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Electron-induced reactions in 3-bromo-pyruvic acid

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Abstract

3-Bromopyruvic acid (3BP) is a potential anti-cancer drug, whose action on the cellular metabolism is not entirely clear so far. The presence of a bromine atom suggests that it is also reactive towards low-energy electrons, which are produced in large quantities during tumour radiation therapy. A detailed knowledge of the interaction of 3BP with secondary electrons is a prerequisite to gain a complete picture of the effects of 3BP in different forms of cancer therapy. Here, we studied dissociative electron attachment (DEA) to 3BP in the gas phase both experimentally using a crossed beam setup and theoretically using scattering and quantum chemical calculations. These results are complemented by a VUV absorption spectrum. The main fragmentation channel is formation of Br⁻ close to 0 eV and within several resonant features at 1.9 eV and at 3 – 8 eV. At low electron energies Br⁻ formation proceeds through a σ^* and a π^* shape resonance and at higher energies through core-excited resonances. We find that the electron capture cross section is clearly increased compared to the non-brominated pyruvic acid, but at the same time fragmentation reactions through DEA are significantly altered as well. The 3BP transient negative ion is subject to a lower number of fragmentation reactions than pyruvic acid indicating that 3BP could indeed act by modification of the electron transport chains within oxidative phosphorylation. At the same time it could act as radiosensitizer.

Introduction

Pyruvic acid is an α -keto carboxylic acid and is an important intermediate in biochemical reaction pathways and the main product of glycolysis. The brominated analogue 3-bromopyruvic acid (3BP; BrCH₂C(O)COOH, mass 166 u, see Fig. 1 for molecular structure) is a potential anticancer and strong alkylating agent.^[1] The anti-cancerous effect of 3BP is based on the fact that it inhibits hexokinase type II (HK II), which is the key enzyme for maintaining increased glycolysis in cancer cells.^[2] HK II is overexpressed in cancer cells, because they have an aberrant energy metabolism in

which they consume more glucose than normal cells and rapidly convert it to lactate. Additionally, 3BP inhibits mitochondrial oxidative phosphorylation, leading to a decrease in ATP formation which will lead to cell death.^[3] Oxidative phosphorylation (and the ATP formation by proton pumping across the mitochondrial inner membrane) is based on delicate electron-transport chains, which are apparently disturbed by 3BP. Due to the presence of the Br atom at C3 of pyruvic acid, 3BP might have favourable electron capturing properties (*i.e.* it might act as an electron acceptor) inducing a leakage of electrons from the electron transport chains as it was suggested recently *e.g.* for polychlorinated compounds.^[4] Electron capture leads to the formation of transient negative ions (TNIs), which may dissociate *via* dissociative electron attachment (DEA).^[5] In this process a negatively charged fragment anion and at least one neutral fragment are formed.

DEA is a process that is also relevant for DNA damage by high energy radiation used in cancer radiation therapy.^[5,6] Halogenated compounds are frequently used as radiosensitizers making the tumour tissue more sensitive to the radiation.^[7,8] This effect can at least partly be ascribed to an increased reactivity of critical cellular components towards low-energy electrons, which are formed in large amounts as secondary particles (having mostly kinetic energies below 10 eV) in the interaction of the energetic radiation with the cell tissue.^[9] Basically, 3BP could also act as a radiosensitizer, however no data on its reactivity towards low-energy electrons have been reported yet. Nevertheless, 3BP has already been used as an alternative cancer treatment. In 2016 it was reported that treatment of several cancer patients with 3BP might have caused their death shortly after 3BP administration.^[10] The actual reason for the deaths could not be revealed, but this case suggests that compounds such as 3BP could have different and maybe less obvious biological, chemical and physical effects. For example, potential radiosensitizing properties of 3BP could become important in conjunction with its role in HK II and oxidative phosphorylation inhibition. As was pointed out above, in all these processes the reactivity towards low-energy quasi-free electrons must be considered.

