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Evidence for reversible internal hydride/deuteride transfers from sodiated deprotonated fructose-6-phosphate and arginine complex with solvated salt structure

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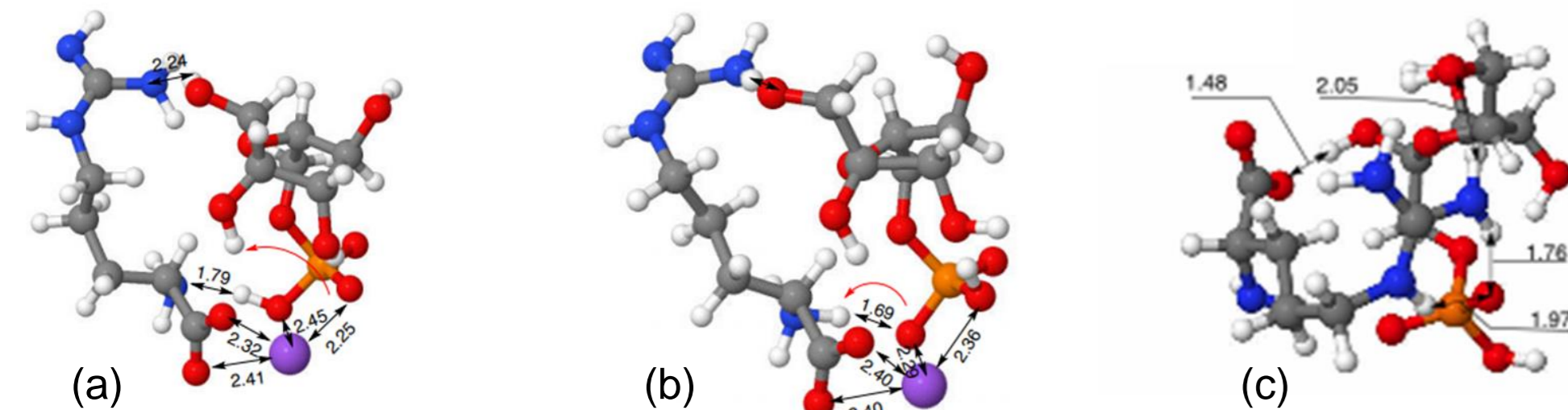


OBJECTIVE

To understand competitive cleavages occurring from fructose-phosphate/sodiated arginine complex anion (i.e., [(F6P/R+Na-H)-H]⁻) leading to the free [F6P-H]⁻ anion and [(F6P/R-2H+Na)-C₃H₆O₃]⁻. For that, to rationalize the formation of the corresponding labeled fragment species from [(F6P_{d6}/R_{d7}-D+Na)-D]⁻ complex (mobile protons being exchanged by deuterons) with a D-enrichment of the free fructose phosphate anion and a D-depletion from the fragment ion related to the C3 neutral loss giving evidence (indicating, suggesting) that the corresponding mechanism implies a stepwise process via the F6P ring opening in the complex.

INTRODUCTION

Recently, it has been shown that prepared under electrospray (ESI) in negative mode, non-covalent sodiated anionic complex (SNCC) consisting of fructose (F6P) and arginine (R) dissociates in CID mainly by (i) the sodiated arginine loss by releasing the [F6P-H]⁻ anion (m/z 259) and in competition, (ii) the F6P ring part loss (covalent bond cleavages, CBC), in low abundance, yielding the fragment [(F6P/R-2H+Na)-C₃H₆O₃]⁻ ion (m/z 365). These decompositions can be respectively representative for various interactions within the complex with arginine considered as salt (i.e., [R-H+Na]), namely: (i) only hydrogen bond(s) (HB-SNCC) with free salt part and (ii) hydrogen bond (HB) together with solvated salt (SS-SNCC) (Scheme 1(a), and (b)). This behavior differs from that of the non-covalent non-cationized complex (NCC) which mainly displays arginine loss (related to the HB-NCC form) but also several small neutral losses by covalent bond cleavages (related to the SB-NC



Scheme 1. Calculated conformations of sodiated [(F6P/R-H+Na)-H]⁻ non-covalent complex maintained by HB and SS interactions with Na⁺ being chelated by (a) both the -CO₂⁻ and -OPO₃H⁻ groups and (b) both the -CO₂⁻ and -OPO₃H₂ groups with an alkoxide site, the form (b) being less stable by 44 kcal/mol. A more compact non-sodiated deprotonated [F6P/R-H]⁻ complex with SB interaction (c).

