}\) with the use of the purified kidney enzyme reported earlier (1). The uniformly labeled inositol-C\(^{14}\) used in this experiment was a generous gift of Professor Friedrich Weygand. L-Gulonate-C\(^{14}\) and L-gulonolactone-C\(^{14}\) were prepared from uniformly labeled D-glucuronate-C\(^{14}\) as described later in this paper. n-myo- and L-myo-isosose-1, and \(\alpha\)-epi-inosose-2 were furnished by Dr. Laurens Anderson. A reductone of \(\alpha\)-epi-inosose-2 (m.p. 152\(^{\circ}\)) was prepared according to the method of Euler and Glaser (5). Hz\(\text{O}^{18}\) was extracted with ether and purified by sublimation.1 The resulting CO\(_2\) was determined as described above. Pure, dry CO\(_2\) was used as standard. Under the conditions used in the mass spectrometry the reproducibility of a ratio of the same sample was \(\pm 0.3\%\).

Mass Spectrometry—Analyses for O\(^{18}\) content of various samples were performed in collaboration with Dr. Frank Eisenberg, Jr., and Mr. William E. Comstock of the National Institutes of Health. The mass spectrometer used was the Consolidated Engineering Corporation model 21-401. The O\(^{18}\) content of the gas phase from the incubation flasks was determined from the ratio of mass 36 to masses 34 and 32. The method used to sample the gas phase is described later in this paper. In the case of organic compounds their O\(^{18}\) content was determined as follows: The isolated pure compounds were dried and pyrolyzed for 1 hour at 400\(^{\circ}\) with dried HgCl\(_2\) as catalyst according to the method of Rittenberg and Ponticorvo (6). The CO\(_2\) formed (containing the O\(^{18}\)) was analyzed in the mass spectrometer and the enrichment in O\(^{18}\) was obtained from the formula:

\[
\text{Atom % O}^{18} = \frac{100\left(\frac{R}{2}ight)}{1 + \frac{R}{2}}
\]

where R is the ratio of mass 46 over masses 44 plus 45 and R' is the ratio of mass 45 over mass 44. The O\(^{18}\) content of water of the incubation mixture was determined as follows: Water was obtained by distillation of an aliquot of the incubation mixture from the frozen state under reduced pressure. Two milliliters of this water and 0.3 ml of pure benzoil chloride were introduced into a Pyrex tube. The tube was sealed and heated in an oven at 120\(^{\circ}\) for 3 hours. The resulting benzoic acid containing the O\(^{14}\) was extracted with ether and purified by sublimation.2 The crystalline benzoic acid was pyrolyzed and the O\(^{18}\) content of the resulting CO\(_2\) was determined as described above. Pure, dry tank CO\(_2\) was used as standard. Under the conditions used in the mass spectrometry the reproducibility of a ratio of the same sample was \(\pm 0.3\%\). For a sample with 0.2 atom % excess of O\(^{18}\) the maximal error is of the order of 0.5%. The procedure used to introduce the CO\(_2\) from the pyrolysis tubes into the mass spectrometer was the same as described by Rittenberg and Ponti-

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1 A preliminary communication on this aspect of our studies has appeared (2).

2 I am indebted to Dr. Frank Eisenberg, Jr., for making this method available before publishing it.
corvo (6) except that the CO₂ was passed through an additional Dry-Ice-acetone trap before it was admitted to the inlet system of the mass spectrometer. Liquid nitrogen noncondensible gases were absent.

Experiments with Inositol-2-C₁⁴—In order to determine the site of cleavage of inositol, experiments were performed with inositol-2-C₁⁴ as substrate and the resulting isolated glucuronates were degraded and the distribution of C₁⁴ determined. Two such experiments were performed in which the purified enzyme that cleaves inositol exclusively to D-glucuronate as well as the crude dialyzed enzyme extract which forms both D- and L-glucuronates were used separately.

Isolation of Radioactive Glucuronates—The radioactive glucuronates formed from inositol were isolated by ion exchange chromatography and were converted to their lactone (7). Approximately 20 μmoles of each lactone were isolated and the optical rotations were estimated. The lactones were quantitatively recovered from the polarimeter tubes and an aliquot was plated and counted in order to determine their specific activity (counts per minute per μmole.)

