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ENERGETICS AND POSSIBLE MECHANISMS OF ION-BEAM PROTEIN TRITIATION BASED ON AB INITIO POTENTIAL SURFACES

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Ab initio self-consistent field potential energy surfaces for the approach of T, T_2, T^+, T_3^+ and HeT^+ to glycine in the gas phase have been determined and this data used to obtain insight into mechanisms of experimental ion-beam protein tritiation processes. Results of these calculations show that the ionic species T^+, T_3^+ and HeT^+ can form stable adducts with glycine (Gly) and that each functions as a tritiation agent forming the complex $GlyT^+$. Neutral T and T_2 experience a purely repulsive interaction with Gly and do not form an intermediate complex. These neutral species are expected to be less effective tritiation agents than the respective ions, in agreement with experimental observations. The fate of the stable $GlyT^+$ complex is discussed and it is proposed that this species is neutralized by electron capture to give GlyT which spontaneously dissociates to either Gly+T or tritiated glycine (Gly*)+H, with the latter reaction product channel favored statistically. The most likely site of exchange is predicted to be at the amine nitrogen although significance exchange is expected to occur at the α-carbon site by a somewhat more complex reaction mechanism.

1. Introduction

A number of techniques for tritiating organic molecules have been developed [1], such as chemical synthesis, catalytic hydrogen exchange, Wilzbach labelling and biosynthetic techniques, but there are practical disadvantages associated with each when they are applied to the tritation of proteins. However, Bush et al. [2] recently reported a promising new experimental ion-beam method which overcomes these problems and efficiently exchanges tritium (T) for hydrogen (H) in proteins, peptides and other fragile biological molecules. This ion-beam approach involves a heterogeneous reaction between solid-phase protein and tritium species produced by exposure of tritium gas to an electron beam. The experimental conditions are such that the solid protein molecules are subjected to impact by T_3^+, T_2^+ and smaller quantities of T^+, T, fast T_2 from charge-transfer reactions, and HeT^+ from the natural radioactive decay of T_2. At present there is no direct experimental information regarding the interactions and T-H exchange mechanisms for the above species with solid proteins. Relevant observations in this regard are that shutting off the electron and ion beams results in more than a 100-fold reduction in the tritium exchange rate and that the specific radioactivities of the tritiated products are dependent on ion kinetic energy. These observations strongly imply that charged species are essential to the tritiation process.

In the present paper we employ ab initio self-consistent field (SCF) calculations to determine the potential energy surfaces for the approach of T^+, T, T_2, T_3^+ and HeT^+ to the amino acid glycine which serves as a model system for heterogeneous protein tritiation. It is hoped that insight derived from these studies, together with new experimental information, will ultimately result in elucidation of the detailed mechanism of the heterogeneous protein tritiation reaction, and thence lead to modified experiments leading to site-selective tritiation.

2. Methods

A split-valence 6-31G Gaussian basis set [3] has been used for all atoms with the exception of helium where a (5s)/ [1] basis [4] has been employed. Previous calculations on glycine and other amino acids have shown that the 6-31G basis set gives SCF energies estimated to be within 0.13% of Hartree-Fock values [5]. Numerical data which are needed for the present investigation have been presented [6] as ab initio SCF calculations describing the approach of each of the species H^+, H, H_2, H_3^+, and HeH^+ to neutral or zwitterionic glycine along various reaction paths. Since within the Born-Oppenheimer approximation, these interactions are expected to be the same for the tritium species T as for the corresponding protium species H, the ab initio data are also applicable to the interactions of T^+, T, T_2, T_3^+ and HeT^+ with Gly.

All calculations have been performed with internal dimensions of the target glycines frozen at their free-molecule equilibrium values illustrated in figs. 1 and 2. The orientations of the attacking tritium species relative to the glycine targets are illustrated in fig. 3. The attacking tritium species have been allowed to approach along one of the directions A, B, C, D in neutral glycine and directions A′, B′, C′, D′ and E′ in glycine zwitterion. The only geometrical parameters varied were the interaction distance r.