Deuterated sodiated [(F6P_{d6}/R_{d7}-D+Na)-D]⁻ complex with all mobile protons exchanged by deuterons in solution, dissociates by unusual pathways. Indeed, complementary enrichment(+D)/depletion(-D) is observed for both the main product ions related to each partner (intact or not) favored under resonant excitation conditions compared to non-resonant mode (i.e., HCD). Generation of the main [F6P_{d6}-D]⁻ product ion probably takes place after F6P_{d6} ring opening and possible hydride/deuteride exchanges rather than directly from intact NCC.

Methods

Mixture of F6P and R (Sigma-Aldrich, USA) in D₂O (at a final concentration of 100μM) was analysed by direct injection (7 μL/min) in ESI negative mode on an Orbitrap Elite (Thermo Fisher Scientific, Courtaboeuf, France). The inter-(with residual water in linear trap) and intra-complex (between partners of ion-dipole complex) HDX for [(F6P_{d6}/R_{d7}-D+Na)-D]⁻ were explored by resonant (CID in LTQ) and non-resonant (HCD in ion routing multiple) excitations using variable ExcT and NCE values for CID and HCD and variable ExcQ for CID. Ion spray (IS) was set at -3.5 kV, resolution was set at 60,000. Variable values of excitation time (ActTime) between 10 ms and 3000 ms for CID and 0.1 ms to 3000 ms for HCD were applied for ion selection and excitation. ERMS and sequential MSⁿ experiments were performed on both the labeled and unlabeled precursor ions and product ions. The same conditions were applied (except the limit of ActTime at 1000 ms) when Orbitrap Fusion (Thermo Fischer Scientific) was used.

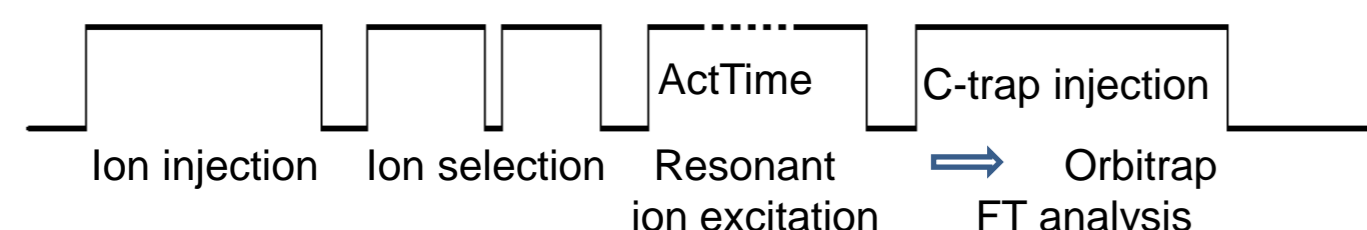


Figure 1. Sequence used for variation of ActTime for both the CID (R) and HCD (NR) experiments

RESULTS

1. Correlation between D-enrichment of the [F6P_{d6}-D]⁻ and D-depletion of the product ion related to the "C3" loss from the F6P_{d6} ring within the survival complex: evidence of the intra-complex gas phase HDX within sodiated [(F6P_{d6}/R_{d7}-D+Na)-D]⁻ complex anion

