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Furan-based acetylating agent for the chemical modification of proteins

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ABSTRACT

We have synthesized a furan-based acetylating agent, 2,5-bisacetoxymethylfuran (BAMF) from carbohydrate derived 5-hydroxymethylfurfural (HMF) and studied its acetylation activity with amines and cytochrome *c*. The results show that BAMF can modify proteins in biological conditions without affecting their structure and function. The modification of cytochrome *c* with BAMF occurred through the reduction of heme center, but there was no change in the coordination property of iron and the tertiary structure of cytochrome *c*. Further analysis using MALDI-TOF-MS spectrometer suggests that BAMF selectively targeted lysine amino acid of cytochrome *c* under our experimental conditions. Kinetics study revealed that the modification of cytochrome *c* with BAMF took place at faster rates than aspirin.

1. Introduction

Protein aggregation and misfolding is a serious concern in the recent time. Many neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and prion diseases are associated with this.[1,2] Hydrophobic interaction between amino acids in different globin chains causes hemoglobin polymerization and results sickle cell disease (SCD).[3] Sometimes abnormal change in protein structure can lower the solubility of protein in the body fluid which causes supersaturation. Example includes sickle cell disease where sickle hemoglobin aggregates due to structural change in β-globin unit.[4] To overcome this problem, the only remedy is the modification of protein with proper modifying agent.

Protein modification is of major interest in chemical biology. In this regard, post-translational modification (PTM) is essential for regulating the function, stability, localization and targeting of many eukaryotic proteins.[5,6] Phosphorylation, farnesylation, ubiquitination, glycosylation, acetylation, formylation, amidation, sumoylation, biotinylation are the examples of PTM. Many chemical methods have been developed for such purpose by carefully balancing the reactivity and selectivity.[7–9] Site-selective chemical modification of a protein requires an efficient reaction and an interesting molecule to attach. Experimental facts have proved that Nt-acetylated proteins are more stable in vivo than non-acetylated proteins.[10] Also the N-terminus of a protein has unique pH-dependent reactivity and is thus an attractive target for single-site modification.[11] Blocking of the N-terminus by Nt-acetylation potentially prevents N-terminal ubiquitination, and thus stabilizes the protein.[12,13] Lysine contains a primary amine group which is protonated under biological pH, but it can still react as a nucleophile. Acetylation of lysine groups in proteins has been extensively used for modifying enzymatic properties, immunological reactivity, and proteolytic digestion patterns. Lysine acetylation has emerged as a major post-translational modification for histones in modulating chromatin-based transcriptional control. It is a reversible posttranslational modification (PTM), which neutralizes the positive charge of this amino acid, changing protein function in diverse ways.[14,15] Lysine acetylation is also important for p53 functions and interactions and for microtubule stabilization.[16]

Enormous number of enzymatic acetylating agent is known these days for chemical acetylation of biomolecules. However, only a few numbers of chemical acetylating agents is reported. Acetic anhydride is the simplest example of acetylating agent which can modify protein molecules by N-terminal acetylation of lysine residues.[17,18] But it is very hard to control its action due to its rapid reaction in aqueous environment. High reactivity also decreases the selectivity towards the target molecules. Aspirin is currently one of the most frequently used drugs which has been shown to acetylate proteins and biomolecules such as hemoglobin, DNA, RNA and histones, as well as several plasma constituents, including

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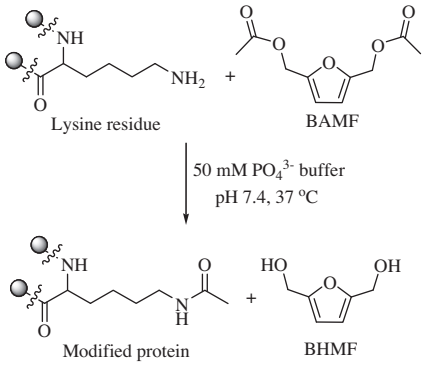

Scheme 1. Modification of lysine residues in protein leading to adduct formation via amidation reaction.

