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Study of interaction between lysozyme-aminoglycosides and penicillin

Anand Krishnamurthy[1]*, Sudarsanam D[1], Anantharamanan R.[3] and Najmeh Alirezaie[2]

[1]Department of Advance Zoology and Biotechnology, Loyola College, Chennai, India.
[2]Research Center of Otolaryngology, Head and Neck Surgery, Hazrat Rasoul Akram Hospital Complex, Iran University of Medical Sciences, Tehran, Iran.
[3]Institute of Computational Biology, India.

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The interaction between aminoglycosides-lysozyme and penicillin-lysozyme was studied by using UV and IR spectroscopy. The study was started with the purification of lysozyme from hen, quail, duck and pigeon and designated as HEWL, QEWL, DEWL and PEWL, respectively. Aminoglycosides (kanamycin and streptomycin) and penicillin (ampicillin) interactions with lysozyme were explored in this investigation. The study provides useful clinical information for the compatibility and use of drugs via the reaction mechanism at the molecule level.

Key words: UV and IR spectroscopy, lysozyme, aminoglycosides, penicillin.

INTRODUCTION

The aim of the study was to investigate the molecular interactions that stabilize the antibiotic lysozyme complex. The studies on the interactions and characterization of the antibacterial enzyme lysozyme C (chicken type) found predominantly in the egg white of poultry birds and other avian sources are of utmost importance. Egg white isolated from *Gallus gallus*, *Columbia livia*, *Coturnix japonica* and *Anas platyrhynchos* have been partially purified using gel filtration chromatography and elution profile recorded after purity detection using a 10% polyacrylamide gel electrophoresis. Penicillin is extensively studied for its binding to lysozyme. Hence, there is an attempt to predict the interactions of aminoglycosides (kanamycin and streptomycin) and aminopenicillin (ampicillin) with lysozyme *in silico* and further validate the molecular binding by UV and IR spectroscopy. The study was started with the purification of lysozyme from hen, quail, duck and pigeon lysozyme and designated as HEWL, QEWL, DEWL and PEWL, respectively. The molecular interactions of these antibiotics against each of the lysozyme purified was studied in detail and interpreted. The enzyme from the four sources (HEWL, QEWL, DEWL and PEWL) after interaction with aminoglycosides (kanamycin and streptomycin) and penicillin (ampicillin) was used as an input in identifying protein-ligand interactions by using UV and Infra red spectroscopy.

MATERIALS AND METHODS

Fresh eggs of hen (*G. gallus*), quail (*C. coturnix japonica*), duck (*A. platyrhynchos*) and pigeon (*C. livia*) eggs were used for the analyses. Besides, lysozyme of purified hen egg white (HEWL) was purchased from Himedia Labs. Purification of lysozyme was achieved by standard kit provided by Bangalore genie. The kit comprised of buffers and chromatographic columns using sephadex G-100. Antibiotics, both aminoglycosides (kanamycin and streptomycin) and penicillin (ampicillin) were purchased from Himedia Labs. Other reagents used were of the highest grade available. LYSO with a concentration of 7.8×10^{-5} mol L^{-1} and 7.8×10^{-5} mol L^{-1} of kanamycin (KC), streptomycin (SC) and ampicillin (AC) were

*Corresponding author. E-mail: anand1459@yahoo.com.

confected with Tris–HCl (0.1 mol L^{-1}) buffer solution of pH 7.4 (Chong-qiu Jiang et al., 2004). The above chemicals were of analytical reagent grade and double distilled water was used throughout the experiment.

Purification of lysozyme

Lysozyme from egg white of avian species was purified after extraction (physical and chemical extraction), isolation (centrifugation) and purification by chromatography. Hen's egg white lysozyme (HEWL) was purified according to the protocol described by Stenesh (1984). Accordingly, pigeon egg white lysozyme (PEWL), duck egg white lysozyme (DEWL) and quail egg white lysozymes (QEWL) were purified.

Molecular weight estimate

To examine the purity of lysozyme preparations and to obtain a molecular mass, polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) was performed as described by Laemmli (1970) using 10% acrylamide gel. The protein bands were stained with Coomassie brilliant blue R-250 (CBB). Marker protein of low molecular weight was obtained from bangalore-genei for standardization.

Protein determination and concentration

During purification, the protein was monitored at 280 nm using a Hitachi UV spectrophotometer (Japan) and the protein concentration was determined by the Lowry (1951) method.

Absorption spectra and elution profiling

The elution profiles of protein in the column chromatography steps were followed by measuring the absorbance at 280 nm using a Hitachi U-2010 spectrophotometer.

