Synthetic Fragments of Calmodulin Calcium-binding Site III

A TEST OF THE ACID PAIR HYPOTHESIS*

Ronald E. Reid
From the Faculty of Pharmacy, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada

The acid pair hypothesis describing the interaction of calcium with the helix-loop-helix conformation of EF hands in calmodulin and related proteins predicts that these calcium-binding sites will have increased affinity for calcium if the anionic amino acid dentates in the loop region which interact directly with the cation are paired on the axial vertices of the resulting octahedral arrangement of chelating residues about the cation. As a test of this hypothesis, synthetic 33 residue analogs of bovine brain calmodulin calcium-binding site III have been prepared by the solid-phase method and analyzed for calcium affinity. The native sequence has a $K_d$ of 735 μM for calcium and contains three anionic ligands which assume the $+x, +y,$ and $-z$ coordinates of the octahedral arrangement about the cation, thus precluding any pairing of the anionic ligands. This dissociation constant is 26 times weaker than that obtained from a synthetic analog of the sequentially homologous calcium-binding site III of rabbit skeletal TnC ($K_d = 28$ μM) which has four anionic ligands paired on the $x$ and $z$ axes. An analog of calmodulin site III with substitutions in the chelating residues at positions 1, 3, 5, 7, 9, and 12 of the 12-residue loop region to make these positions identical to those of rabbit skeletal tropolin C site III decreased the calcium dissociation constant of the calmodulin peptide to 19 μM, similar to the tropolin C peptide. Two synthetic analogs of calmodulin site III which contain three anionic ligands with two ligands paired on the $x$ axis and two on the $z$ axis have a $K_d$ for calcium of 524 and 59 μM, respectively. This study provides strong support for and a better definition of the acid pair hypothesis and further demonstrates the usefulness of synthetic calcium-binding fragments in delineating the mechanism of calcium regulation of calmodulin and related proteins.

Crystal structures of the major calcium-binding regulatory proteins (1–4) have made significant contributions to the knowledge of the events at the molecular level which lead to calcium regulation of biochemical systems. In spite of this abundance of data at the molecular level, several questions remain unanswered. The sequence and structural similarities of calcium-binding sites in the majority of these proteins along with the fact that several orders of magnitude separate the calcium affinity of some of these sites, both between different proteins and within the same protein, invites such questions as: What are the molecular features defining calcium affinity in these proteins? What are the molecular changes resulting from calcium binding which lead to regulation of other biochemical events?

Tufty and Kretsinger (5) noticed the helix-loop-helix conformation of the calcium-binding units in these proteins which he termed the “EF hand.” Studies on calcium-induced conformational change in these proteins led to the concept of calcium-induced $\alpha$-helix formation as the first step in the translation of information carried by cellular calcium to biochemical activity (6). The demonstration of high and low affinity calcium sites in rabbit skeletal tropolin C (STnC) by Potter and Gergely (7) provided a link between calcium-induced structural change and intracellular levels of calcium. The amphiphilic nature of the $\alpha$-helices induced by calcium led to further refinement of the concept of information transfer and resulted in hypothetical molecular explanations for a variety of calcium-induced interactions between the calcium-binding proteins and other molecules (6, 8).

The fact that the calcium-binding units are a linear sequence of 30–36 amino acids coupled with the fact that the sequences are highly homologous led to the examination of amino acid sequences in search of a sequence factor involved in the description of calcium binding and subsequent events. Vogt and colleagues (9) first noticed a high correlation of $\beta$ turn propensity with the occurrence of a calcium-binding loop region using the $\beta$-turn forming residue probabilities of Lewis et al. (10). The high propensity consisted of two overlapping tetrapeptides in the first 6 residues of the 12-residue loop region of the calcium-binding unit.