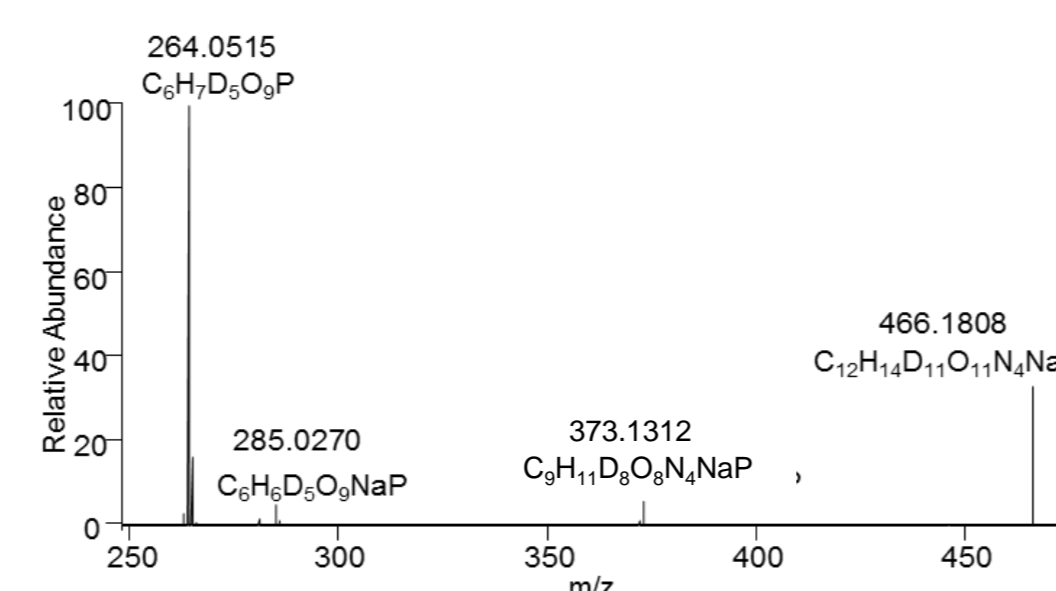


Figure 2. Product ion spectrum (NCE=15% in resonant excitation during 10 ms, CID mode with LMCO at m/z 115) of the d11-labelled sodiated complex (m/z 466).

It appears product ions which mainly carry the expected labeling :
 - 5D in the m/z 264 product ion corresponding to [F6P_{d6}-D]⁻
 - 4D in the m/z 285 ion related to [(F6P_{d6}-D+Na)-D]⁻
 - 8D in the m/z 373 ion resulting to the d3-"C3" loss (i.e., C₃H₃D₃O₃) from the m/z 466 precursor complex anion.