Reduction of Radioactive Glucuronic Acids to Corresponding Gulonic Acids—In order to determine the distribution of C₁⁴ in the radioactive glucuronolactones, the degradation scheme outlined in Diagram 1 was followed. Radioactive glucuronolactone, 20 μmoles, isolated from the incubation mixture containing the purified enzyme (D-glucuronolactone), was dissolved in 1 ml of water and diluted with a 10-fold excess of nonlabeled D-glucuronolactone. The lactone was converted to sodium glucuronate by careful addition at 0⁰ of an equivalent amount of 0.1 M NaOH. In a similar manner, the radioactive glucuronolactone formed by the crude enzyme system (DL-glucuronolactone) was diluted with a 10-fold excess of nonlabeled, DL-glucuronolactone (prepared from a similar experiment with nonradioactive inositol) and was converted to the sodium glucuronate as described above. The two samples of sodium glucuronate were crystallized from ethanol to constant specific activity. The purity of the crystalline glucuronates was further confirmed by paper chromatography (7). The two samples of radioactive glucuronates, 162 μmoles each, were dissolved separately in 2 ml of 0.1 M boric acid, and 2 ml of a freshly prepared, ice-cold solution of 0.130 M NaBH₄ in 0.1 N NaOH was added. The reaction was allowed to proceed at 22⁰. Reduction of the glucuronates to the gulonates was complete by the end of 1 hour. To each reaction mixture, 0.5 g of Amberlite IR 120 (H⁻) was added in order to destroy excess NaBH₄ and the resin-free solutions were concentrated to approximately 2 ml under vacuum from a bath temperature of 40⁰. The boric acid present in the concentrated solutions was removed by the addition of 10 ml of methanol followed by evaporation to dryness. The addition of methanol and the evaporation to dryness were repeated four more times. To each flask containing the respective gulonolactone, 4 ml of ethanol were added and the flasks were manually swirled until crystallization was complete.

**Diagram 1**

Chemical degradation and distribution of C₁⁴ in D-glucuronic acid derived from enzymatic cleavage of inositol-2-C₁⁴

The numbers indicate the carbon atoms of inositol and the figures in parentheses indicate the yields at each step. The same C₁⁴ distribution was obtained in the case of DL-glucuronate.
Degradation of Glyoxylate—The two samples of radioactive glyoxylates, 113 μmoles each, were separately dissolved in 3 ml of water and titrated with the equivalent amount of a 0.1 M solution of NaOH. The solutions were allowed to stand at room temperature for 30 minutes and 2.6 ml of an ice-cold 0.2 M solution of periodic acid was added. The reaction mixtures were incubated at 18° in the dark for 40 minutes. It was shown in preliminary experiments that, under these conditions, 1 mole of glyoxylate is oxidized to 1 mole of formaldehyde, 1 mole of glyoxylic acid, and 3 moles of formate with the consumption of 4 moles of periodic acid. These results are similar to those reported by Fleury et al. (8) for the periodic acid oxidation of gluconic acid. The oxidation products were isolated after removal of excess periodate and iodate salts as insoluble barium salts. Each of the two supernatant fluids containing the formaldehyde, the formate, and the glyoxylate was treated with Amberlite IR 120 (H+). To remove excess barium ions and the mixture was concentrated to a sirup by vacuum distillation at a bath temperature of 40°. The glyoxylic acid remaining in the distillation flask was determined colorimetrically and was isolated as the crystalline sodium salt. Its purity was determined as described elsewhere (9). A small amount of the salt was dissolved in water and used for radioactivity measurements. Approximately 96 μmoles of glyoxylate were obtained from each sample (85% yield). The distillate from the distillation mentioned above was used for the estimation and isolation of formaldehyde (10) and of formic acid (9). Appropriate amounts of the isolated crystalline (m.p. 191°) dimedon derivative of formaldehyde and of sodium formate were plated and counted. The yields of formaldehyde and formate were 98 and 100%, respectively.

Since all the radioactivity of the degraded gluconates was found in the fraction containing the glyoxylate, the 2, 4-dinitrophenyl-hydrazone of the radioactive glyoxylates was prepared with 50 μmoles of each of the two glyoxylic acid samples (m.p. 191°). The specific activity of this derivative agreed very well with that of the original sodium glyoxylates.