hormones and enzymes.[19] Aspirin is reported as an antisickling agent which modifies sickle hemoglobin through βLys-82 acetylation.[20] Walder et al. reported a bromo aspirin derivative, acetyl-3,5-dibromosalicylic acid (dibromoaspirin), as an antisickling agent which demonstrated potential acetylating ability for intracellular hemoglobin in vitro.[21] The bromoaspirin effectively targets the amine groups of Hb S unit to get acetylated, which increases the oxygen affinity to exert the antisickling effects. But sometimes the use of aspirins can cause fatal gastrointestinal bleeding, hemorrhagic strokes, nephrotoxicity, and adverse effects on the central nervous system. Thus investigations for new acetylating agents will remain continue in the context of suitable application with minimum side-effects.

In the present work we report the synthesis of a furan-based acetylating compound 2,5-bisacetyloxymethylfuran (BAMF) from 5-hydroxymethylfurfural (HMF) which is a dehydration product of hexose sugars.[22,23] Although BAMF was first isolated as a natural product from an ethyl acetate extract of the terrestrial *Streptomyces* species,[24] the chemical synthesis of this compound has not been reported yet. We have shown that this compound acetylates primary amine groups in the biological pH range. The preliminary results have motivated us to further study its efficiency in the acetylation of cyclohexylamine and L-lysine (Scheme 1). Cytochrome *c* and lysozyme were selected as model proteins because of their different chemical characteristics, high content and easy accessibility of surface lysine residues, hydrophobicity indices and extensive structural information. Cytochrome *c* also plays a key role in the energy production in mitochondria and also has so many enzymatic activities in animals. BAMF has successfully modified both the proteins without changing their structure and activity.

2. Experimental

2.1. Materials

5-Hydroxymethylfurfural, cytochrome *c* (from bovine heart) and lysozyme (from chicken egg white) were purchased from Sigma–Aldrich. Sodium borohydride and sodium acetate were purchased from SRL, India. Acetic anhydride, ethyl acetate and dichloromethane were supplied by S D Fine-Chem, India. Potassium dihydrogen phosphate, dipotassium hydrogen phosphate and potassium chloride were obtained from Merck. Guanidinium chloride (GdmCl) was the ultrapure sample from MP Biomedicals. Unless otherwise stated, all chemicals were used without further purification and distilled water was used as aqueous phase.

2.2. Synthesis of 2,5-bishydroxymethylfuran (BHMF)

In a 50 mL round bottom flask 0.63 g (5 mmol) HMF was dissolved in 10 mL distilled water. In another flask, 0.19 g (5 mmol) $NaBH_4$ was dissolved in 3 mL distilled water. Then $NaBH_4$ solution was added drop wise to the HMF solution with continuous stirring. The reduction process was very fast. After the complete reduction, the product (BHMF) was isolated by extracting with ethyl acetate (4 × 20 mL). Here the aqueous phase was saturated with NaCl for the quantitative extraction of BHMF. After evaporation of ethyl acetate, BHMF was obtained as white solid product (0.64 g, 100% yield) and characterized by NMR studies. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.16 (s, 2H, 2C*H*), 4.50 (s, 4H, 2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.0, 108.5, 57.4.

2.3. Synthesis of 2,5-bisacetyloxymethylfuran (BAMF)

In a 10 mL round bottom flask 0.512 g (4 mmol) BHMF was dissolved in 1.89 mL (20 mmol) of acetic anhydride. Then sodium acetate (0.066 g, 0.8 mmol) was added into the mixture as a catalyst and it was stirred for 6 h at room temperature. After the reaction, 2 mL of distilled water was added into the reaction mixture very slowly to consume the excess acetic anhydride. Reaction product was isolated by extracting with dichloromethane (5 × 8 mL). After evaporation of dichloromethane BAMF was obtained as white solid product (0.611 g, 72% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.35 (s, 2H, 2C*H*), 5.01 (s, 4H, 2CH_2), 2.06 (s, 6H, 2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5 (*C*=O), 150 (*C*–O), 111.4 (*C*H), 57.9 (CH_2), 20.7 (CH_3). Melting point = 62 °C.