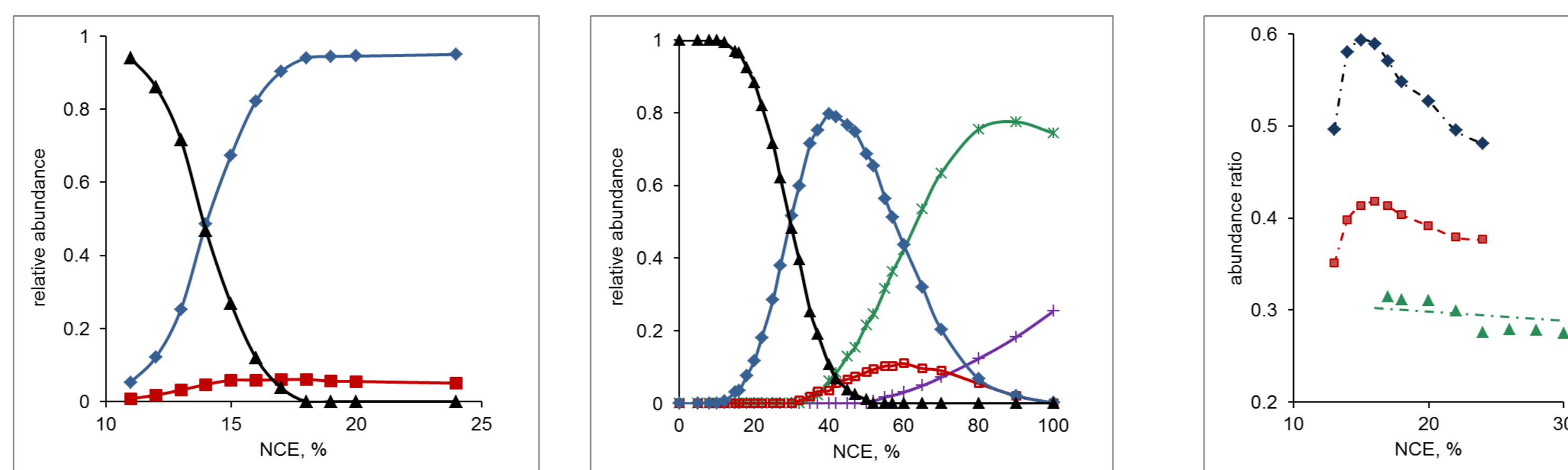


Figure 3. ERMS breakdown curves of sodiated [(F6P/R_{d7}-D+Na)-D]⁻ complex (a) in resonant excitation (CID mode: slow heating and competitive processes) and (b) in non-resonant excitation (HCD mode: fast heating and consecutive processes (ion abundance relative to the total ionic current for \blacktriangle labeled complex precursor ion, \blacklozenge $\sum_{n=6,7,8} [F6P_n-D]^-$ (n=6, 7 and 8), \blacksquare $\sum_{m=1,2,3} [(F6P/Rd7-2D+Na)-C_3H_6D_mO_3]^-$ (m=1, 2 and 3), \square (191+192), \star $\sum_{r=0,1,2} [PO_3H_{2-r}D_r]^-$ (r=0, 1 and 2), \blacktriangle PO₃⁻). (c) Evolution of the \blacklozenge (I₂₆₅+I₂₆₆)/I₂₆₄ and \blacksquare (I₃₇₁+I₃₇₂)/I₃₇₃ product ion abundance ratios with the NCE values from the CID experiment and \blacktriangle (I₂₆₅+I₂₆₆)/I₂₆₄ from the HCD experiment (weak HDX and slow decrease with the increase of NCE value).