Degradation of Sodium Glyoxylates—The radioactive glyoxylate, 30 μmoles each, were further oxidized by periodic acid to equimolar amounts of CO2 and formate which were isolated and counted (9).

Experiments with O18—In the conversion of inositol to glucuronate one atom of oxygen is bonded enzymatically on carbon atom 1 of inositol which subsequently becomes the carboxyl of glucuronate. From the standpoint of the mechanism of this reaction it is important to know whether the enzyme activates molecular oxygen and incorporates it into the carboxyl group or whether at a certain stage the mechanism involves hydration followed by dehydrogenation. In order to determine the origin of the oxygen of the carboxyl group of glucuronate, experiments were performed in which O18 and H2O18 were used separately. In those experiments in which H2O18 was used as the source of O18, all the reagents, with the exception of the enzyme, were dissolved in H2O18. Control vessels containing sodium glucuronate but with no added inositol were run at the same time. These control vessels served to detect any possible exchange of O18 between H2O18 and glucuronate. All vessels were run in duplicate. The glucuronates formed were isolated from the reaction mixtures as described earlier in this paper except that the ion exchange chromatography was performed at 4° and the solutions containing the eluted glucuronates were concentrated by lyophilization in order to prevent lactonization of the glucuronates with resulting loss of O18 content. Experiments confirming that no lactonization occurs under these conditions are described below.

When O18 was employed as the sole source of O18, the composition of the incubation mixtures was identical to that described for the H2O18 experiments except that the reagents were dissolved in ordinary distilled water and the gas phase was oxygen containing 95.5 atom % excess of O18. Special reaction vessels were used in these experiments in order to allow introduction of the O18 gas and sampling of the gas phase at the end of the incubation. Briefly, the reaction was carried out in round bottom, 15-ml flasks each with two necks fitted with high vacuum stopcocks. One of the stopcocks was connected through a glass ground joint to an adapter leading to the O18 reservoir. By means of a side arm on the adapter the entire system could be evacuated. The various solutions, with the exception of the enzyme, were pipetted into the reaction flasks and were frozen by immersing in Dry-Ice-acetone. The flasks were evacuated for 10 minutes and then were allowed to thaw. Freezing and evacuation of the flasks followed by thawing was repeated three more times. The evacuated flasks were placed in ice and the enzyme solution was introduced, care being taken to prevent air from entering the vessels. The flasks containing the cold but unfrozen solutions were evacuated once more for 10 minutes. No bumping occurred during this period. The flasks were then attached to the reservoir containing the O18 oxygen and were filled with the gas at a positive pressure of 5 cm of mercury. Incubation was carried out at 35° for 50 minutes and the reaction was stopped by immersing the closed vessels in a Dry-Ice-acetone bath. Sampling of the gas phase of the incubated vessels was carried out by attaching each vessel to the adapter carrying a “sampling tube.” The latter was an 8-ml Pyrex tube with a break-seal. After evacuating the adapter and the sampling tube, gas from the reaction vessel was admitted into the sampling tube, which was then sealed and used for analysis in the mass spectrometer as described earlier for the CO218 analyses. Duplicate gas samples of each reaction vessel could be obtained without difficulty.

RESULTS

Experiments with Inositol-2-C14.—The conversion of radioactive inositol to glucuronate by the crude enzyme extract and the purified enzyme is demonstrated in Table I. The distribution of radioactivity in the two samples of glucuronic acid was determined after degrading them by the procedure outlined in Diagram 1 and the results obtained are summarized in Table II. It is seen that all the radioactivity of both samples of glucuronic acid was found in the glyoxylate fraction which was derived from carbon atoms 5 and 6 of glucuronic acid (carbon atoms 2 and 1 of inositol). The further oxidation of the radioactive glyoxylates by HIO3 to equimolar amounts of formate and CO2 revealed that all the C14 was contained in the formate fraction and none in the CO2 fraction. Thus, the present findings show that the
C\textsuperscript{14} of the glucuronates formed from inositol-2-C\textsuperscript{14} is located in
 carbon atom 5 (next to the carboxyl) of both the dl- and d-glucu-
ronate.