Consequently, in the present work DEA to 3BP is studied both experimentally and theoretically. We focus on the anion formation and fragmentation of gas phase 3BP within an energy range of 0 – 12 eV. The experimental results are accompanied by scattering calculations revealing details about TNIs formed from 3BP. Albeit our discussion will address mainly low energy features below 7 eV, a high-resolution VUV photoabsorption measurement in the photon energy range of 3.8 – 10.8 eV is presented for 3BP in order to gain knowledge on possible electronic excitation during anion formation. The present results indicate that 3BP is highly susceptible towards low energy electron attachment close to zero eV. DEA at this electron energy leads to the formation of the Br⁻ anion, which is by far the most abundant anion formed for this compound.

Results and Discussion

Molecular structure of 3BP

In our present study on 3BP, we have initially analysed the molecular structure of 3BP by DFT calculations with the B3LYP exchange-correlation functional and the cc-pVDZ basis set. Four planar conformers have been identified, which are displayed in Fig. 1 (denoted A – D) and which differ by their rotation around the C2 – C3 and C3 – O axes. In the energetically most favourable conformer a hydrogen bond is established between the hydrogen of the carboxyl group and the α carbonyl group. In this conformation 3BP possesses a moderate dipole moment of 0.62 D, as obtained from the DFT calculations. For native pyruvic acid the two lowest identified conformers possess a similar geometry; however, it should be noted that already the energetically lowest structure has a considerably higher

dipole moment (2.3 D)^[11] compared to the brominated compound studied here. We note that the dipole moment for the 3BP conformer A is subcritical in view of a dipole bound anion and hence not sufficiently high to bind an excess electron. In contrast, the dipole moment in the other, energetically less favourable conformations is considerably higher (up to 4.41 D, see Fig. 1). However, it should be noted that the calculated Boltzmann population at 328 K, which corresponds to the sublimation temperature used in the experiment, suggests that the great majority of molecules (i.e. > 95 %) is present as conformer A when transported into the gas phase by thermal sublimation. Nevertheless, we investigated the possibility of dipole bound states (DBS) for these conformers, since they may be involved in the formation of the dehydrogenated anion as previously observed for biomolecules.^[12] We employed the aug-cc-pVDZ basis set augmented with diffuse functions located on the hydrogen atom of the hydroxyl group, as suggested by Skurski et al.^[13] Since structure A has a low dipole moment (0.62 D) and structure D a very low Boltzmann population (0.54 eV above structure A), we only investigated the structures B and C. Although the former has a supercritical dipole moment, we did not obtain a DBS with the models employed in the present study. In case a DBS exists for the structure B, its binding energy would probably be very small. While the contribution from structure C to the measurements should be modest, in view of the low population, a DBS bound by 19.3 meV was obtained from Second-Order Møller-Plesset perturbation theory (MP2). Improving the description of electronic correlation increases the binding energy to 36.7 meV (singles and doubles coupled cluster, CCSD) and 37.9 meV (CCSD with non-iterative triples, CCSD(T)). Apart from vibrational corrections, which have not been taken into account, the DBS binding energy should therefore be close to 40 meV. The dipole moment vector for structure C is shown in Fig. 2 (left panel) and indicates that the positive end of the dipole is pointing towards the hydroxyl group. Therefore, when plotting the DBS orbital (Fig. 2, right panel), most wave function density can be found close to this group. Hence, the DBS may act as a doorway state^[14] for the experimentally observed loss of hydrogen (see below). The contribution from the dipolar interaction should significantly increase the cross section magnitude for the conformer C, and also couple ν_{OH} stretch vibrations. The large dipole moment therefore favors H-elimination reactions and could compensate the low population of the conformer C to some extent. From a pure dipole scattering model, in which the cross section scales with the square of the dipole moment magnitude, we could expect the cross section of the conformer C to be about 40 times larger than that of conformer A at low energies.

Fig. 1: Four planar structures of 3BP were optimized with density functional theory (DFT), employing the B3LYP functional and the cc-pVDZ basis sets implemented in GAMESS.^[15] The structures are labeled A to D in order of increasing energy. The relative energies, magnitude of dipole moments, and the relative Boltzmann populations (p), with respect to the ground state, for 328 K ($k_B T = 28.3$ meV) are given below each structure.

Fig. 2: The dipole moment vector of the conformer C (left panel), and the singly occupied orbital of the dipole bound state (right panel). The plots were generated from CCSD(T) calculations with the aug-cc-pVDZ basis set.