An attempt to correlate the amino acid sequence of the loop region of the helix-loop-helix calcium-binding unit with affinity for calcium in an effort to isolate features of the amino acid sequence which may be responsible for high and low calcium affinity recognized two important features of the loop sequence (11). The first concerned the number and arrangement of acidic side chains located in chelating positions 1, 3, 5, 7, 9, and 12 of the 12-residue loop. It was suggested that high affinity sites would have four acidic side chains in chelating positions of the loop, paired on the vertices of the regular octahedral arrangement of liganding atoms. This later became known as the acid pair hypothesis (12, 13). The second feature concerned the hydrophobic nature of the residue in the $-y$ coordinate of the octahedral arrangement of liganding atoms. It was suggested that the sites with high affinity for

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calcium will contain a hydrophobic residue (preferably with an aromatic side chain) in the \(-\gamma\) coordinate which would affect the cation hydration sphere such that chelation by the liganding atoms would be assisted. This hypothetical dehydration mechanism was also suggested to be cooperative between paired calcium binding sites. More recently, this cooperative interaction between the paired sites has been developed to produce an algorithm which ranks pairs sites according to calcium affinity constants based on net charge on the ligands, \(\beta-\gamma\) sheet hydrophobicity, and helical hydrophobicity (14).

Boguta et al. (15) used the method of Garnier et al. (16) for structure prediction and correlated \(\alpha\)-helix, \(\beta\)-turn, and random coil propensities with known calcium affinities of single helix-loop-helix conformations to produce an algorithm that predicts calcium affinity of the single or paired calcium-binding sites based on the propensity of the sequence to form the proper helix-loop-helix conformation.

Richardson and Richardson (17) noticed that the \(\alpha\)-coiled supersecondary structure of Efimov (18) could be applied to the calcium-binding EF hands, and they concluded that the so-called lap-joint is ideally suited to cation-regulated conformational change because cation-induced conformational change of the loop would cause a change in the helix contact angle therefore coupling ion binding at the loop to overall tertiary structural change. This principle of a pivotal change in helix orientation is also central to the calcium trigger hypothesis (19) which relates the conformational change induced by calcium binding to the loop region to a small twist in the \(\beta\)-sheet formed between the loop regions of paired calcium-binding sites which in turn results in a rotation and displacement of the four helices.

The majority of mechanistic descriptions of calcium binding outlined above are concerned with the conformational contribution of the individual amino acids in the single or paired EF hands. Although the importance of conformational changes induced by calcium in the proteins cannot be underrated, the physicochemical properties of individual amino acids in particular positions in the sequence will play major roles in determining that the correct conformational changes are elicited as well as stabilized by the cation and that the events subsequent to the conformational change are also of the correct nature. The importance of the correct number and location of acidic residues in the calcium-binding loop region has been addressed by the acid pair hypothesis (11-13) and is the only known mechanism addressing the physicochemical properties of specific amino acid residues in the EF hand sequences.

The present study is designed to further elucidate the nature of the effect that the number and location of acidic residues in the loop region has on cation affinity and how these effects may be extended to include the nature of the conformational parameters required for proper response of the protein to calcium.

**EXPERIMENTAL PROCEDURES**

**Synthetic Protocol.** The peptides were synthesized semiautomatically using the Vega 1000 peptide synthesizer with a totally sequential solid-phase strategy as previously described (12, 13). The only difference is that all amino acids were coupled in HPLC grade dimethylformamide using benzotriazol-1-yl-oxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) with 1-hydroxybenzotriazole as the coupling reagent (20, 21).

**Peptide Purification.** The peptides were purified using \(\text{C}^18\) and \(\text{C}^4\) reverse-phase and ion exchange high pressure liquid chromatography as previously described (12, 13). The son exchange purification step was not used in the purification of the NDL/\(3\times\text{CaM}\) and NDL/\(\text{ZCaM}\) peptides.

**Circular Dichroism Spectroscopy.** Calcium interaction with the synthetic peptides was examined using CD spectroscopy on a Jasco J500 CD spectrophotometer as previously described (22).