**Experiments with O\textsuperscript{18}**—When the purified enzyme was incu-
bated with inositol in the presence of H\textsubscript{2}O\textsuperscript{18} the isolated glu-
curonate contained no O\textsuperscript{18}. However, when oxygen gas was used
as the sole source of O\textsuperscript{18}, 1 atom of O\textsuperscript{18} was found per molecule of
 glucuronate isolated. No O\textsuperscript{18} was fixed in the glucuronate when
in Table III. In the experiments with O\textsuperscript{18} it was necessary to demonstrate that
no lactonization of the glucuronate occurred during its isolation.
Lactonization would cause loss of incorporated O\textsuperscript{18} which could
amount to as much as 50\% of the original content of O\textsuperscript{18}. To test
this possibility, two kinds of experiments were performed with
the use of the isolation procedure of glucuronate described for

**Lack of Lactonization of Glucuronate-O\textsuperscript{18} during its Isolation—**
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**Degrada
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<table>
<thead>
<tr>
<th>Isolated compound</th>
<th>Yield</th>
<th>Specific activity</th>
<th>Melting point</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude†</td>
<td>Purified†</td>
<td>Crude†</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>c.p.m./μmole</td>
</tr>
<tr>
<td>Gulonolactone</td>
<td>90</td>
<td>89</td>
<td>20,702</td>
</tr>
<tr>
<td>Glyoxylate</td>
<td>85</td>
<td>85</td>
<td>21,105</td>
</tr>
<tr>
<td>2,4-Dinitrophenyl hydrazine of glyoxylate</td>
<td>52</td>
<td>45</td>
<td>21,000</td>
</tr>
<tr>
<td>Formate</td>
<td>90</td>
<td>100</td>
<td>38</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>98</td>
<td>98</td>
<td>0</td>
</tr>
</tbody>
</table>

* c = 1 in H\textsubscript{2}O, 1 = 1 dm.
† This refers to the crude dialyzed extract and the purified enzyme respectively. The purified enzyme had a specific activity (units per mg of protein) of 300 (1).

**Poss
eble Intermediates in Conversion of Inositol to Glucuronate—**
In an attempt to identify intermediates formed in the conversion
of inositol to d-glucuronate, uniformly labeled inositol-C\textsuperscript{14} was
incubated with the purified enzyme in the presence of various
amounts (1 to 30 μmoles) of each of the following compounds:
D-glucose, L-gulose, L-gulonolactone, L-gulonate, L-ascorbate myo-
inosose-2, D-myco-inosose-1, L-myco-inosose-1, D-epi-inosose-2, and
dL epo-inosose 2. The isolated radioactive glucuronate from
each of these incubations was counted and its specific activity
was determined. In all cases it was found that the specific
activity of the isolated glucuronate did not differ significantly from
that of the radioactive inositol. In other experiments in which
the above compounds were incubated individually with the en-
zyme in the absence of inositol no evidence for the formation of
glucuronate was obtained.

**Enzyme Inhibition by Compounds Related to Inositol—** In some of the experiments mentioned above it was observed that the presence of various inososes in the incubation mixture caused a marked inhibition of the formation of glucuronate. A systematic study of this phenomenon was undertaken and the results are summarized in Table IV. It is seen that these inososes cause marked inhibition of the enzymatic activity. D Inositol, L ino-
sitol, scyliitol, and pinitol were without effect.

**Lack of H\textsubscript{2}O\textsubscript{2} Formation during Cleavage of Inositol to Glu-
Incorporation of O\textsuperscript{18} into d-glucuronate formed from inositol

One milliliter of purified enzyme was incubated in 0.1 M phosphate buffer, pH 7.2, at 35\textdegree C for 50 minutes. Inositol (0.1 M) or glucuronate (3.3 X 10\textsuperscript{-3} M) was added separately in the respective flasks. Final volume was 6 ml.