Dissociative electron attachment to 3BP

Experimentally we investigated DEA to 3BP using a crossed electron-molecular beam setup (see Methods for details). Within the detection limit of the apparatus, we obtained nine different fragment anions in DEA to 3BP. The ion yield curves of the main fragmentation channels are displayed in Fig. 3. Table 1 summarizes all observed fragment anions along with the corresponding peak positions. The anion signals displayed in Fig. 3 can be attributed to the following fragmentation reactions:

Fig. 3: Ion yield curves obtained by DEA to 3BP at m/z 79 (Br^-), 165 ($(3\text{BP-H})^-$), 87 ($(3\text{BP-Br})^-$) and 126 ($\text{CO}_2\text{H}_3\text{Br}^-$).

Table 1: Peak maxima for observed fragment anions as determined from DEA spectra.							
Anion	Peak position / eV						
Br^-	0.1	1.9		3.9	5.0	6.2	7.3
$(3\text{BP-Br})^-$	0.1	1.8					
$(3\text{BP-H})^-$	0	~0.75	1.3		4.8		8.6
$\text{CO}_2\text{H}_3\text{Br}^-$	0.1						
HCOO^-					5.0		
COO^-			2.3	3.3			
CH_2CO^-			2.6		4.4		
OH^-					4.9	5.8	
O^-					4.8	5.4, 6.4	

By far the most intense fragment ion is the bromide ion Br^- , which is observed through a sharp peak at 0.1 eV. A second, much weaker feature appears at 2 eV and a series of about four resonances is observed at 3 – 8 eV. Experiments with radiosensitizers based on the substitution with a halogen atom like bromine frequently show an increased cross section for dissociative electron attachment.^[8,16] In particular, the high electron affinity of bromine (3.363 eV^[17]) may lead to exothermicity of the dissociation reaction involving the cleavage of the C–Br bond (2.954 eV bond dissociation energy) and efficient capture of low-energy electrons. We calculated DEA thresholds from the energy difference between the fragments and the parent neutral molecule. The results for Br^- are given in Table 2 for the three lowest-energy conformers of 3BP (*i.e.*, A – C). Accordingly, the reaction is exothermic for release of Br^- (about -0.45 eV, slightly depending on the conformer considered). We also considered the neutral hydrogen affinity of the de-brominated pyruvic acid radical, $\text{P} = (3\text{BP-Br})$. From gas-phase G4MP2 calculations, we estimate the $\text{D}(\text{PH})$ dissociation energy to be 4.85 eV for the isomer A. The latter energy would be high enough to favor H-atom abstraction from biomolecules, which can be the initial step of detrimental reactions, as suggested by Gregory.^[18]

The cleavage of the C–Br bond not only results in Br^- , but also in the corresponding fragment anion $(3\text{BP-Br})^-$ with release of neutral bromine. This fragment anion forms within a sharp signal close to zero eV and a slightly broader signal peaking at 1.8 eV, but with much weaker intensity than Br^- (about a factor of 750). The $(3\text{BP-H})^-$ ion is also formed with considerably lower intensity compared to Br^- (about 4.4% of the peak maximum), within a signal peaking at 0.75 eV. This feature might be composed of three distinct features with additional and weaker contributions close to 0 eV and at 1.3 eV. Additionally, $(3\text{BP-H})^-$ appears at 4.8 and 8.6 eV with rather low intensities. The second most intense fragment anion is observed at m/z 126 and is assigned to the $\text{CO}_2\text{H}_3\text{Br}^-$ ion, which appears within a signal peaking at 0.1 eV. We note that this anion cannot arise from a simple bond cleavage reaction. Instead, this fragment can only be formed by strong structural rearrangement within the TNI, and due to high thermodynamic thresholds, we cannot rule out that this is due to a Br containing impurity.

Very recently, Zawadzki et al.^[11] reported 11 fragment anions in a DEA study with pyruvic acid using two different crossed beam experiments. Apart from the dehydrogenated parent anion, all fragment anions form only at electron energies > 2 eV. For example, the most abundant fragment anion OH^- was observed with strongest intensities at about 6 and 8 eV. These studies further revealed an interesting property of the intermediate pyruvic acid anion: it seems to possess an unusually high cross section for anion – molecules collisions. This led to the efficient formation of

additional anionic products (like for example the long-lived parent anion and the dehydrogenated dimer anion) close to zero eV electron energies, even at modest pressures of pyruvic acid in their apparatus.