2.4. Acetylation of cyclohexylamine

In a 10 mL glass vial 19.8 mg (0.2 mmol) cyclohexylamine and 21.2 mg (0.1 mmol) BAMF were taken. To it 1 mL of 50 mM potassium phosphate buffer (pH = 7.4) was added and the reaction mixture was stirred at 37 °C for 6 h. After the completion of reaction, the aqueous phase was saturated with NaCl and the product was isolated by extracting with ethyl acetate. Product was characterized by ^{1}H NMR (Fig. S3) and yield of acetylated cyclohexylamine was calculated by using mesitylene as an external standard. Yield = 82%.

2.5. Acetylation of L-lysine

In a 10 mL glass vial 29.2 mg (0.2 mmol) L-lysine and 21.2 mg (0.1 mmol) BAMF were taken. To it 1 mL of 50 mM potassium phosphate buffer (pH = 7.4) was added and the reaction mixture was stirred at 37 °C for 6 h. Due to high solubility of lysine and its amide in aqueous phase, it was not possible to characterize the product by ^{1}H NMR. In this case UV–vis spectroscopy was used since the lysine amide shows an absorbance at 215 nm, while free lysine does not show any absorbance in this range (Fig. S4). Absorbance of free BAMF was subtracted from the acetylated lysine.

2.6. Preparation of protein samples

Lysozyme and cytochrome *c* solutions were dialyzed extensively against 0.1 M KCl at pH 7.0 at ~4 °C. Protein stock solutions were filtered using 0.22-μm millipore filter paper. All the proteins gave a single band during polyacrylamide gel electrophoresis. Concentration of the protein solutions was determined experimentally using the molar absorption coefficient (ε) values (3.9×10^4 M^{-1} cm^{-1} at 280 nm for lysozyme, and 1.06×10^5 M^{-1} cm^{-1} at 410 nm for cytochrome *c*). The concentration of GdmCl stock solution was determined by refractive index measurements. All solutions for optical measurements were prepared in the desired degassed buffer. For desired pH range, 50 mM phosphate buffer (pH 7.4) was used. Since pH of the protein solution may change on the addition of co-solvents, pH of each solution was also measured after the denaturation experiments.

2.7. Modification of cytochrome *c* and lysozyme by BAMF

Acetylation of amino groups of cytochrome *c* and lysozyme was performed in the following way. Cytochrome *c* and lysozyme (50 μM both) were incubated overnight with different concentrations of BAMF (0.3 mM, 0.6 mM, 0.9 mM and 1.2 mM) at 37 °C in 50 mM phosphate buffer at pH 7.4. Protein samples modified in this manner were used for the subsequent experiments. The ratio of protein/BAMF was varied as per needed throughout the experiment. For the reference experiment the protein (cytochrome *c*) was treated with aspirin under the same conditions.

2.8. Tryptic digestion for mass spectroscopic analysis

The pH of native and modified cytochrome *c* was adjusted to 7.5–9.0 by adding 10 mM ammonium bicarbonate containing 1 mM $CaCl_2$. The lyophilized trypsin was solubilized in 1 mM EDTA and added to the protein solution to a final concentration of ~1:50 trypsin/protein (w/w). After overnight incubation at 37 °C, the samples were cooled to room temperature, and the pH was adjusted to 3–4 with formic acid to stop the digestion process.

2.9. Thermal denaturation study

Thermal denaturation studies of cytochrome *c* were carried out in a Jasco V-660 UV–visible spectrophotometer equipped with a Peltier-type temperature controller at a heating rate of 1 °C per minute. This scan rate was found to provide adequate time for equilibration. Each sample was heated from 20 to 85 °C. The change in absorbance with increasing temperature was followed at 400 nm for cytochrome *c*. About 650 data points of each transition curve were collected. Measurements were repeated three times. After denaturation, the protein sample was immediately cooled down to measure reversibility of the reaction.