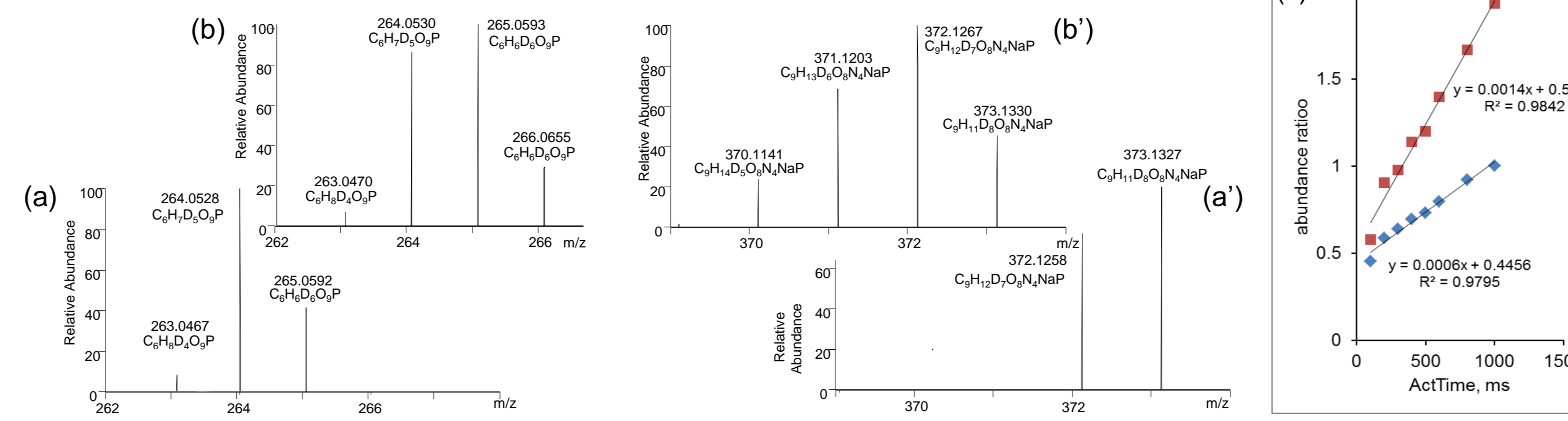


Figure 4. Regions of interest (related to both the main product ions characterized by complementary HDX) of CID spectra (NCE=8%) of the sodiated complex [(F6P/R_{d7}-D+Na)-D]⁻ for ActTime=50 ms (for (a) and (a')) and for ActTime=3000 ms value (for (b) and (b')). (c) Like linear evolution of the ion abundance ratios (related to unexpected over expected HDX between F6P and R within sodiated NCC): the \blacklozenge (I₂₆₅+I₂₆₆)/I₂₆₄ and \blacksquare (I₃₇₁+I₃₇₂)/I₃₇₃ vs. the ActTime logarithm.

These results show that the D-enrichment and D-depletion during HDX are almost complementary and increase with ActTime. However, the observed reinforced D-depletion (higher slope in Figure 4 (c)) is due to the back HDX with residual water in ion trap.

2. Non homogeneous structural population of the [F6P-H]⁻ ions formed from dissociation of the precursor [(F6P/R-H+Na)-H]⁻ complex anion evidenced by the HDX experiments