Free calcium concentrations were determined using a modified Perrin and Sayce computer program (23) with \(\log[\text{Ca}]\) of the association constant (24) for the differing species of nitritotriacetic acid (NTA) at: \(\text{H}^+\) to NTA\(^-\), 0.73; NTA\(^-\) to HNTA\(^-\), 2.40; \(\text{H}^+\) to HNTA\(^-\), 1.89; Ca\(^2+\) to NTA\(^-\), 6.50. To correct the free metal concentration for peptide-bound calcium, it is assumed that 1 mol of calcium is bound/mole of peptide, and an estimate of this association constant is inserted into the modified Perrin and Sayce program for two ligands. Free calcium concentrations are calculated, and these values are used to determine an association constant for calcium as described below. This value is then used to revise the estimated association constant in the modified Perrin and Sayce program. The procedure is repeated until the calculated association constant equals that inserted in the Perrin and Sayce program. The \(\text{H}^+\) to peptide log; association constant was set at 4.00 to correspond roughly to the \(\text{pK}_a\) of the acid side chains involved in calcium chelation.

The association constant of peptide for calcium was calculated using a nonlinear regression computer program which fits the CD calcium titration data to an equation of the form:

\[
f = \frac{K_C[\text{Ca}^{2+}]/\left[1 + K_C[\text{Ca}^{2+}]\right]}{1 + K_C[\text{Ca}^{2+}]}
\]

where \(f\) is the fraction of peptide molecules in the calcium-chelated state and is determined as the ratio of the change in ellipticity at 222 nm to the maximum change in ellipticity which can be elicited by calcium at 222 nm. \(K_C\) is the apparent association constant of the peptide for calcium and \([\text{Ca}^{2+}]\) is the concentration of free calcium calculated as described above. This calculation assumes all activities are equal to concentrations and that the peptide has a single independent calcium-binding site.

It is acknowledged that the deviations seen in the calcium-binding curves at high and low calcium concentrations may suggest some degree of heterogeneity in the binding process. However, the fact that the calcium-induced UV difference spectrum of one of the peptides (A\(^{\text{AStNC}}\) (22) is blue-shifted, which may indicate the movement of tyrosine into a more hydrophilic environment plus the fact that all the calcium titration data fit the theoretical curve of a single independent calcium-binding site with correlation coefficients greater than 0.996, are taken to indicate that calcium-induced aggregation of the peptides is unlikely in these experiments.

A previous study (25) has shown the \(\alpha\)-helix content obtained from \([\Theta]_{222}\) for a pure \(\alpha\)-helix with an added refinement allowing for differences in the lengths of helical segments (26) can be used to determine the \(\alpha\)-helix content for a fragment of CaM in the presence and absence of calcium. By difference between these two values in good agreement with the value derived from the CD difference spectrum by the curve fitting procedure. Hence, this simplified procedure was adopted to estimate the helical content of the synthetic peptides. The fraction of \(\alpha\)-helix (\(f_\alpha\)) present was calculated as follows:

\[
f_\alpha = \left[\frac{[\Theta]_{222}}{[\Theta]_{210}}\right][1 - k/n]
\]

where \([\Theta]_{222}\) and \([\Theta]_{210}\) are calculated constants (26) which are 39,500 and 2,57, respectively, and \(n\) is the average helical length taken as 9 in these cases (22, 25).

The CD studies were carried out using a 100 mM MOPS, pH 7.2, 150 mM KCl, 1 mM NTA buffer and a 3:1 (v/v) mixture of trifluoroethanol with this buffer. The calcium-free solutions were prepared from demineralized water prior to use. Nanolec laboratory equipment was used in place of glassware to avoid contamination of solutions with calcium leached from glass. The concentration of the calcium solutions used in the titrations were determined by titration with EGTA as the primary standard using murexide as indicator. The peptides were quantitated from amino acid analysis of an aliquot of the solutions taken prior to the assay.

**RESULTS AND DISCUSSION**

The acid pair hypothesis relates calcium affinity to chemical properties of the amino acid residues in positions 1, 3, 5, 7, 9, and 12 of the loop region in the helix-loop-helix calcium-binding unit. Previous studies on synthetic calcium-binding units from rabbit skeletal TnC (13) and bovine brain calmodulin (12) indicated a 26-fold difference in calcium affinity. In order to determine if this large difference in calcium affinity...
could be attributed to the above residues in the loop region, a hybrid of the two peptides was prepared and examined for calcium affinity. The hybrid (NDL/4XZ) differed from the calmodulin peptide (L^109CaM) in positions 3 (Asp changed to Asn), 5 (Asn changed to Asp), and 9 (Ser changed to Asp) (Fig. 1). These changes produced a loop region identical to the rabbit skeletal TnC peptide (A'^STnC) in positions 1, 3, 5, 7, 9, and 12 (Fig. 1). This hybrid has an affinity for calcium (K_d = 19 pM) similar to that of the A'^STnC peptide (K_d = 28 pM) and 39-fold greater than the L^109CaM peptide (K_d = 735 pM) (Table I and Fig. 2).