Fig. 4: Symmetry-resolved elastic scattering cross section (ICS) indicating two shape resonances at 0.4 eV (A' component) and 2.7 eV (A'' component), respectively. The orbital plots were generated with the compact 6-31G basis (not used in the scattering calculations). The ICS peaks are assigned to σ_{CBr}^* and π_2^* shape resonances. A stable valence bound π_1^* anion was found with a vertical binding energy of about 1 eV.

In order to obtain more information about the TNIs formed upon DEA to 3BP, we have performed scattering calculations. The diagonalization of the scattering Hamiltonian represented in the square-integrable CSF bases allows for the inspection of bound anion states and resonance characters. In fact, we obtained a stable valence bound anion state (π_1^*), lying 0.9 eV below the neutral ground state (vertically). Experimentally no parent anion of 3BP is observable within the detection limit of the apparatus. Therefore, in the present experiments carried out under single collision conditions this valence bound anion cannot be stabilized within the detection time and most likely decays upon spontaneous emission of the excess electron. The symmetry-resolved elastic integral cross section (ICS) is shown in Fig. 4. The orbital plots shown in the panels in Fig. 4 were generated with the compact 6-31G basis (not used in the scattering calculations). They are consistent with the pseudo-eigenstates of the scattering Hamiltonian and provide insight into the resonance characters. The calculations point out two shape resonances around 0.4 eV (A' component) and 2.7 eV (A'' component), respectively labeled σ_{CBr}^* and π_2^* . We note that the strong Br^- signal is found around 0 eV and hence may not be directly assignable to the σ_{CBr}^* resonance. However, Br^- elimination triggered by the formation of the σ_{CBr}^* resonance is possible when taking into account that (i) dissociation could start from vibrationally excited states, and (ii) errors around ~ 0.3 eV in the calculated resonance positions are possible.

The π_2^* resonance at 2.7 eV is expected to initiate several DEA processes. An indirect mechanism involving a $\pi_2^*/\sigma_{\text{CBr}}^*$ coupling can result in both the Br^- and $(3\text{BP}-\text{Br})^-$ signals observed around 2 eV. Other DEA reactions around 2.0 eV (see below), which for example lead to fragment anions from the carboxyl group, can also be triggered by the π_2^* resonance. The gap between the

calculated energy of the π_2^* state (2.7 eV) and the DEA peaks (~ 2.0 eV) is a bit larger than observed in previous studies (< 0.4 eV). Consistency checks performed with scaled virtual orbital energies suggest that the present scattering calculations actually overestimate the π_2^* resonance position. The procedure described by Modelli^[19] indicates energies around 2.0 eV for the isomers A, B and C. We also employed the empirical expression suggested by Pshenichnyuk et al.^[20] to estimate the energy of the σ_{CBR}^* anion state. We obtained 0.64 eV for the A isomer, and 0.98 eV and 0.88 eV for the B and C isomers, respectively.

Table 2: Calculated DEA thresholds for the generation of $(3\text{BP-H})^-$ and Br^- .

Fragment	Parent	$\Delta H_{\text{thr}}(\text{eV})$
$(3\text{BP-H})^-$	A	+0.55
$(3\text{BP-H})^-$	B	+0.46
$(3\text{BP-H})^-$	C	+0.40
Br^-	A	-0.46
Br^-	B	-0.47
Br^-	C	-0.42