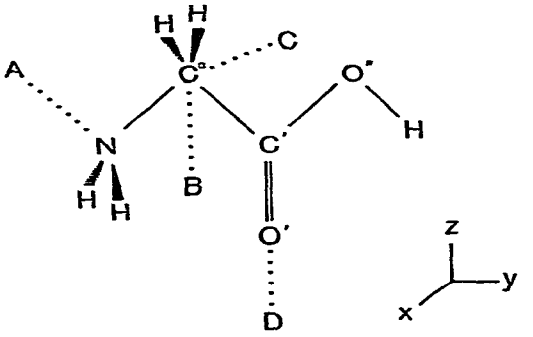

Fig. 1. Approach directions of the tritiating species to neutral glycine at positions A, B, C, D.

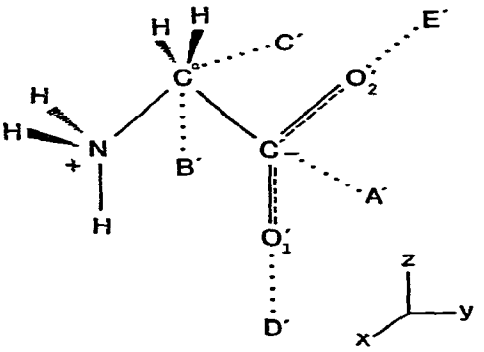

Fig. 2. Approach directions of the tritiation species to the glycine zwitterion at positions A′, B′, C′, D′, E′.

3. Results and discussion

Potential energy curves for the interactions $T^+ \cdots X$, $T \cdots X$ and $T_2 \cdots X$ (see fig. 3) are given in figs. 4–6 as a function of distance r. Energy minima and equilibrium distances for attack of T^+, HeT^+ and T_3^+ along the various approach directions (figs. 1 and 2) are presented in table 1. From the data presented in this table it is to be noted that the ionic species T^+, T_3^+ and HeT^+ experience a significant attractive interaction with glycine and glycine zwitterion. However, the neutral species T and T_2 do not show a significant tendency to bind to Gly or +Gly− at any of the attack sites considered. These features are illustrated in figs.

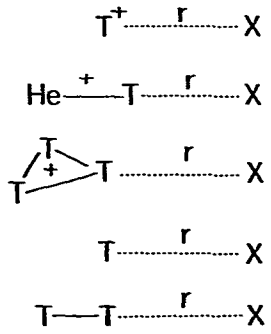

Fig. 3. Orientations of tritiating species approaching the various sites of glycine along the internuclear distance (r).

Table 1

Equilibrium interaction energies (D_e) and internuclear distances (r_e) for the interaction of T^+, HeT^+, T_3^+ along directions of approach to neutral glycine (fig. 1) and glycine zwitterion (fig. 2)

Molecule	Direction of approach	T^+		HeT^+		T_3^+	
		D_e (kJ/mol)	r_e (Å)	D_e kJ/mol)	r_e (Å)	D_e (kJ/mol)	r_e (Å)
Neutral glycine	A	930.5	1.01	528.9	1.13	228.9	1.38
	B	683.3	2.33	321.3	2.82	148.5	3.20
	C	306.3	1.35	41.8	1.78	–	–
	D	715.1	0.95	425.9	1.08	184.9	1.33
Glycine zwitterion	A′	783.7	1.50	518.8	1.62	343.9	1.84
	B′	721.7	2.37	353.1	2.76	177.0	3.13
	C′	205.9	1.30	–	–	–	–
	D′	912.5	0.92	592.5	0.98	314.2	1.08
	E′	956.0	0.91	657.3	0.97	378.2	1.06

4–6 where detailed curve shapes are given as a function of r for several typical interactions. The strength of the attractive interaction between T^+, T_3^+, HeT^+ and glycine is seen from table 1 to be strongly dependent on attack site, and one sees that for a given attack site the strength of attraction is always in the order $Gly\cdots T^+ > Gly\cdots HeT^+ > Gly\cdots T_3^+$.