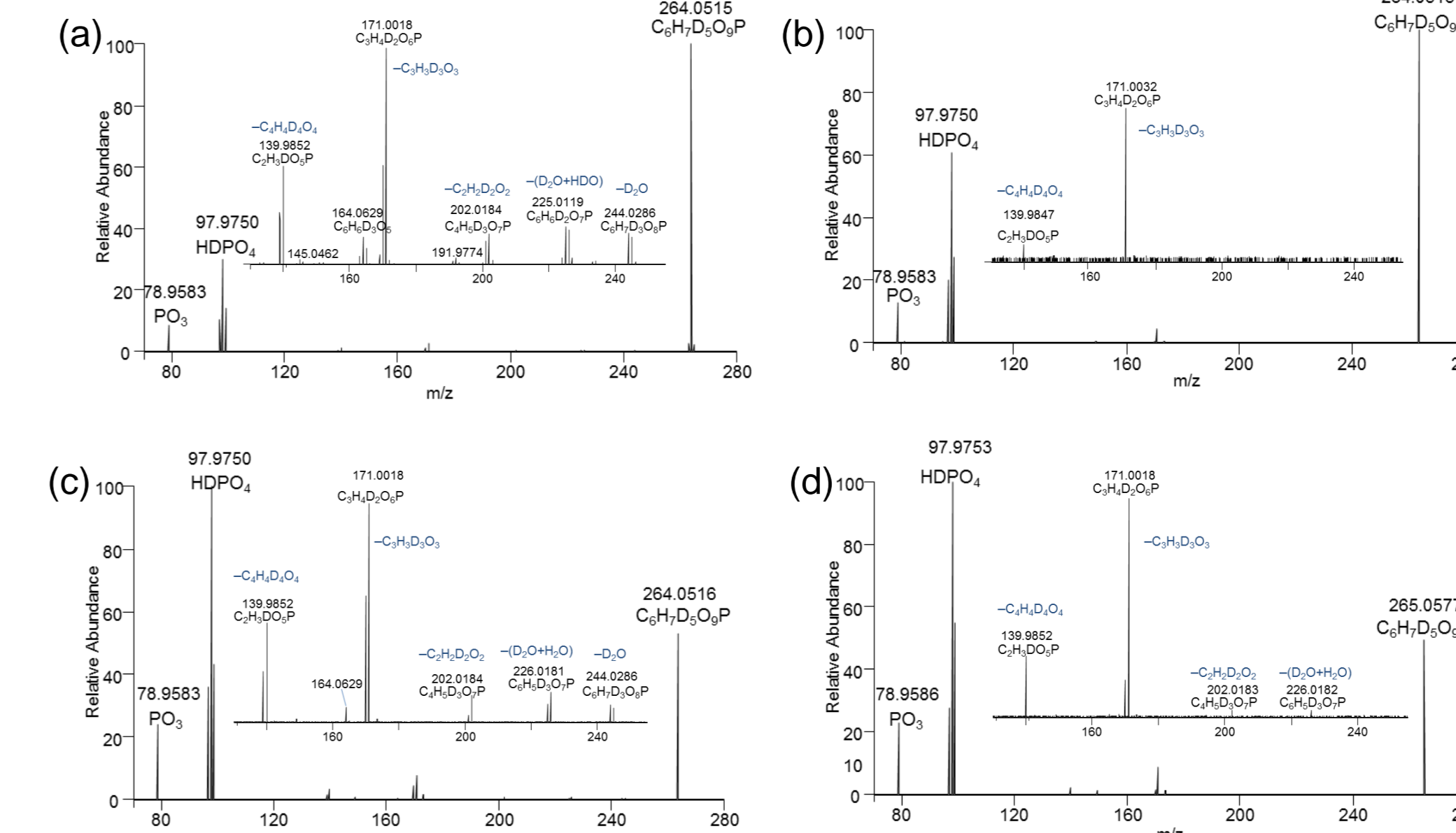


Figure 5. Product ion spectra of m/z 264 generated (a) in ESI source (MS²: CID NCE=17%), (b) by CID-MS² (NCE=14%) of non-sodiated [F6P/R_{d7}-D]⁻ ion (m/z 445) (MS³ exp. m/z 445 → m/z 264, NCE=17%), (c) by CID-MS² (NCE=18%) of sodiated [(F6P/R_{d7}-D+Na)-D]⁻ ion (m/z 466) (MS³ exp. m/z 466 → m/z 264, NCE=18%), (d) Product ion spectrum of m/z 265 generated by CID-MS² (NCE=18%) of sodiated [(F6P/R_{d7}-D+Na)-D]⁻ ion (m/z 466) (MS³ exp. m/z 466 → m/z 265, NCE=18%).

These CID spectra are informative on the structural differences of fructose-6-phosphate anions related to their origin:

- the m/z 264 formed in source differs from that produced in CID spectra of non-sodiated complex anion (m/z 445) but is very similar to that formed from dissociation of sodiated complex anion (m/z 466)

- The m/z 265 displayed in CID spectra of sodiated complex anion (m/z 466) differs to that of m/z 264 of same origin but is similar to that formed from non-sodiated complex.

Additional information may enlighten the D number remaining on the arginine moiety in the product m/z 373, m/z 372 and m/z 371 ions diagnostic to the D-depletion. Sequential MS³ experiments of these product ions formed from the dissociation of the m/z 466 precursor ion show that a common neutral C₃H₃D₂O₂ loss occurs to yield sodiated arginine phosphate NCC ion at m/z 300 (7D), m/z 299 (6D) and m/z 298 (5D). Thus, the D-depletion takes place specifically at the sodiated arginine partner of the complex anion.

3. Non homogeneous structural population of the [F6P-H]⁻ ion formed from dissociation of the precursor [(F6P/R-H+Na)-H]⁻ complex anion evidenced by the HDX experiments