<table>
<thead>
<tr>
<th>Source of O\textsuperscript{18}</th>
<th>Substrate added</th>
<th>Dilution factor</th>
<th>Atom % excess of O\textsuperscript{18} in glucuronate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Observed</td>
</tr>
<tr>
<td>O\textsuperscript{18} (35.8 atom % excess)</td>
<td>Inositol</td>
<td>56</td>
<td>1.6912</td>
</tr>
<tr>
<td></td>
<td>Inositol</td>
<td>56</td>
<td>1.7084</td>
</tr>
<tr>
<td></td>
<td>Glucuronate</td>
<td>0</td>
<td>zero</td>
</tr>
<tr>
<td>H\textsubscript{2}O\textsuperscript{18} (10.0 atom % excess)</td>
<td>Inositol</td>
<td>64</td>
<td>zero</td>
</tr>
<tr>
<td></td>
<td>Inositol</td>
<td>64</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>Glucuronate</td>
<td>0</td>
<td>zero</td>
</tr>
</tbody>
</table>

* This expresses the degree of dilution that the incorporated atom of oxygen into glucuronate underwent by the remaining oxygen atoms of the biosynthetic as well as the carrier glucuronate.

† Calculated from the atom % excess of the O\textsuperscript{18} source and the dilution factor.

Inhibition of formation of glucuronate from inositol by various Inososes

The incubation mixture contained 100 pmoles of inositol, 100 pmols of phosphate buffer, pH 7.2, 75 pg of purified enzyme, and additions as indicated, in a final volume of 1 ml. Incubation was carried out at 35\textdegree C for 15 minutes in an atmosphere of oxygen. Glucuronate was determined colorimetrically.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Concentration, final molarity</th>
<th>Enzymatic activity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>d-myo-Inosose-1</td>
<td>3.6 \times 10\textsuperscript{-4}</td>
<td>76</td>
</tr>
<tr>
<td>d-myo-Inosose-1</td>
<td>4.0 \times 10\textsuperscript{-4}</td>
<td>0</td>
</tr>
<tr>
<td>L-myo-Inosose-1</td>
<td>4.0 \times 10\textsuperscript{-4}</td>
<td>82</td>
</tr>
<tr>
<td>L-myo-Inosose-1</td>
<td>5.0 \times 10\textsuperscript{-4}</td>
<td>10</td>
</tr>
<tr>
<td>myo-Inosose-2</td>
<td>1 \times 10\textsuperscript{-4}</td>
<td>0.4</td>
</tr>
<tr>
<td>myo-Inosose-2</td>
<td>4 \times 10\textsuperscript{-4}</td>
<td>48</td>
</tr>
<tr>
<td>DL-\textsuperscript{2}epi-Inosose-2</td>
<td>1 \times 10\textsuperscript{-4}</td>
<td>68</td>
</tr>
<tr>
<td>DL-\textsuperscript{2}epi-Inosose-2</td>
<td>1.5 \times 10\textsuperscript{-4}</td>
<td>0</td>
</tr>
</tbody>
</table>

In formulating a reasonable mechanism for the conversion of inositol to glucuronic acid, the following experimental findings must be taken into account: (a) the purified enzyme requires the participation of iron and SH groups for activity (1); (b) the enzymatic reaction involves the loss of 2 hydrogen atoms from inositol and the “fixation” of 1 atom of oxygen which appears in the product; (c) the fixed oxygen must come from oxygen gas and not from water; (d) formation of H\textsubscript{2}O\textsubscript{2} during the reaction is not observed; (e) familiar coenzymes as well as oxidation-reduction dyes cannot replace oxygen as the hydrogen acceptor. These observations indicate that the enzyme catalyzes two different types of reaction: (a) oxidation of 2 hydrogen atoms by 1 atom of oxygen to form water, thus resembling an oxidase and (b) the consumption of 1 atom of gaseous oxygen which appears in the product, a reaction similar to but not identical with that catalyzed by oxygenases.

Mason (11), in a comprehensive discussion of oxygen metabolism, distinguished three different classes of enzymes: (a) oxygen transferases which catalyze the consumption of 1 molecule of oxygen per molecule of substrate and both atoms of consumed oxygen appear in the product; (b) mixed function oxidases which catalyze the consumption of 1 molecule of oxygen per molecule of substrate; 1 atom of the oxygen molecule appears in the product, the other is reduced by a hydrogen donor other than the substrate; and (c) Electron transfer oxidases which catalyze 2-electron or 4-electron reductions of oxygen, hydrogen peroxide or water being formed from the oxygen consumed.