Dissociation energies of the bound $GlyT^+$, $GlyHeT^+$ and $GlyT_3^+$ are presented in table 2 for a number of different dissociation pathways. Examination of these data indicates that although the 'complexes' $Gly\cdots HeT^+$ and $Gly\cdots T_3^+$ are predicted to be stable relative to dissociation to $Gly + HeT^+$ and $Gly + T_3^+$ (except for T_3^+ attack at the C^α position), these species are unstable relative to dissociation to $GlyT^+ + He$ and $GlyT^+ + T_2$, respectively. Thus, the species $GlyT^+$ can be formed, via an energetically favorable route, as a result of attack by any of the three ionic species (T^+, HeT^+, T_3^+) on glycine. The $GlyT^+$ ion is therefore expected to be an important intermediate present in the ion-beam tritiation of amino acids and proteins, since T and H are bound with nearly equal strength in this intermediate. Similar energy considerations hold for the attack of various tritiation species on the glycine zwitterion.

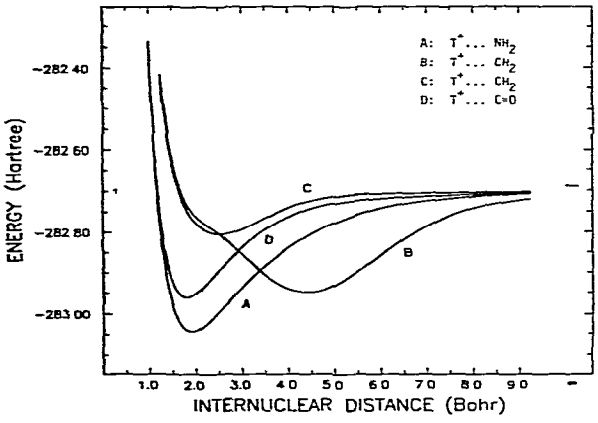

Fig. 4. Interaction potentials between tritons and glycine along the directions of approach illustrated in fig. 1.

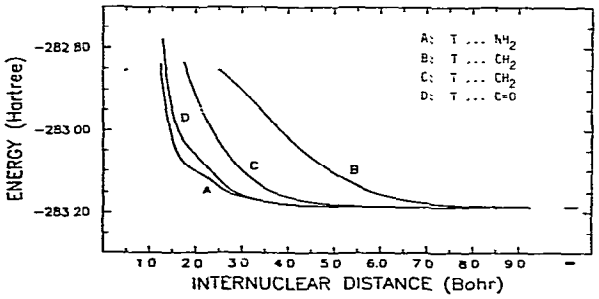

Fig. 5. Interaction potentials between tritium atoms and glycine along the directions of approach illustrated in fig. 1.

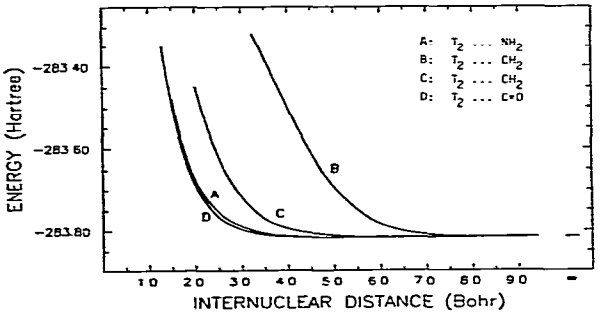

Fig. 6. Interaction potentials between tritium molecules and glycine along the directions of approach illustrated in fig. 1.

with $GlyT^+$ again emerging as the energetically favored intermediate.

We now consider the fate of the $GlyT^+$ intermediate established in the preceding discussion. In this connection it is necessary to examine the various tritiation sites separately. For tritium attack at $-NH_2$ or at $>C=O$ the data in table 2 indicate that the $GlyT^+$ complex represents a true energy minimum, these species being stable with respect to all possible dissociation channels, including the charge-transfer channel $Gly^+ + T$. Under the conditions of a typical ion-beam tritiation experiment it is possible for neutralization of the positively charged $GlyT^+$ to occur. When $GlyT^+$ ions are neutralized by electron capture from the environment, the neutralized complex dissociates along the purely repulsive potential curve displayed in fig. 5. The resulting sequence of events for $-NH_2$ or $>C=O$ attack is

$$\begin{matrix} Gly+T_3^+ \\ Gly+T^+ \\ Gly+HeT^+ \end{matrix} \longrightarrow GlyT^+ \xrightarrow{c} GlyT \rightleftarrows \begin{matrix} Gly+T \\ Gly^*+H \\ \text{(tritiated)} \end{matrix}$$