It is possible that the difference in calcium affinity (Fig. 3) as well as the differences in structure (Table I and Fig. 2) between the apo-, calcium saturated, and TFE calmodulin analogs (NDL/4XZ and L^109CaM) may be due to the difference in the number of anionic chelating amino acid residues and not the location of the acidic residues in the loop region. This problem is addressed through the synthesis of two peptide analogs of calmodulin which have 3 acid residues in chelating positions of the loop region (Fig. 1; NDL/3XCaM and NDL/3ZCaM).

NDL/3XCaM was prepared by changing Asp^{59} of L^{109}CaM to Asn and Ser^{101} to Asp. The NDL/3XCaM peptide has 3 acidic residues in chelating positions with one acid pair on the x axis (Fig. 1). The calcium affinity of this peptide is 524 pM which is slightly better than the natural L^{109}CaM sequence (K_d = 755 pM). It would appear that the cation affinity is not greatly affected by pairing the acid residues on the x axis, and if we compare the affinity of NDL/3XCaM (K_d = 524 pM) with NDL/4XZCaM (K_d = 19 pM) we find that the latter has 28-fold greater affinity for calcium than the former (Fig. 3, Table I). This suggests that the high calcium affinity of NDL/4XZCaM is due to the number of acidic residues in chelating positions as opposed to positioning of the acidic residues on x and z axes. However, if we compare the calcium affinity of L^{109}CaM and NDL/4XZCaM with the NDL/3ZCaM peptide (K_d = 59 pM), we find the calcium affinity of NDL/3ZCaM to be 12-fold higher than the natural L^{109}CaM sequence and only three times less than the NDL/4XZCaM peptide (Fig. 3; Table I). The NDL/3ZCaM sequence is also 3-fold higher in affinity for calcium than the NDL/3XCaM peptide (Fig. 3; Table I). The NDL/3ZCaM sequence is also 3-fold higher in affinity for calcium than the NDL/3XCaM peptide (Fig. 3; Table I). The NDL/3ZCaM peptide has 3 acid residues in chelating positions with an acid pair on the x axis.

The majority of crystal structures of calcium-binding proteins with an acid pair on the x axis indicate that the -x coordinating residue interaction with the cation is mediated by a water molecule, and there are not two negative charges interacting with the cation to stabilize the complex but one negative charge from the +x coordinating residue and a water molecule which is expected to be a less efficient interaction due to the incomplete dehydration of the cation. Such an inefficient interaction of the acid pair with the cation may lead to a repulsive interaction during the cation-binding process between the +x acidic residue and other acidic residues located in any of the remaining five chelating positions. This would explain the high affinity of both calcium sites in the parvalbumin calcium-binding proteins. The crystal structure of parvalbumin shows that a water molecule is not involved in the -x coordinating position of the calcium cation in site II but is involved in the -x coordinate of site III. In the case of site III, high affinity can be attributed to the fact that there is a z axis acid pair which interacts directly with the cation resulting in a stable, efficient interaction (Fig. 4). The high affinity of site II can be attributed to the fact that a Glu residue is located in the -x coordinating position of all known parvalbumin site II sequences, and therefore the side chain is long enough to interact directly with the cation and mediation by water is not needed to maintain proper structure in the site. Therefore, parvalbumin site II will interact directly with the cation through an acid pair on the x coordinate axis which results in complete dehydration and a stable interaction with the cation.