The inositol-cleaving enzyme does not seem to belong to any of the three classes of enzymes discussed by Mason. It is apparently unique in that it catalyzes the “fixation” of 1 atom of oxygen per molecule of substrate.

**TABLE III**

**Incorporation of O\textsuperscript{18} into d-glucuronate formed from inositol**

**TABLE IV**

**Inhibition of formation of glucuronate from inositol by various Inososes**
oxygen, which appears in the product, in contrast to the oxy-
genases which catalyze the incorporation of 2 atoms of oxygen.
At the same time it functions as an oxidase in a manner similar
to ascorbic acid oxidase which is a copper enzyme. It cannot
be classified as a "mixed function oxidase" because in the latter
case the hydrogen donor is a compound other than the substrate,
usually DPNH or TPNH.

The following sequence of events is proposed as a possible
mechanism to account for the experimental observations. It is
proposed that inositol is first oxidized to an enediol by loss of two
hydrogen atoms from carbon atoms 1 and 6. This is followed by
cleavage of the double bond with the concomitant "fixation" of
one oxygen atom in carbon atom one of inositol which becomes
the carboxyl of glucuronic acid. The following postulated reac-
tions appear to be consistent with the observed events. The iron
of the enzyme could be linked by way of an SH group to the
apoenzyme as indicated in Reaction 1.

\[ E-\text{SH} + \text{Fe}^{+++} \rightarrow E-S-\cdot\text{Fe}^{++} + H^+ \]  

The \( E-S-\cdot\text{Fe}^{++} \) would represent the active form of the enzyme.
It explains why reducing substances such as glutathione protect
the enzyme against the chelating action of cyanide, azide, and
others. Glutathione by reducing the SH groups of the enzyme
would drive Reaction 1 towards the right with the reduced iron
now exhibiting lower affinity for chelating agents, a situation
which is also true with the hemin-iron enzymes. It also explains
why denaturation of the enzyme by acid causes the quantitative
release of iron while denaturation by heat coagulation releases
only 5% of the iron (1). If we represent the active form of the
enzyme \( (E-S-\cdot\text{Fe}^{++}) \) as \( E \), we can suggest the following reac-
tions:

1. \( E + O_2 \rightarrow E.O_2 \)  

2. \( E-O_2 \rightarrow E \cdot O-O^- \rightarrow E-O \cdot O^- + 2H^+ \)  

3. Inositol

4. \( E \cdot O \cdot O^- + 2H^+ \rightarrow E \cdot O \cdot O^- + H_2O \)  

5. \( E \cdot O \cdot \rightarrow E + \text{d-glucuronic acid} \)

In Reaction 2 the enzyme forms a complex with oxygen followed
by a 2-electron oxidation of inositol as indicated in Reaction 3,
leading to the formation of an enediol and release of 2 \( H^+ \). In
Reaction 4 the enzyme-oxygen-enediol complex reduces the 2
protons to form water by means of a 2-equivalent reduction.
Finally in Reaction 5 the enediol cleaves to glucuronic acid and
free enzyme. In the above reactions the enzyme-oxygen complex
is thought to occur with the iron of the enzyme \( E \cdot S \cdot \text{Fe}^{+++} + O_2 \rightarrow E-S \cdot \text{Fe}^{++}O_2 \) in a manner similar to that taking
place between oxygen and uncomplexed ferrous iron (12).

The proposed mechanism of oxygen activation and cleavage of
inositol serves only to illustrate one out of perhaps many differ-
et mechanisms. It does, however, explain the experimental
findings in a reasonable manner.

SUMMARY

The mechanism by which a purified enzyme of kidney cleaves
inositol to d-glucuronic acid has been investigated with the aid
of inositol-2-C\textsuperscript{14} and O\textsuperscript{18}. The results indicate that:

1. Inositol is enzymatically cleaved between carbon atoms 1
   and 6.

2. One atom of oxygen from \( O_2 \) is fixed into position 1 of inosi-
tol and appears in the carboxyl of glucuronic acid.

3. The two hydrogen atoms removed from inositol reduce 1
   atom of oxygen to form water.

4. The participation of SH group(s) and of the iron of the en-
zyme in the catalytic process is discussed and a possible mecha-
nism for the activation of oxygen and the cleavage of inositol is
presented.

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