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Theoretical study on pegylation reaction mechanisms of IFN-α-2a, IFN-α-2b and IFN-β-1a

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Abstract: Ab initio and DFT calculations have been carried out to study the reaction mechanism between interferons (IFNs) α-2a, α-2b and β-1a and polyethylene glycol (PEG) group. The calculations show that the mechanisms are concerted, in agreement with the results of experimental works. However, although it appears that there is one single transition state, the characteristics of its structure reveal a very synchronous reaction mechanism. The reactions are clearly exothermic and as well have feasible activation energies. Our computational study shows that the lowest transition state energies are related to Lys 134, His 34 and Met 1 of IFN-α-2a, IFN-α-2b and IFN-β-1a, respectively.

Keywords: IFN-α-2a; IFN-α-2b; IFN-β-1a; pegylation; transition state; DFT.

INTRODUCTION

Interferons (IFNs) are currently a class of human proteins most widely used as therapeutic agents and approved to treat various types of cancer and viral diseases.[1–6] They are divided into type I (α and β IFNs) and type II (γ or immune interferon). Alpha interferons are used in the treatment of chronic hepatitis B and C infections, in combination with antiviral drugs.[7–11] Beta interferon reduces the relapse rate in subgroups of patients with multiple sclerosis.[7] The necessity of a more effective and tolerable drugs caused the introduction of some innovative methods such as drug delivery and carriers. One of the most effective methods is polyethylene glycolation (pegylation). This modification is a standard procedure to increase the solubility, stability, efficacy of a drug, and half-life, and it is applied in the preparation of several drugs.[12] As pegylation increases a protein's half-life in the vascular circulation, it reduces the dose of proteins required for the treatment and consequently reduces the drug's side effects. Accordingly, the pegylation of IFNs has been successfully used to improve the pharmacokinetic properties and efficacy of the drug PEGylated IFNs (PEG–IFNs) are the covalent

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conjugate of recombinant IFNs with a monomethoxy polyethylene glycol (PEG) in a 1:1 mole ratio[13,14], originally designed to improve pharmacokinetic profiles. PEG–IFN-α-2b (Pegintron/Sylatron) has been approved for the treatment of chronic hepatitis B and hepatitis C virus (HCV) infection[15] and so PEG–IFN-α-2a (Pegasys/Peginterferon) is approved for the treatment of HCV, hairy cell leukemia, and chronic phase Philadelphia chromosome-positive chronic myelogenous leukemia.[16,17] pegylated IFN-β-1a (Plegridy) was developed by the attachment of a polyethylene glycol side chain to the parent interferon molecule and has been approved for the treatment of relapsing multiple sclerosis in adult patients to slow the progression of disability and decrease the frequency of relapses.[18,19]

In pegylation process, the conjugation of PEG can occur conceivably at any of the numerous nucleophilic sites of the primary amino acids, potentially available in protein structure. These sites generally include lysine (Lys), serine (Ser), tyrosine (Tyr), histidine (His), threonine (Thr), methionine (Met) and N-terminal cysteine (Cys) in different types of interferon.[14,20–23]

The IFN-α-2a protein contains 11 lysine residues plus the N-terminus that are the potential sites for pegylation theoretically.[24] It has been shown that not all 11 pegylation sites on IFN-α-2a are accessible for the interaction with the PEG moiety and no pegylation was observed at the N-terminus.[25] In fact, purified peginterferon (40PEG-IFN-α-2a) is composed of nine bioactive isomers contained 95–99% monopegylated conjugates.[26,27] There are 6 Lys (Lys 83, Lys 134, Lys 131, Lys 121, Lys 70 and Lys 31) in suitable positions for interaction with PEG group, and so 94% of PEG attachment take place in Lys 134, Lys 131, Lys 121 and Lys 31 and the other lysine residues are in inner cavity of protein.[26]

Theoretical pegylation sites in IFN-α-2b protein are numerous nucleophilic groups containing the ε-amino groups of the 10 lysines, the α-amino group at the N-terminal cysteine, the imidazolylnitrogens of the 3 histidines, and the hydroxyl groups at the 14 serines, 10 threonines, and 5 tyrosines. Cys 1–Cys 98, Cys 29––Cys138, His 7, His 57 and His 34 are positional of pegintron, of which His 7, His 57 and His 34 are major positional of pegintron.[14,28]

The IFN-β-1a protein has numerous nucleophilic amino groups due to its constitutive amino acid residues. In Plegridy (20PEG–IFN-β-1a) the N-terminal α-amino group of methionine is modified.[29] Two important potential sites of IFN--β-1a contain Met 1 and Cys 17 were the objects of this investigation.