We suggest another reaction mechanism for the dehydrogenated parent anion which is slightly endothermic by 0.40 – 0.55 eV (depending on the conformer considered). Basically, it could be expected that the $(3\text{BP-H})^-$ ion is formed through a σ_{OH}^* shape resonance. However, other σ^* shape resonances than the one discussed above do not have clear signatures in the calculated cross sections. These signatures only become clear when heavier atoms, such as Br and S, are present. While the present calculations do not point out σ_{OH}^* resonances, often too broad and hidden in the dipolar background, inspection of the virtual orbitals (VOs) calculated with the compact 6-31G basis set suggests that such a resonance could exist. Nevertheless, as is pointed out above we assign the $(3\text{BP-H})^-$ signal to arise from vibrational Feshbach resonances (VFRs) rather than a $\pi_2^*/\sigma_{\text{OH}}^*$ coupling mechanism. From the frequency of the ν_{OH} stretch mode (0.45 eV) we could estimate VFR energies for H elimination, by assuming that the ν_{OH} frequency would be the same for the DBS anion state. Harmonic-approximation estimates point out VFRs around 0.23 eV (closed channel), 0.68 eV, 1.13 eV and 1.58 eV, for the $\nu_{\text{OH}} = 0, 1, 2, 3$ vibrational levels. In these estimates, a binding energy of ~ 0 eV was assumed for the DBS, which would be consistent with the B structure. Since the DBS has a binding energy of 0.038 eV for the C structure, the VFRs would be slightly lower lying, but nothing would really change. Consequently, we assign the $(3\text{BP-H})^-$ signals at 0 – 1.3 eV to DBS.

Three ion yields shown in Figure 3 also indicate weak formation above the electron energy of ~ 3.0 eV. While the low energy features can be assigned to single particle resonances, where the initial electronic configuration of the molecule remains unchanged, we assign those features above this energy to core-excited resonances, where an electron of the molecule is promoted to a higher lying orbital in the electron capture event. This applies to the ion yields shown in Figure 5 and 6, where abundant ion yield is observed in the electron energy regime of core excitation. We associate the ion yields shown in Figure 5 with fragment anions formed from the carboxyl group or the central carbonyl group:

The corresponding ion yield curves are shown in Fig. 5. COO^- is formed from the π_2^* resonance located at 2.3 eV and a shoulder is visible at 3.3 eV, which is attributed to a core-excited

anion. HCOO^- is formed within a single feature with maximum at 5.0 eV. Both fragment anions arise from loss of the negatively charged carboxyl group. CH_2CO^- is generated through two resonances, the π_2^* resonance located at 2.6 eV, and a core-excited resonance peaking at 4.4 eV. This anion forms by simple bond cleavages, where the CH_2 group remains at the carbonyl group. We also detected atomic oxygen anion and the diatomic hydroxyl anion upon DEA to 3BP. OH^- and O^- are observed with considerable intensity within a single core-excited resonance located at around 5.0 eV (Fig. 6). Since the calculations indicate hydrogen bond formation between the hydrogen of the carboxyl group and the α carbonyl group, OH^- may involve oxygen from the carboxyl group or carbonyl group. Several DEA studies on organic acids^[21] and amino acids^[22] containing the carboxyl group have been reported already. In these studies, the formation of OH^- , O^- and HCOO^- was commonly reported, while, to the best of our knowledge, the COO^- anion was reported to form only in very low abundances compared to the dehydrogenated parent anion or HCOO^- .^[23] The CO_2 molecule has a negative electron affinity; however, a small barrier between the ground state of the anion and the vibrationally excited electronic ground state of the neutral extends the lifetime of the anion up to ms.^[24] In addition to several other fragment anions, OH^- , O^- and HCOO^- were also reported to form in DEA to pyruvic acid, while COO^- and CH_2CO^- exclusively form for the brominated compound. Further, we note that if a fragment anion is formed for both compounds, the resulting ion yield looks considerably different. This indicates that the electronic structure as well as the fragmentation dynamics are strongly modified by the presence of the bromine atom.

Fig. 5: Ion yield curves showing the formation of COO^- , HCOO^- and CH_2CO^- by DEA to 3BP.

Fig. 6: Ion yield curves showing the formation of OH⁻ and O⁻ by DEA to 3BP.

The assignment of ion yields to core-excited resonances discussed above is also supported by VUV photoabsorption studies. A high resolution photoabsorption VUV spectrum is presented in Fig 7. Below 7 eV two broad excited states centered at 5.3 eV and 6.4 eV can be distinguished with local cross sections of 2 Mb and 6.2 Mb, respectively. The calculated excited states by means of three different exchange correlation functionals, are in good agreement (Table 3). The lowest excited state peaking below 3.8 eV (not visible in the VUV spectrum) can be assigned to a $n \rightarrow \pi^*$ transition from the bromine lone pair or oxygen lone pair to π^* . As mentioned above, the energy dependence of the COO⁻ ion yield shows a shoulder centered at 3.3 eV attributed to a core-excited resonance. Since COO⁻ was not observed in DEA studies from pyruvic acid, this dissociation channel is most probably related to the excitation from a Br lone pair to a π^* orbital leading to the COO⁻ fragment.