2.10. Circular dichroism (CD) study

CD measurements were made in a Jasco J-810 spectropolarimeter equipped with peltier controller at 25 °C with six accumulations. Protein concentration used for the CD measurements was 0.5 g/L. Cells of 1.0 cm path length were used for the measurements of near-UV spectra in the range of 240–320 nm. Necessary blanks were subtracted. The CD instrument was routinely calibrated with D-10-camphorsulfonic acid.

2.11. Extrinsic fluorescence study

Fluorescence spectra of the protein samples were measured in a Perkin Elmer LS 55 Spectrofluorimeter in a 3 mm quartz cell, with both excitation and emission slits set at 10 nm. Protein concentration for all the experiments was 5 μM. For ANS-protein binding experiments the excitation wavelength was 360 nm, and emission spectra were recorded from 400–600 nm. ANS concentration was kept 16 fold higher than that of protein concentration. Necessary blanks were subtracted for each sample. Each spectrum was repeated at least three times.

2.12. Activity study of lysozyme

Lysozyme hydrolytic activity was determined turbidimetrically by measuring the decrease in absorbance at 450 nm of a suspension of bacterial cells. The effect of BAMF adduct formation on the function of lysozyme was studied by the treatment of different concentrations of BAMF (0.3, 0.6, 0.9 and 1.2 mM) in phosphate buffer at pH 7.4. Each study was done for 1500 s. Specific activity of lysozyme was then plotted against the different concentrations of BAMF used.

2.13. ESI-MS analysis

ESI-MS was performed on native and BAMF-modified peptides using a Waters Corp. (QTOF) II MS. 5 μL of the sample solutions were separated by HPLC on a 15 cm × 75 μm i.d. reverse-phase C_{18} column using a 20 min linear gradient of 5–100% acetonitrile in 0.1% formic acid and at a flow rate of 0.2 mL min^{-1}.

2.14. MALDI-TOF-MS analysis

Protein molecular ions were analyzed in linear, positive ion mode using a SCIEX TOF/TOF 4800 Plus Analyzer (Applied Biosystems). The trypsin digested mixtures (13 μL) were acidified with 0.7 μL of 10% (v/v) trifluoroacetic acid (TFA). A C4 ZipTip was washed with 50% (v/v) aqueous acetonitrile containing 0.1% (v/v) TFA and then equilibrated with 0.1% (v/v) TFA solution. The acidified sample (10 μL) was extracted with the prepared ZipTip and washed with 0.1% (v/v) TFA. Finally, the sample was eluted with 75% (v/v) aqueous acetonitrile containing 0.1% (v/v) TFA (1.2 μL). The prepared samples were mixed 1:10 (vol/vol) with a saturated solution of sinapinic acid in 50% aqueous acetonitrile/0.1% aqueous trifluoroacetic acid (1:1, vol/vol) and 1 μL spotted onto the stainless steel MALDI target plate and allowed to dry before the analysis. Using an acceleration voltage of 25 kV and a laser intensity of 2500 V, each spot was analyzed a minimum of three times, accumulating spectra composed of approximately 2500 laser shots in total.

3. Results and discussion

3.1. UV–vis spectroscopic study

The simple synthesis of BAMF comprises two steps, (i) reduction of HMF to BHMF and (ii) acetylation of BHMF to BAMF. To study the activity of synthesized compound (BAMF) in aminoacetylation reaction we chose a primary amine compound, cyclohexylamine which was treated with BAMF in the same reaction conditions as that of biological media. The ^{1}H NMR spectrum (Fig. S3) shows almost quantitative transfer of acetyl groups from BAMF to cyclohexylamine to form its corresponding amide. After achieving this success we sought to find out the acetyl group transferring ability of BAMF to L-lysine. The UV spectra showed similar result in the same condition which promoted us to explore the BAMF for protein modification.