However, many fundamental issues remain unclear about the reaction mechanism of the formation of PEG–IFNs. To the best of our knowledge, no theoretical studies have yet been reported on the investigating of the reaction mechanism of PEG–IFNs. Therefore, in this paper, we carried out a detailed density functional theory (DFT) study aimed at gaining a deeper insight into the mechanism of the interaction between IFN-α-2a, IFN-α -2b and IFN-β-1a with PEG group. Based on computational studies in this work we proved that the major

positions of IFN-α-2a, IFN-α-2b and IFN-β-1a were pegylated at Lys 134, His 34 and Met 1, respectively.

METHODOLOGY

All calculations were performed with Gaussian 09,[30] geometries of the structures were optimized without any symmetry constraints at B3LYP/6-31g(d,p) level theory.[31] The structures were drawn in the Gaussview 5.0 software. Each optimized structure was confirmed by frequency analysis at the same level, to confirm the stationary point as a true minimum (no imaginary frequency). Intrinsic reaction coordinates (IRC) calculations were performed to confirm that the transition states connect to the right starting materials and products. Stationary points located through IRC were then completely optimized. We present potential energy surfaces for the reactions and provide characterization of the transition states and intermediates involved.

In this study, we present the theoretical investigation of the interaction between probable sites of IFN-α-2a, IFN-α-2b and IFN-β-1a with PEG group. The stability and the electronic properties of the products have been unveiled by DFT calculation. This shall help to understand more thoroughly the origin of the interactions between residues in IFNs with PEG group. However, the analyzing the nature of interactions provides a good basis for the development and support of a chemical model. Accordingly, in the present work, the strength of the interactions between active sites of IFNs and PEG group has been unveiled. Within this work, several *a priori* plausible mechanisms for PEG–IFNs have been tested.

RESULTS AND DISCUSSION

Geometries of IFN-α-2a, IFN-α-2b and IFN-β-1a

In the present study, we selected B3LYP function to test suitability for the structures under consideration. The crystal structures of the IFN-α-2a, IFN-α-2b and IFN-β-1a with PDB codes: 1ITF, 1RH2 and 1AU1, respectively, were retrieved from the RCSB Protein Data Bank (http://www.rcsb.org), and water molecules were removed. The mechanisms for the reactions between PEG group and IFN-α-2a, IFN-α-2b and IFN-β-1a have been calculated. According to the obtained results of experimental studies,[14,26,32] there are various sites of IFNs for PEGylation which are shown in Fig. 1. In this work, we investigated the reaction pathways and stability of the main sites in PEGylation of IFNs. At first, for each of the IFNs an active space is considered the one for which optimizations were conducted without any constraint, and the important bond lengths are shown in Fig. 2. The selected residues (Fig. 2) were truncated so that generally only the side chains were kept in these models. To keep the optimized structures close to those obtained experimentally, the truncation of atoms, except those residues which participate in reaction, was fixed after optimization.

Reaction mechanisms of the formation of PEG–IFN-α-2a

The calculating studies for the investigation the energy of the transition state (TS) and reaction mechanism in the field of PEG–IFNs has not been done. Our study commenced with the proper choice of the computational models. Figure 3