The excited state centred at 5.3 eV in the VUV spectrum can be associated with either a $\pi \rightarrow \pi^*$ or an $n \rightarrow \pi^*$ transition. However, according to the calculations the $n \rightarrow \pi^*$ transition is essentially dark due to a negligible oscillator strength. The CH₂CO anion is formed *via* a core-excited resonance peaking at 4.4 eV. This can be as well assigned to an excitation from the π orbital at the carboxyl group to a π^* . Nevertheless, this fragment is only detected in DEA to 3BP. Basically, a vibronic (non-adiabatic) coupling between the $n \rightarrow \pi^*$ (involving the Br lone pair) and the $\pi \rightarrow \pi^*$ states is possible as the energies are very close to each other. The fragment ions HCOO⁻, O⁻ and OH⁻ are also formed through core excited resonances peaking at around 5 eV. These fragments are observed as well in DEA to pyruvic acid,^[11] therefore can be associated with transitions from the oxygen lone pair or π orbitals. These transitions can be associated with excitations observed at 6.4 eV in the VUV spectrum.

Table 3. Vertical electronic excitation spectrum calculated with TDDFT employing the exchange-correlation functionals CAM-B3LYP, wB97XD and M06-2X. Only the singlet excited states are shown along with the positions of the measured VUV absorption peaks and the respective cross sections.

Energy (eV)	Symmetry	Character	Functional	VUV position (eV)	Cross section (Mb)
3.366	A''	$n \rightarrow \pi^*$	M06-2X	< 3.8	< 0.10
3.555	A''	$n \rightarrow \pi^*$	CAM-B3LYP		
3.529	A''	$n \rightarrow \pi^*$	wB97XD		
5.219	A''	$n \rightarrow \pi^*$	M06-2X	5.3	2
5.308	A''	$n \rightarrow \pi^*$	CAM-B3LYP		
5.326	A''	$n \rightarrow \pi^*$	wB97XD		
5.505	A''	$n \rightarrow \pi^*$	M06-2X	6.4	6.2

5.434	A''	$n \rightarrow \pi^*$	CAM-B3LYP		
5.434	A''	$n \rightarrow \pi^*$	wB97XD		
5.548	A'	$\pi \rightarrow \pi^*$	M06-2X	6.4	6.2
5.383	A'	$\pi \rightarrow \pi^*$	CAM-B3LYP		
5.396	A'	$\pi \rightarrow \pi^*$	wB97XD		

In the study of electron attachment to native pyruvic acid it was also pointed out that this compound possesses a strong tendency for anion-induced dimerization by formation of an anion-radical pair.^[11] Zawadzki et al.^[11] observed anion-molecule reactions in a similar crossed beam-quadrupole mass spectrometer arrangement like the present one, where the pressure was between one and two orders of magnitude higher, but also in a DEA - time of flight mass spectrometer, where considerably lower pressures must be used due to the multi-channel plate detector present in the chamber. As generally expected for molecules containing a carboxyl group, the most favourable hydrogen-bonded dimer structure was assumed to be a head-to-head configuration of the carboxyl groups.^[25] For 3BP such a dimer configuration would be sterically possible also, since the bromine group is present at the opposite methyl group. However, in the present experiments we did not observe any signature of anion induced dimerization. We rather exclude that this absence arises from specific experimental conditions. The working pressure used was about $3 - 3.8 \times 10^{-5}$ mbar during the present experiments with 3BP. Further, it should be noted that this stated value was determined by a pressure gauge mounted in the vacuum chamber. The actual density of molecules in the collision zone with the electrons can be expected to be higher since a capillary was used to form the molecular beam. Furthermore, anions are extracted parallel to the neutral beam in the present crossed beam arrangement. Therefore, experimental conditions do not provide a proper explanation for the absence of anionic clustering effects for 3BP. Instead, we ascribe this lower reactivity of the anion to the considerably lower dipole moment of 3BP compared to pyruvic acid (0.62 D and 2.3 D, respectively). As discussed in ^[11], the cross section for the collision between the transient negative ion and the neutral strongly depends on the long-range interactions, which are dominated by anion-permanent dipole interactions in this case. Another reason for the strong occurrence of secondary reactions mentioned in ^[11] was the stabilization by a double hydrogen bond in the dimer, i.e. short-range interactions. For 3BP a similar dimer structure may be observed. However, for 3BP those interactions would not be operative in a comparable manner due the lower strength of the long-range interaction. Furthermore, the presence of the Br atom might change the interaction of anion and neutral considerably due to the localization of the σ^* resonance on the Br atom, and due to strong Br-H interactions leading to altered geometries compared to pyruvic acid.