To demonstrate the feasibility of our newly prepared BAMF for the protein modification, we have employed UV–vis spectroscopy to confirm that the cytochrome *c* has been modified by BAMF. The protein cytochrome *c* was treated by different concentrations of BAMF in phosphate buffer at pH 7.4 (Fig. 1a). Since heme center is the most essential part of cytochrome *c* for folding state,[25] we have concentrated on its detail study. The typical absorption peak at 410 nm corresponds to the Soret band of heme protein, while a broad band with maximum at 530 nm is for Fe^{+3} center in ferricytochrome *c*. The adduct formation by the BAMF can be assessed by studying the reduction of heme iron of cytochrome *c* from Fe^{+3} to Fe^{+2} state. The disappearance of peak at 530 nm and appearance of new peaks at 522 and 551 nm indicate the reduction of iron center in cytochrome *c* as a result of modification. The same results were found when cytochrome *c* was subjected to modify using homocysteine thiolactone.[26] The reduction of the iron center is occurred most probably due to the change in ligation property. The Soret band also showed a red shift to 414 nm for BAMF and

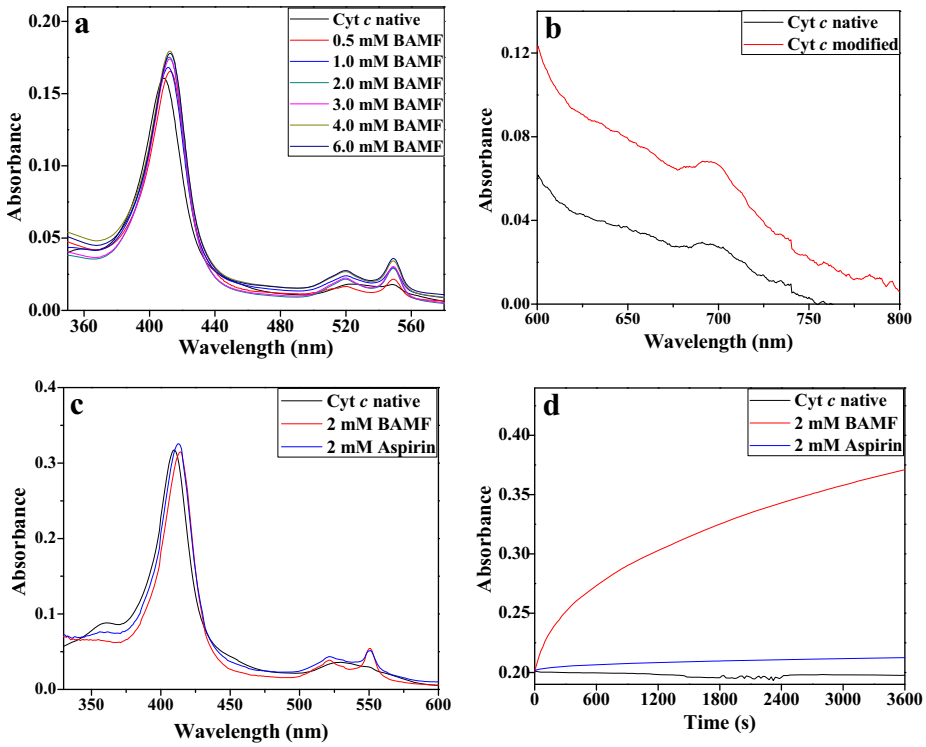

Figure 1. (a) UV–vis spectra of modified cytochrome *c* with different concentration of BAMF. (b) 695 nm band in modified cytochrome *c* (red line) and native cytochrome *c* (black line). (c) UV–vis spectral changes in cytochrome *c* (50 μM) modified by 2 mM BAMF and aspirin. The protein was incubated for overnight at 37 °C, pH 7.4. Soret band of modified protein (red and blue line) and native cytochrome *c* (black line). (d) Redox kinetics of cytochrome *c* at 550 nm wavelength treated with 2 mM BAMF and 2 mM aspirin.