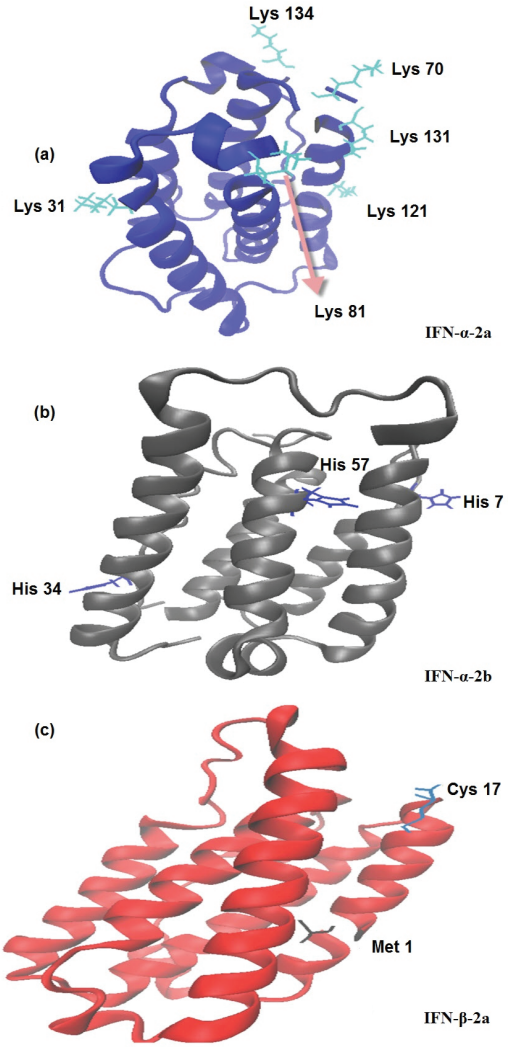

Fig. 1. Positional assignment map for PEG isomers of: a) IFN-α-2a, b) IFN-α-2b and c) IFN-β-2a.

was used as the model for the investigation of the reaction profile, when NH_2 groups of IFNs participate at pegylation reaction, reaction profile follows the a mechanism. If the imidazolyl nitrogen of IFNs reacts with PEG group, reaction pathway follows the b mechanism.

The transition states for the nucleophilic attack by the NH_2 groups of Lys residues of IFN-α-2a on the PEG group were optimized and confirmed to be the