As the neutral GlyT dissociates, either T or H may be ejected, with the latter more likely on statistic grounds. Thus, for example, in the free amino group adduct $-NH_2T^+$, where calculations [6] on the $-NH_3^+$ analog show that all three protons are equivalent, the probability of H leaving in the charge-neutralized adduct would be two-thirds and the probability of T leaving would be one-third. Alternatively, in the case of the complex ions formed at a free carboxyl end $-CO_2HT^+$ or at a peptide-bound nitrogen $-NHT^+$- the statistical probability of tritium incorporation following electron-capture neutralization would be 1/2, since T and H are bound with nearly equal strength in the intermediate. Tritiation at the amine site leads to water-exchangeable tritium in the molecule. Finally, formation of the adduct at a peptide-bound

$$\begin{matrix} O-T^+ \\ \| \\ -C- \end{matrix}$$

carbonyl group yields zero probability of

Table 2

Dissociation energies of tritiated glycine ion intermediates

Reactant	Products	Dissociation energy (kJ/mol)					
		Neutral glycine position			Glycine zwitterion position		
		O	N	C^α	O_1'	O_2'	C^α
$GlyT^+$	$Gly+T^+$	715.1	930.5	306.3	912.5	956.0	205.9
	$Gly^+ + T$	386.0	601.1	−23.2	450.6	494.0	−256.7
$GlyT_3^+$	$Gly^+ + T_2 + T$	242.2	285.6	56.9	238.3	302.0	−76.2
	$Gly+T_3^+$	184.9	228.9	0.0	314.2	378.2	0.0
	$GlyT^+ + T_2$	−144.7	−316.5	79.1	−213.2	−193.0	179.5
$GlyHeT^+$	$Gly+HeT^+$	425.9	528.9	41.8	592.5	657.3	0.0
	$Gly^+ + He + T$	208.4	311.7	−175.6	242.2	306.8	−350.2
	$GlyT^+ + He$	−177.5	−290.4	−152.5	−208.4	−187.2	−94.6

isotopic exchange following neutralization, at least in the simplest model where no proton/triton migration is allowed to occur during the lifetime of the complex.

When the $GlyT^+$ adduct forms at the α-carbon of glycine to yield $-CH_2T^+-$, the situation is more interesting because of the desirability of incorporating tritium at this nonlabile position. The mechanism of tritium exchange is more complex at this site, since the $-CH_2T^+-$ adduct can dissociate spontaneously without electron transfer. Although the complex $GlyT^+$ can be formed along energetically favourable routes by attack of T^+, T_3^+, or HeT^+, the complex itself may be relatively short lived and decompose spontaneously to $Gly^- + T$ or Gly* (tritiated) $+ H^+$. Another possible mechanism may be envisioned depending on the lifetime of the $GlyT^+$ complex. If $GlyT^+$ lives long enough to be neutralized by an electron to produce a dissociative neutral GlyT as discussed in the $-NH_3T^+-$ and $\geq CO_2HT^+$ cases above, the mechanism at $-CH_2-$ would be the same as that postulated at those sites.

Reactions between T_3^+ and glycine amine nitrogen leading to charged, tritiated glycine are exothermic and are expected to occur readily. The corresponding reactions at the α-carbon site, leading to nonexchangeable tritium, are endothermic and require energy for the formation of tritiated glycine. The energetic considerations are consistent with the experimental observation that the probability of ion-beam tritiation of proteins at exchangeable sites is much greater than the probability of tritiation of nonexchangeable sites. In addition, it has been found [2] that nonexchangeable tritium incorporation into proteins is maximized by the use of high-velocity tritium ions which is consistent with the presence of an energy barrier to reaction of the type found in the case of glycine.

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