413 nm for aspirin modified cytochrome *c* respectively (Fig. 1c). Generally a blue shift of Soret band means unfolding of proteins.[27] So in this study protein structure remains in its folding state which does not cause any aggregation after modification. Another wavelength from 600 to 800 nm was selected in the UV–vis experiment to evaluate the sixth iron-heme ligand, Met-80 (Fig. 1b). The peak at 695 nm remained intact which suggests that there was no change in the tertiary structure of cytochrome *c* as well as no loss of ligand from heme center. The reported compound BAMF was compared with the existing acetylating agent aspirin in terms of activity. Figure 1c shows the UV–vis spectra of similarly modified cytochrome *c* in both cases when treated with 2 mM BAMF and 2 mM aspirin. To compare the rate of adduct formation by BAMF and aspirin we studied the reduction kinetics of cytochrome *c* in presence of the two compounds. The redox kinetics of heme center in cytochrome *c* was performed at 550 nm for 1 h duration. Results shown in Figure 1d confirms that the rate of adduct formation by BAMF is many fold higher than aspirin.

3.2. CD Study

As we have showed that the potential of BAMF to form adduct with the lysine residues of the protein is very high, we were then interested in investigating the effect of BAMF on the structure of cytochrome *c*. For this, we performed near UV CD measurements and here we did not find any change in the near UV CD signal of modified cytochrome *c* with different concentrations of BAMF used. The characteristic negative peaks of cytochrome *c* at 282 nm and 289 nm of tyrosyl side chains did not show any change. These results (Fig. 2a) showed that there is no effect of adduct formation by BAMF on the tertiary structure of cytochrome *c*. We were unable to obtain far UV CD spectra because of the high noise level due to high absorption of BAMF in far UV CD range. We also procured near UV CD spectra of lysozyme which showed no significant change in CD signal upon BAMF treatment as shown in Figure 2b and hence no perturbation in the tertiary structure of lysozyme. So by the CD study it is again proved that BAMF has no such effect on the structure of protein molecules as revealed by the UV–vis spectroscopy earlier.

3.3. ANS-binding study

Whenever there is structural perturbation in protein, its hydrophobic core is exposed. ANS (1-anilino naphthalene-8-sulfonate) is a fluorescent dye and being hydrophobic in nature it always tends to bind the hydrophobic core of the protein. After binding with the hydrophobic core of the protein there is increase in the emission fluorescence of ANS along with a shift in emission peak from 520 nm to 475 nm, which confirms the exposure of hydrophobic residues of proteins to outside and hence the unfolding of the protein. In our study we also sought to find out the effect of BAMF adduct formation upon protein structure. So to check whether Nt-acetylation can perturb conformation of cytochrome *c*, we carried out extrinsic fluorescence study using ANS dye. Figure 3a shows ANS binding study of cytochrome *c* treated with different concentrations of BAMF which revealed no exposure of hydrophobic core of cytochrome *c* and hence no binding of ANS. ANS binding study was also done to confirm that there was any change in the structure of lysozyme (Fig. 3b). The same results were obtained to prove that BAMF has no effect on the proteins for the exposure of hydrophobic core.

3.4. Thermal denaturation study

Heat induced denaturation of cytochrome *c* was carried out to test the effect of BAMF adduct formation on thermodynamic

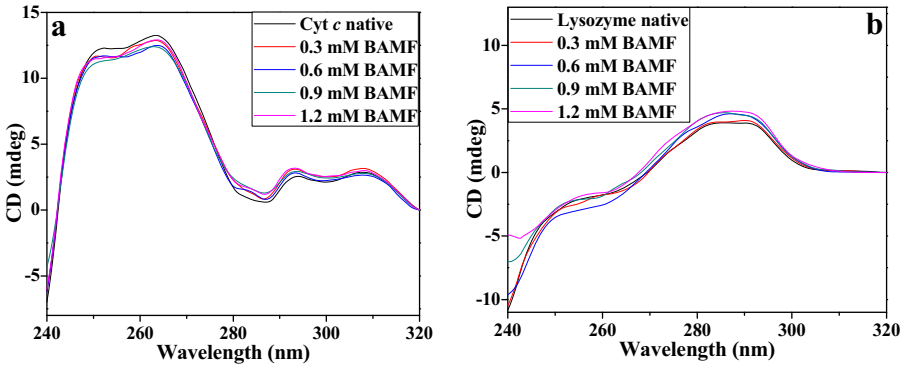

Figure 2. Near UV CD spectra of (a) cytochrome *c* and (b) lysozyme treated with different concentrations of BAMF.