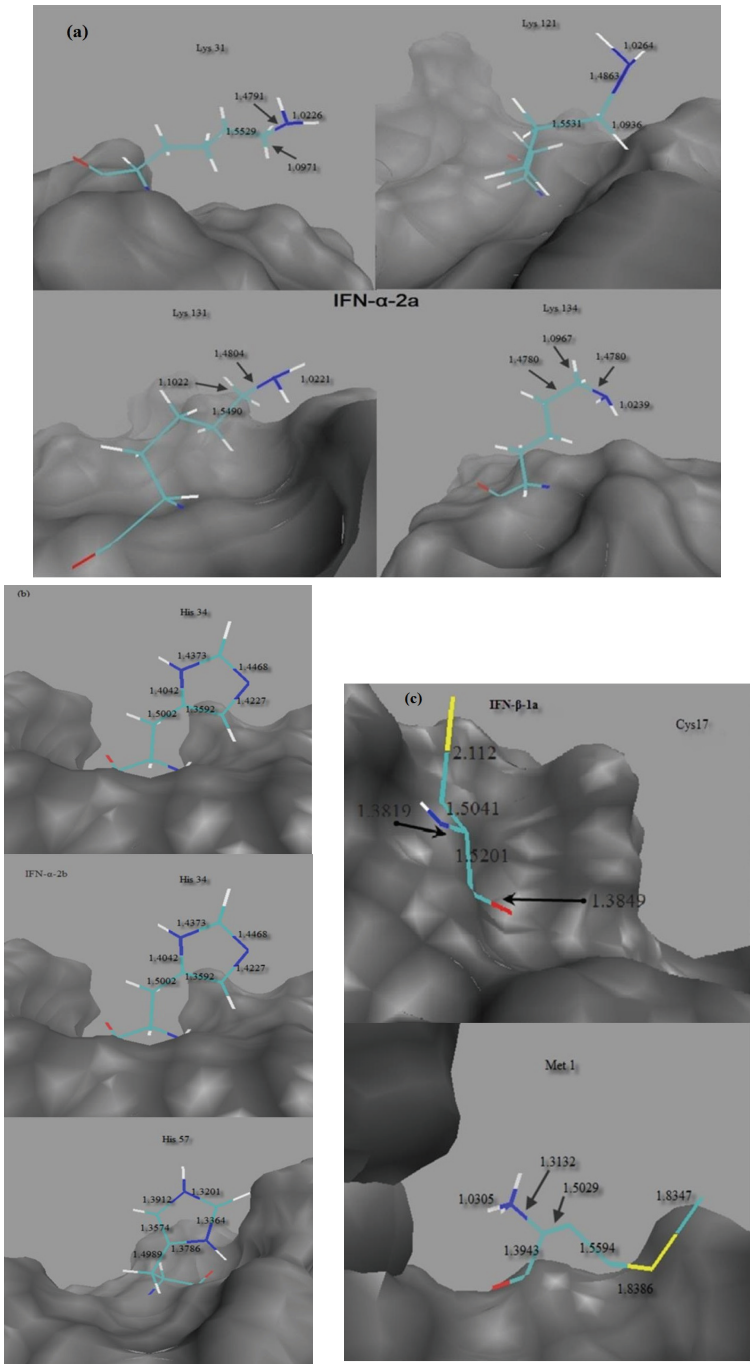

Fig. 2. Optimized structures for: a) IFN-α-2a, b) IFN-α-2b and c) IFN-β-1a with PEG group, the bond distances are in Å.

first-order saddle point with only one imaginary frequency for Lys 31, Lys 121, Lys 131, Lys 134, Lys 83 and Lys 70 (104i, 100i, 120i, 83i, 118i and 101i cm^{-1}).

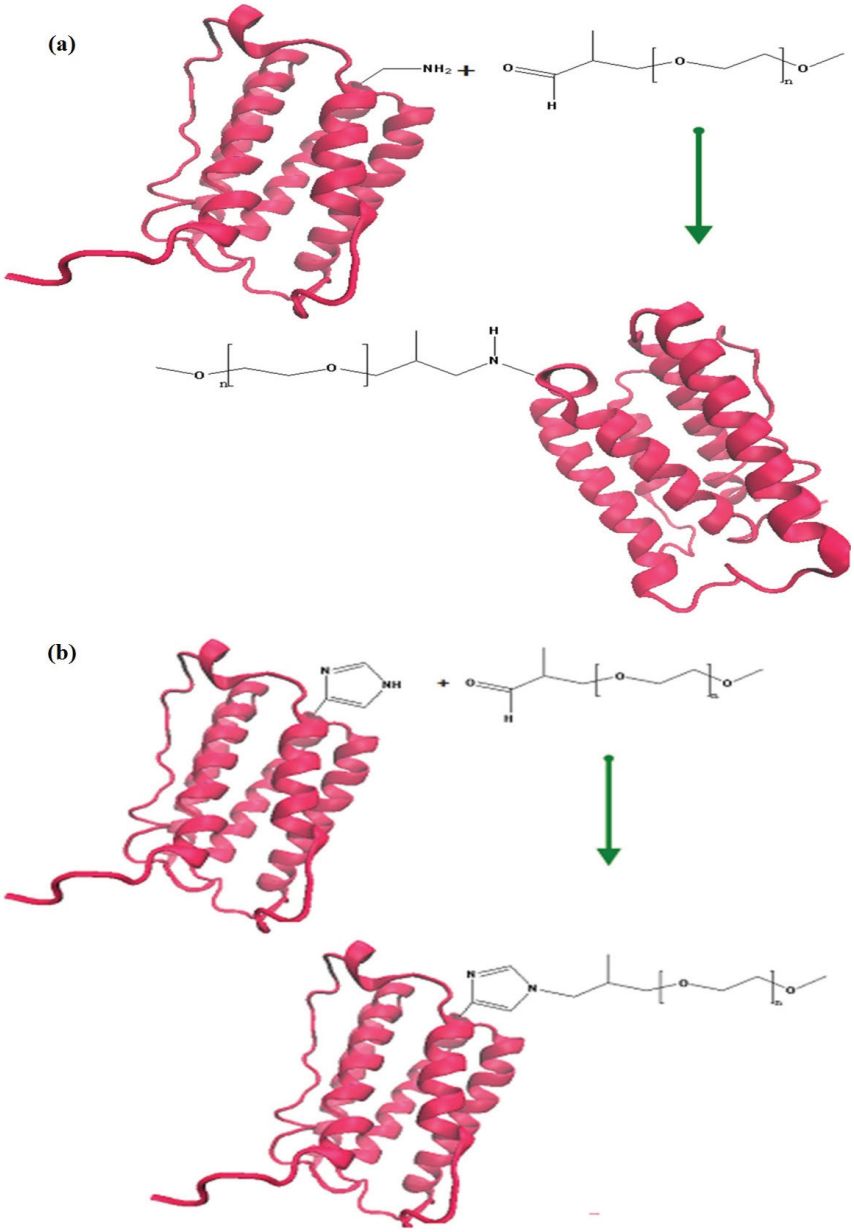

Fig. 3. The suggested reaction paths for: a) NH_2-terminal group of amino acids and b) NH group of imidazoleof IFNs with PEG.