This study has provided considerable refinement of the acid pair hypothesis. Originally, the hypothesis stated that maximum calcium affinity in the helix-loop-helix unit would require four acidic residues paired on the x and z coordinate axes of the tetrahedral arrangement of chelating ligands. It would now appear that high affinity can be had by helix-loop-helix units with 3 acid residues provided 2 of the residues are paired on either an x or z coordinate axis. This study seems to indicate the superiority of the z axis for the acid pair provided the -x position is occupied by a charged aspartic acid residue or uncharged polar residue. The x axis acid pair may be just as important as the z axis acid pair, provided that -x position is occupied by a glutamic acid residue as occurs in parvalbumin site II (Fig. 4). Under these circumstances the -x dentate will not be mediated by a water molecule and the

**Fig. 1.** Amino acid sequence of A'^STnC, L^{109}CaM, NDL/4XZCaM, NDL/3XCaM, and NDL/3ZCaM. The N- and C-terminal helical regions flanking the calcium-binding loop region are indicated by horizontal bars. The loop region is indicated by a horizontal bar, and the sequence positions in the loop region are numbered 1 through 12 starting with the N-terminal residue. The amino acid residues with side chains interacting with calcium are circled and the position of the chelating residue in the octahedral arrangement of dentates is indicated. The -y position is assumed to be the peptide carbonyl oxygen of the residue in position 7 of the loop region.
Acid Pair Hypothesis

TABLE I
CD and $K_D$ values of the synthetic peptides

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Apo peptide $\Delta_0$</th>
<th>Ca$^{2+}$ peptide $\Delta_0$</th>
<th>TFE peptide $\Delta_0$</th>
<th>$K_D$ $\mu M$</th>
</tr>
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<tbody>
<tr>
<td>$A^{88}STnC$</td>
<td>8,079 ± 273 (9)$^a$</td>
<td>17,223 ± 248 (20)</td>
<td>20,872 (24)</td>
<td>28.2 ± 1.7</td>
</tr>
<tr>
<td>NDDL/4XZCaM</td>
<td>6,573 ± 98 (8)</td>
<td>17,187 ± 183 (20)</td>
<td>20,902 (24)</td>
<td>19.1 ± 0.1</td>
</tr>
<tr>
<td>$L^{109}CaM$</td>
<td>5,470 ± 145 (6)</td>
<td>13,755 ± 60 (16)</td>
<td>19,760 (20)</td>
<td>755 ± 61</td>
</tr>
<tr>
<td>NDL/3XCaM</td>
<td>5,715 ± 247 (7)</td>
<td>15,251 ± 197 (18)</td>
<td>27,599 (32)</td>
<td>524 ± 16</td>
</tr>
<tr>
<td>NDL/3ZCaM</td>
<td>7,095 ± 291 (8)</td>
<td>19,540 ± 423 (23)</td>
<td>23,450 (27)</td>
<td>58.8 ± 0.1</td>
</tr>
</tbody>
</table>

$^a$ Mean residue ellipticity at 222 nm is expressed in millidegrees ± S.D. (n = 3).
$^b$ $K_D$ is the calcium dissociation constant expressed as $\mu M$ concentration ± S.D. (n = 3).
$^c$ The data indicated are the result of a single experiment. The TFE is 3:1 (v/v) in 100 mM MOPS, pH 7.2, 150 $\mu M$ KCl, 1 mM NTA.

In order to examine the applicability of the acid pair hypothesis to the natural calcium-binding proteins, an examination of the 12 residue loops from bovine brain calmodulin (Fig. 4) utilizing the premises of the acid pair hypothesis was undertaken. Analysis shows that the four sites in this protein will have two high and two low affinity sites for calcium. Sites I and IV will have high affinity for calcium based on the four acidic residues present in chelating positions and one acid pair on the $z$ axis. These sites reside in different pairs of sites and therefore will be independent but need not be identical.
Fig. 3. CD-monitored calcium titration of the synthetic peptides. Cell path length is 0.1 cm. Data points from a single experiment are indicated as follows: NDDL/4ZCaM (peptide conc. = 0.6 mg/ml (C)); A°STnC (peptide conc. = 0.7 mg/ml (A); NDL/ 3ZCaM (peptide conc. = 0.5 mg/ml (B); NDL/3XCaM (peptide conc. = 1.5 mg/ml (C)); and 1°3CaM (peptide conc. = 0.4 mg/ml (D). f is the ratio of the calcium-induced change in ellipticity at 222 nm to the maximum change in ellipticity that can be elicited by calcium at 222 nm. The lines are the computer-generated fits described under "Experimental Procedures." The correlation coefficients of all fittings to the single site model were greater than 0.996 indicating excellent correlation between the data and the model.