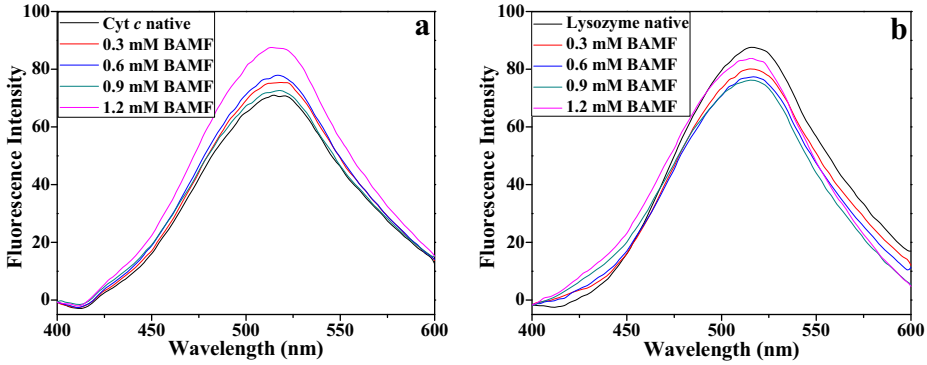

Figure 3. ANS binding study of (a) cytochrome *c* and (b) lysozyme treated with different concentration of BAMF.

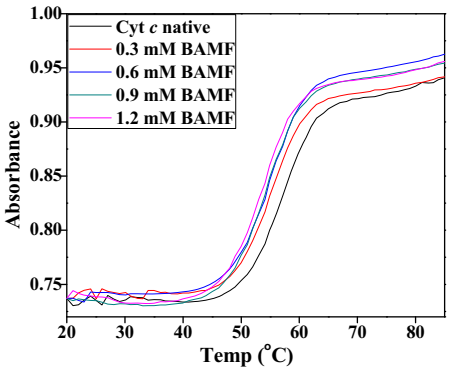

Figure 4. Effect of BAMF on thermal denaturation of cytochrome *c*.

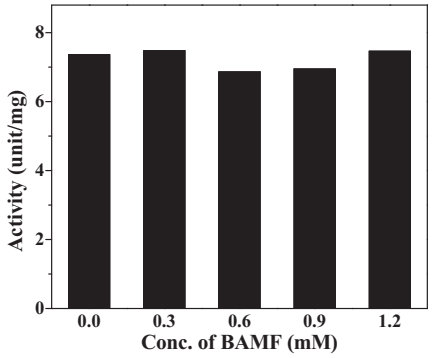

Figure 5. Effect of BAMF on the enzymatic activity of lysozyme.

Table 1
Thermal denaturation study of native and modified cytochrome *c*

Sample	T_m (°C)	H_m (kJ/mol)
Cytochrome *c* native	56.50 ± 0.10	124.6 ± 3.3
0.3 mM BAMF	54.81 ± 0.10	125.3 ± 3.2
0.6 mM BAMF	54.67 ± 0.08	120.2 ± 2.5
0.9 mM BAMF	53.81 ± 0.07	112.6 ± 2.0
1.2 mM BAMF	53.03 ± 0.10	113.2 ± 3.0

stability of proteins upon treatment with different concentrations of BAMF at pH 7.4. The change in absorbance was measured at 400 nm. 1.5 M Gdmcl was added to each set to obtain complete thermal transition. Each heat-induced transition curve was analyzed for T_m (midpoint of denaturation) and ΔH_m (denaturational enthalpy change at T_m) using a non-linear least-squares method according to the relation[28] (Eq. 1),

$$y(T) = \frac{y_N(T) + y_D(T)\exp[-\Delta H_m/R(1/T - 1/T_m)]}{1 + \exp[-\Delta H_m/R(1/T - 1/T_m)]} \quad (1)$$

where $y(T)$ is the optical property at temperature T (K), $y_N(T)$ and $y_D(T)$ are the optical properties of the native and denatured protein molecules at T (K), respectively, and R is the gas constant.