The active sites of the important residues (Fig. 2a) and PEG group were set as the zero-point of the potential energy surface. At TS, the key distances between NH_2 group of Lys residues and the C atom which lie at the end of a carbon chain of PEG group are 2.23, 2.18, 2.30, 2.16, 2.38 and 2.41 Å. Calculating energetic barriers for these reactions reveals a feasible energy barrier. The calculated energetic barriers for these reactions are 15.7, 14.5, 19.2, 11.3, 66.1 and 57.2 kcal*/mol (Fig. 4). According to the reaction profile, the activation barrier for Lys 31, Lys 121, Lys 131 and Lys 134 are lower than Lys 70 and Lys 83. Therefore, it is of interest to obtain a larger quantity of PEG-IFN-α 2a that includes Lys 31, Lys 121, Lys 131 and Lys 134.

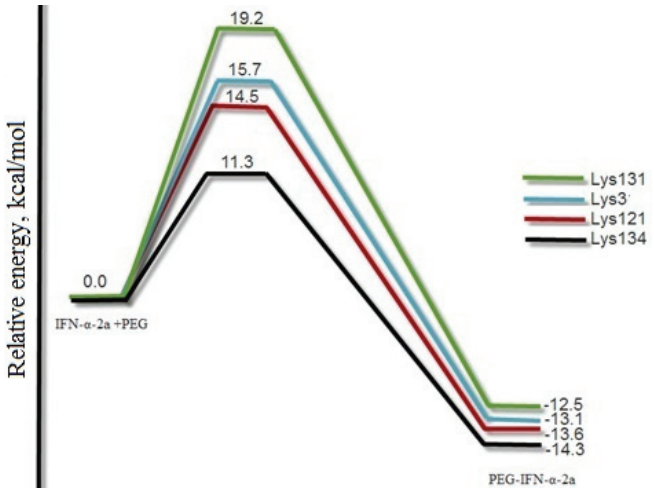

Fig. 4. The calculated energy profile along the suggested reaction path for PEG–IFN-α-2a.

Reaction mechanisms of the formation of PEG–IFN-α-2b

In IFN-α-2, the lone electron pair of the imidazolyl nitrogen of His has more nucleophilic strength than the CH of the ring. A useful aspect of the natural bond orbital (NBO) method is that it gives information about the interactions in both filled and virtual orbital spaces, that could enhance the analysis of intra and intermolecular interactions. The electron densities for this functional group have been analyzed using NBO, and it reveals that the values of electron densities on C–H of the ring are 0.206, –0.0301 and –0.141, the value of electron density on NH is –0.542, so that NH group participates in PEGylation. The transition states for the nucleophilic attack by the N of His on the PEG group were optimized and confirmed to be the first-order saddle point with only one imaginary frequency for His 7, His 34 and His 57 (98i, 32i and 86i cm^{-1}). The active sites of the important residues (Fig. 2b) and PEG were set as the zero-point of the potential energy

* 1 kcal = 4186 J

surface. At TS, the key distances between NH and the C atom which lie at end of a carbon chain of PEG are 2.54, 2.22 and 2.49 Å. The calculated energetic barriers are very feasible 34.4, 17.1 and 29.6 kcal/mol (Fig. 5). According to the barrier activation, we expect more product contains PEG group connected to His 34.

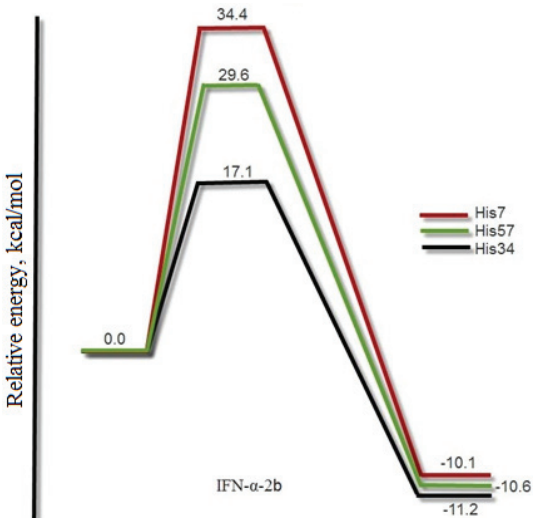

Fig. 5. The calculated energy profile along the suggested reaction path for PEG.IFN-α-2b.