PROTEIN | C2 BINDING SITE NO. | C2 BINDING LOOP SEQUENCE | NO. OF ACID RESIDUES | LOCATION OF ACID PAIRS |
<table>
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<tr>
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<tr>
<td>BOVINE BRAIN CAP</td>
<td>I</td>
<td>x x x 6 7 - y 9 10 11 12</td>
<td>6</td>
<td>2 Axis</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>x x x 6 7 - y 9 10 11 12</td>
<td>6</td>
<td>3 Axis</td>
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<tr>
<td></td>
<td>III</td>
<td>x x x 6 7 - y 9 10 11 12</td>
<td>6</td>
<td>4 Pair</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>x x x 6 7 - y 9 10 11 12</td>
<td>6</td>
<td>2 Axis</td>
</tr>
<tr>
<td>RABBIT SKELETAL TnC</td>
<td>I</td>
<td>x x x 6 7 - y 9 10 11 12</td>
<td>6</td>
<td>2 Axis</td>
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<td></td>
<td>II</td>
<td>x x x 6 7 - y 9 10 11 12</td>
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<td>IV</td>
<td>x x x 6 7 - y 9 10 11 12</td>
<td>6</td>
<td>2 Axis</td>
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In bovine brain calmodulin is similar to that occurring in the four calcium-binding sites of rabbit skeletal troponin C. According to the acid pair hypothesis and in agreement with current dogma, STnC will have two high and two low affinity calcium sites in a situation similar to the paired cooperative model described by Iida and Potter (30) and Cox et al. (31, 32).

Although the acid pair hypothesis is ineffective in suggesting which of the two models (the paired cooperative sites model or the four interactive sites model) is more likely to prevail in calcium interaction with calmodulin, it is useful in suggesting the order in which the sites are filled by calcium in both models.

The situation in bovine brain calmodulin is similar to that occurring in the four calcium-binding sites of rabbit skeletal troponin C. According to the acid pair hypothesis and in agreement with current dogma, STnC will have two high and two low affinity calcium sites in a situation similar to the paired cooperative model described in calmodulin. The two high affinity sites are paired on the C terminal half of the molecule and carry both x and z acid pairs (Fig. 4). There is potential for cooperative interactions between the two high affinity sites in TnC although it is not readily apparent which of the two sites will bind calcium first. The two low affinity sites in TnC are not identical by the acid pair hypothesis. Although both sites contain 4 acid residues in chelating positions, site I contains an Asp residue in the -y coordinate which does not interact with the cation via the amino acid side chain. If we adhere to the hypothesis, then site I will have 3 acid residues which interact with the cation and none of the anionic residues are paired on a chelating axis (Fig. 4). Therefore site I will be lower in affinity for calcium than site II which has 4 acid residues with an acid pair on the x axis and the sequence of filling the TnC sites should proceed IV = III > II > I.
Acid Pair Hypothesis

The synthetic calcium-binding peptide analogs described in this study are useful tools for the investigation of calcium interaction with calmodulin and related calcium-binding proteins. They have been used to provide strong support and to further elaborate upon the major premise of the acid pair hypothesis which is that cation affinity of the helix-loop-helix calcium-binding unit will be increased if the anionic amino acid dentates in the loop region which interact directly with the cation are paired on the axial vertices of the resulting octahedral arrangement of chelating residues about the cation. Although the evidence thus far suggests that the synthetic peptides bind calcium identically to the sites in the natural proteins, the definitive study on this point will conclude when the crystal structure of the synthetic peptides has been solved.

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REFERENCES
Synthetic fragments of calmodulin calcium-binding site III. A test of the acid pair hypothesis.
R E Reid


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