Fig. 7: Detailed view of the VUV absorption spectrum of 3BP close to the onset of photoabsorption. The inset shows the yield for the whole range of investigated photon energies (3.8 – 10.8 eV).

Conclusion

Utilizing a crossed beam experiment, we investigated dissociative electron attachment to 3-bromopyruvic acid (3BP). Scattering and quantum chemical calculations were also carried out, which allowed the characterization of the intrinsic properties of 3BP with respect to electron capture and the decay. VUV absorption spectra supported the assignment of ion yields at higher electron energies to electronic core-excitation processes. Compared to the native compound, 3BP reveals a significantly different behaviour in electron attachment. The substitution of a hydrogen atom in the methyl group by bromine leads to an enhanced electron capture cross section at electron energies close to about zero eV. This may lead to radio-sensitization effects, when 3BP is used in connection with radiation therapy. Further, we note that the fragmentation pattern of the carboxyl group is also altered upon halogenation, which leads for example to the detection of the COO^- anion. Probably the most important result is significantly lower reactivity of the transient negative ion of 3BP compared to pyruvic acid, which we ascribe to the different dipole moments of these compounds. As mentioned in the Introduction, 3BP was suggested to be a potential anti-cancer agent by disturbing electron transport chains within oxidative phosphorylation. The present results fully support these observations made by ion biochemistry.

Methods

Crossed electron-molecular beam experimental set-up

The experiments were performed in the Siedlce lab in a crossed-beam arrangement consisting of an electron source, an oven and a quadrupole mass analyzer (QMA). The components are housed in a UHV chamber at a base pressure of 2×10^{-8} mbar. An electron beam with well-defined energy (FWHM ≈ 180 meV) is generated by a trochoidal electron monochromator (TEM) and focused into the reaction region. Two halogen bulbs heat the chamber for two purposes: (1) to sublime the solid samples at 328 K, generating an effusive molecular target beam, and (2) to prevent condensation of the sample in the lens system and walls of the chamber. The temperature inside the chamber is controlled by a PT100 resistor. The sublimated molecular beam of 3BP ($\geq 97\%$ purity powder, purchased from Sigma Aldrich) is guided through a capillary towards the collision region. Negative ions formed from the electron-molecule collision are mass analysed by the QMA and detected by a secondary electron multiplier (SEM). The electron energy scale is calibrated by using the SF_6 gas yielding the well-known SF_6^- resonance near zero eV. However, the measurements are performed without the presence of the calibration gas in order to avoid unwanted reactions of dissociative electron transfer.

VUV measurements

The VUV photoabsorption studies were carried out on the AU-UV beam line at the ASTRID2 synchrotron facility at Aarhus University, Denmark. The experimental set-up has been reported in detail by Palmer et al.^[26] In brief, a gas cell with a path length of 15.5 cm is mounted on the output of the monochromator, with a MgF_2 window separating it from the UHV of the beam line. The pressure within the cell is monitored using a heated 1 Torr full-scale capacitance manometer (Chell model CDG100D). The 3BP photoabsorption cross sections were measured at a pressure of 0.02 Torr, with the sample and cell heated to 333K. A photo-multiplier tube (PMT) detector is used to measure