Reaction mechanisms of the formation of PEG–IFN-β-1a

In IFN-β-1a, NH_2 groups attached to Met 1 and Cys 17 are nucleophilic and so they can attack the PEG group. The result of NBO analysis reveals the value of electron density on N atom of NH_2 of Met 1 and Cys 17 are –0.69 and –0.52, respectively. The transition states for the nucleophilic attacks on the PEG group were optimized and confirmed to be the first-order saddle point with only one imaginary frequency for Met 1and Met 36 (35i and 65i cm^{-1}). The active sites of important residues (Fig. 2c) and PEG were set as the zero-point of the potential energy surface. At TS, the distances between corresponding N of Met 1 and Cys 17 with the C atom which lie at end of a carbon chain of PEG are 2.34 and 2.63 Å, respectively. The calculated energetic barriers are the very feasible, 22.3 and 39.8 kcal/mol (Fig. 6). According to the barrier activation, we expect more product contains PEG group connected to Met 1.

CONCLUSIONS

N-Terminally-pegylated IFNs are useful for the treatment of certain cancers, therefore investigation of the reaction mechanism of the formation of pegylated IFNs is important. The theoretical study of the pegylation of the N-terminus of IFN-α-2a, IFN-α-2b IFN-β-2a has been carried out at B3lyp/6-31g(d,p) level theory. The most relevant aspect of this theoretical study concerns the achievement of valuable information on the reaction pathways of PEG–IFNs formation

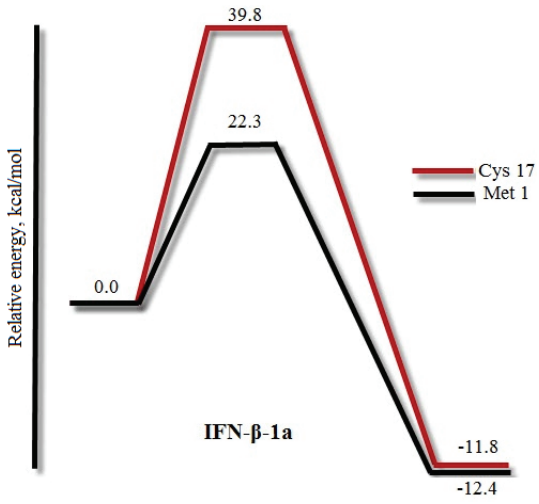

Fig. 6. The calculated energy profile along the suggested reaction path for PEG–IFN-β-1a.

and on the transition state energy, which would be important. We expect that our results will provide the new insight into interaction, reaction pathways and the chemical properties between IFNs and PEG group for developing important performance theoretical calculation for suggesting sites in pegylation of IFNs. There are two possible mechanisms based on the nucleophilic attack of N-terminal residues. The major sites of IFN-α-2a, IFN-α-2b and IFN-β-1a which contribute to the nucleophilic attacks to PEG group are Lys 134, His 34 and Met 1, respectively. The activation barriers in the formation mechanism of PEG–IFNs are easily surpassed under the reaction conditions, therefore, the suggested mechanism is the probable one. The results of investigating TS shows that Lys 134, His 34 and Met 1 have the lowest energy to get to the transition state. Our calculated results are in good agreement with the experimental observations.

И З В О Д

ТЕОРИЈСКО ПРОУЧАВАЊЕ МЕХАНИЗМА РЕАКЦИЈЕ PEG-ИЛОВАЊА IFN-α-2A, IFN-α-2B И IFN-β-1A

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Урађена су *ab initio* и DFT израчунавања да би се проучио механизам реакције између IFN-α-2a, IFN-α-2b и IFN-β-1a и атомских група полиетиленгликола (PEG). Израчунавања показују да се ради о концертованом механизму, што је у сагласности са експерименталним резултатима. Међутим, иако изгледа де постоји само једно прелазно стање; особености његове структуре откривају веома синхронизован механизам реакције. Реакције су веома егзотермне и имају релатвно мале енергије активације. Нумеричко проучавање показује да су најниже енергије прелазног стања повезане са Lys 134, His 34 и Met 1 у IFN-α-2a и IFN-α-2b, односно IFN-β-1a, редом.

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