No significant change in T_m and ΔH_m was observed as shown in Figure 4 and Table 1. This led us to conclude that there is no effect of Nt-acetylation by BAMF on the thermodynamic parameters of cytochrome *c* and hence no change in thermal stability.

3.5. Activity study of lysozyme

To study the effect of BAMF on protein function, we took another protein lysozyme in our study. The effect of BAMF adduct

Table 2
Modification sites identified by tryptic cytochrome *c* fragments

Peptide sequence	Position	Calculated mass (native)	Observed mass (native)	Observed mass (modified)	Acetyl group added	Possible modification site
MIFAGIK	81–87	779.448	778.526	819.943	1	87
IFAGIKKK	82–89	904.598	904.553	988.217	2	87 and 88
GIKKKTERED	85–94	1203.669	1203.775	1287.058	2	87 and 88
FVQKCAQCHTV	11–21	1263.597	1263.853	1305.681	1	14
GIKKKTEREDL	85–95	1316.753	1316.834	1401.092	2	87 and 88
NLHGLFGRKTGQ	32–43	1327.723	1326.811	1368.722	1	40
TEREDLIAYLKKATNE	90–105	1893.992	1894.165	1978.129	2	100 and 101

formation on the function of lysozyme was studied using UV–vis spectrophotometer at 450 nm in phosphate buffer at pH 7.4. When we plotted a bar diagram of the specific activity of lysozyme with the concentrations of BAMF used, no significant change in the enzymatic activity was observed (Fig. 5). There can be two possibilities, either there is no change in the conformation of lysozyme or there is no change in the active site of the enzyme leading to no change in the enzymatic activity.

3.6. Protein analysis by mass spectrometry

We have confirmed the modification of cytochrome *c* with BAMF by using ESI-MS spectroscopic technique (Fig. S5). The mass spectrum of the BAMF-modified cytochrome *c*, obtained from a reaction in the buffer solution at pH 7.4, reveals a series of additional peaks than those of native cytochrome *c*. Further to identify the modification sites, native and BAMF-modified cytochrome *c* were digested with trypsin and the peptide mass fingerprints were analyzed by online Mascot and ExPASy database (Fig. S6). The database contained the acetyl-adduct as a variable modification. Table 2 summarizes the results obtained from the digestion, where a total of 7 peptides were identified to form acetylated product. The peptide molecular mass (MM) obtained from these experiments were compared with the peptide MM predicted for native protein digestion for identification of these modified peptide fragments (increase of $n \times 42$ Da in MM, n = units of acetyl group). As per sequence shown in Table 2, 11–21 residues contain one lysine (Lys) and one histidine (His), and 32–43 residues contain one Lys, one His and one arginine (Arg). The results show that in both residues, there is an increase of ~42 Da mass value which corresponds to one acetyl group. The acetyl group can bind with all the three amino acids because of their basic polar nature. However, nucleophilicity of amine group of Lys is higher than the other two amino acids, which makes it more probable to acetylate selectively by BAMF.

4. Conclusions

In summary we have reported a new furan-based acetylating compound, BAMF, which has modified proteins in the biological conditions. Mass spectrometric analysis showed that BAMF selectively modified the lysine residues excluding histidine and arginine. The modification did not affect in structure and function of proteins. This compound has the ability to replace aspirin particularly for protein modification because of two reasons (i) BAMF has faster reaction kinetics than aspirin and (ii) BAMF is supposed to have the minimum toxicity in living cell, since it releases 2,5-bishydroxymethylfuran (BHMF) after acetyl group transfer. Because of its faster kinetics BAMF can be used as a potent drug in case of sickle cell disease where acetylation of hemoglobin is reported to cure the disease. Nt-acetylation of histone using BAMF as acetylating agent can also be useful in case of chromatin remodeling and gene regulation.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.12.053.

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