the light transmitted through the cell. The vacuum ultraviolet light exits the absorption cell through a MgF₂ transmission window, which sets the lower limit of detectable light to 115 nm. A gap between the PMT and the absorption cell was evacuated using a scroll pump to prevent any absorption of oxygen from the air for measurements at energies above 6.20 eV. For measurements below 6.20 eV, air is allowed into this gap to let oxygen absorb higher orders of light (at half the chosen wavelength) that may be passing through the cell. Absolute photoabsorption cross sections were obtained using the Beer–Lambert attenuation law $I_t = I_0 \cdot \exp(-n\sigma l)$, where I_t is the radiation intensity transmitted through the gas sample, I_0 is the radiation intensity transmitted through the evacuated cell, n is the molecular number density of the sample gas, σ is the absolute photoabsorption cross section, and l is the absorption path length. ASTRID2 is operated in “top-up” mode, keeping the stored electron beam current quasi-constant. The beam current is recorded for each point measured and the final data normalised for the small changes in intensity which occur. The 3BP sample has a very low vapour pressure, with a significant water contamination (around 20%) observed for the sample. The VUV spectrum presented here has been corrected for this contamination and therefore the uncertainty in the absolute cross sections obtained is estimated to be around 10-20%.

Calculations

1) Geometry Optimization. Four planar structures were optimized with density functional theory (DFT), employing the B3LYP functional and the cc-pVDZ basis sets implemented in GAMESS.^[15]

2) Scattering Calculations. Fixed-nuclei scattering calculations were performed at the lowest-lying equilibrium geometry of the target ground state (structure A). The target ground state was described at the restricted Hartree-Fock (HF) level, with the Cartesian Gaussian basis described by Kossoski and Varella.^[27] In both bound-state and scattering calculations the norm-conserving pseudopotentials of Bachelet, Hamann and Schlüter (BHS)^[28] were used to represent the nuclei and core electrons of all atoms but hydrogen. The elastic cross sections were computed with the parallel implementation of the Schwinger multichannel method with pseudopotentials, which was recently reviewed.^[29] The scattering wavefunction was represented with a set of configuration state functions (CSFs) $|\chi_{mn}\rangle = |\Phi_m\rangle \otimes |\varphi_n\rangle$, given by anti-symmetrized products of target states (Φ_m) and single-particle scattering orbitals (φ_n). The calculations were performed in the static-exchange plus polarization (SEP) approximation, which takes into account the dynamical response of the target due to the incoming electron, although neglecting electronic excitation channels. Virtual target states were described by single excitations to modified virtual orbitals (MVOs),^[30] generated in the field of the molecular cation with charge +6. Scattering calculations were performed separately for the A' and A'' symmetry components of the C_s group, and the respective CSF bases were built as proposed by Kossoski and Bettega,^[31] employing the cutoff energy of -3.43 Hartree for the MVOs employed as both particle orbitals (virtual target excitations) and scattering orbitals. The CSF spaces comprised 10644 configurations and 10076 configurations, respectively for the A' and A'' irreducible representations. To highlight the signatures of the resonances, the cross sections were not corrected to account for the dipole potential contribution to the higher partial waves ($l \rightarrow \infty$).

3) Dipole Bound States. We also investigated dipole-bound states (DBSs) for the anion species employing the aug-cc-pVDZ basis set augmented with diffuse functions located on the hydrogen atom of the hydroxyl group, as suggested by Skurski et al..^[13] Electron correlation was described employing Second-Order Møller-Plesset perturbation theory (MP2), Singles and Doubles Coupled Cluster (CCSD), and CCSD with perturbative triples (CCSD(T)). The DBS estimates were calculated with the Gaussian09 package^[32].

4) DEA Thresholds. The DEA thresholds were calculated from the energy difference between the fragments and the parent neutral molecule. The computations were performed with the composite

G4MP2 method implemented in GAUSSIAN09,^[32] which provides a good compromise between accuracy and numerical effort (including vibrational corrections). In general, the thresholds obtained at zero temperature are very close to those obtained from enthalpies (ΔH) at room temperature (298K).

5) Electronic Excitation. The vertical excited state spectrum for structure A was calculated with Time-Dependent Density Functional Theory (TDDFT) in the same geometry used in the scattering calculations. In all computations, the aug-cc-pVDZ basis set was employed, although with different correlation-exchange functionals, namely the range-separated generalized gradient approximations (GGAs) CAM-B3LYP and wB97XD, and the meta-GGA M06-2X. All TDDFT computations were performed with GAUSSIAN09.

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