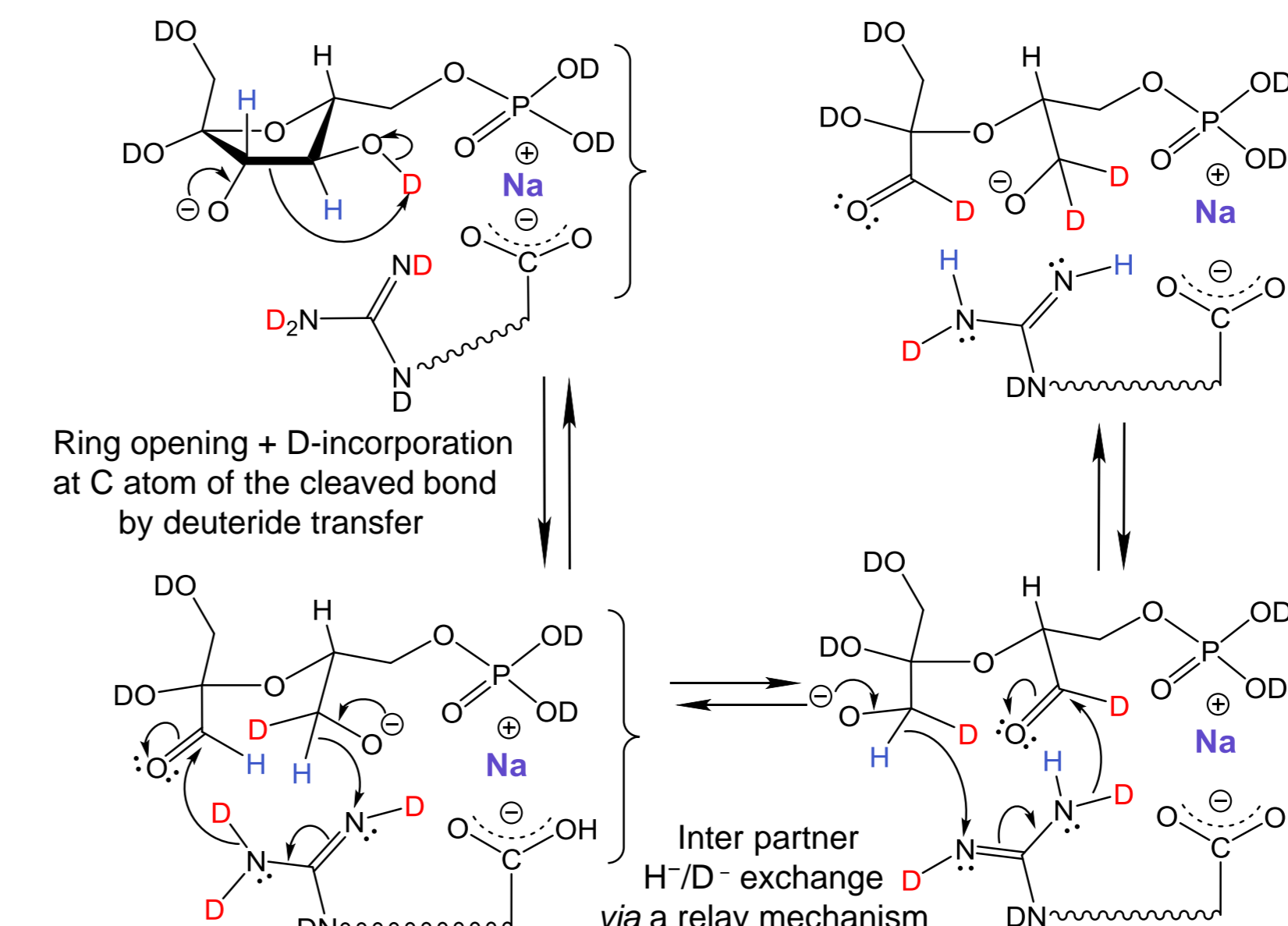


Figure 6. Proposed mechanism to rationalize the multiple hydride/deuteride transfer via ring opening the reversible H/D exchange based on relay pathway.

For the same sample, various structures derived from F6P may co-exist according to the excitation energy, their storage duration and MSⁿ origin. This explain the difficulty to attribute a particular structure to the complex and to interactive F6P.

CONCLUSION

Competitive cleavages (as free [F6P-H]⁻ and [(F6P/R-2H+Na)-C₃H₆O₃]⁻) of fructose-6-phosphate/sodiated arginine complex anion (i.e., [(F6P/R+Na-H)-H]⁻) are more complicated than expected. Indeed, the product ions generated from cleavages of [(F6P_{d6}/R_{d7}-D+Na)-D]⁻ present (i) D-enrichment of fructose-6-phosphate by hydride transfer from the [R_{d7}-D+Na] partner via opened ring intermediate during a stepwise pathway and (ii) complementary D-depletion for the second product ion, reinforced by back HDX. As a consequence, the existence of several intermediates results in an unexpected mixture of isomeric structures for the m/z 259 anion (shifted to m/z 264 and m/z 265 from the d11 complex anion) and not only one which should correspond to [F6P-H]⁻ as provided from a standard.