Protein characterization by UV and IR spectroscopy

Ultraviolet measurements were carried out with Hitachi U-2010 UV spectrophotometer equipped with 1.0 cm quartz cells for the UV spectrum scanning. All pH measurements were made with a digital pH-meter with a combined glass-calomel electrode. To 10 ml comparison tube, 2.0 ml Tris–HCl buffer solution, 1 ml LYSO solution and 1 ml KC solution was added and were diluted to 10 ml with water, then shaken. Absorption spectra were measured after 10 min (Chong-qiu and Ting, 2004). Using the same methods, the absorption spectra of SC and AC with LYSO from QEWL, DEWL and PEWL was obtained. All 16 samples with different permutations of KC (kanamycin), SC (streptomycin) and AC (ampicillin) along with HEWL, QEWL, PEWL and DEWL were lyophilized and prepared for IR spectroscopy. FTIR (Fourier transform infrared) measurements were done for the 16 lyophilized samples according to the protocol of Dong and Caughey (1994).

RESULTS AND DISCUSSION

Purification of the enzyme

HEWL, QEWL, DEWL and PEWL were purified on a gel filtration column using sephadex G-100 column after physical and chemical extractions followed by centrifugal isolation. The protocol for the purification was synchronized with the method of Stenesh (1984) in experimental biochemistry. QEWLs were similar to those of HEWL, indicating a similar overall structure as it showed that only six amino acid substitutions were found between HEWL and Japanese quail. The elution profile suggests a peak at the preliminary fraction that is around the 6th or 7th fraction in all the sources (hen, quail, duck and pigeon) suggesting an easy and fast elution of the purified enzyme, which is a characteristic of gel filtration chromatography (Fernandez-Sousa and Rodriguez, 1977). The gel filtration technique was used because this is one of the chromatography techniques amicable for handling large initial volume or contaminating proteins (Hjorth, 1997).

Protein concentration determination

The concentration of the protein determined by Lowry method showed that in crude extract, HEWL, QEWL, DEWL and PEWL had 27.5, 30.2, 32.5 and 25.23 g per 50 ml which indicated a high concentration of lysozyme in the crude extract.

After the purification, purified lysozyme samples were 49.14 mg/50 ml for HEWL, 55.22 mg/50 ml for QEWL, 60.11 mg.50 ml for DEWL and 42.35 mg/50 ml for PEWL. The protein concentrations were in agreement with typical values for hen, quail duck and pigeon (Alderton et al., 1945; Feeney et al., 1960).

Molecular weight estimate

The molecular weight of the purified enzyme from HEWL, QEWL, DEWL and PEWL were found to be of the order of 14 KDa that was estimated on the basis of the small molecular weight marker and was in agreement with standard values of the molecular weight estimate. The molecular weight approximated was in agreement with the computationally determined; using protparam tool with protein amino acid sequence taken as an input.

Aminoglycosides and penicillin interactions

Aminoglycosides (kanamycin and streptomycin) and penicillin (ampicillin) interactions with lysozyme were explored in this investigation. The aminoglycoside binding sites in the enzymes are composed of acidic amino acid residues that through electrostatic contacts interact with amino groups of the aminoglycoside (Fong and Berghuis, 2002; Pedersen et al., 1995). Thus, several studies imply that aminoglycoside binding to negatively charged pockets could be a general feature by which aminoglycosides interact and interfere with enzymes. The reversible binding of penicillins and other drugs to proteins is

Table 1. UV spectra peaks in nm for lysozyme-antibody complexes.

Lysozyme antibody interaction	Maximum wavelength absorbance
HEWL-Pure	281.0 200.8
HEWL-Ampicillin	281.4 200.8
HEWL-Streptomycin	281.0 202.6 195.2
HEWL-Kanamycin	244.0 239.0 198.8
QEWL-Pure	244.0 241.0 197.8
QEWL-Ampicillin	244.2 241.0 221.0 197.0 195.4
QEWL-Streptomycin	244.0 241.0 199.6
QEWL-Kanamycin	244.2 241.0 199.8
DEWL-Pure	244.0 241.0 221.0 198.4 195.2
DEWL-Ampicillin	281.0 202.8 200.4
DEWL-Streptomycin	244.0 239.2 198.8
DEWL-Kanamycin	244.2 241.0 239.0 200.0
PEWL-Pure	244.2 241.0 199.4 195.6
PEWL-Ampicillin	244.0 241.0 199.2
PEWL-Streptomycin	244.0 241.0 199.6 195.2
PEWL-Kanamycin	251.8 249.2 241.0 198.8

of utmost importance, which is why penicillin binds less effectively as compared to aminoglycosides.

UV-spectra

The peaks determined by the interaction of lysozyme from hen egg white, quail egg white, duck egg white and pigeon egg white had a maximum absorbance after the binding of aminoglycosides and penicillin showing a high absorbance at the same wavelengths. The binding may impart some folding characteristics on the enzymes under study and offers a special opportunity to study the role of intermediates in lysozyme folding mechanisms (Bahman et al., 2008). The UV spectra are primarily due to the absorbance by aromatic residues of tyrosine and tryptophan in the protein chain. Mutated and substituted protein sequences from avian sources suggest a high absorbance for lysozyme containing high aromatic residues (tyrosine and tryptophan) (Rothwarf and Scheraga, 1996). The UV peaks in nm for HEWL, QEWL, DEWL, PEWL and its antibody complex are shown in Table 1.

IR spectra

The infra red spectroscopy is one of the classical methods adopted to validate the efficacy of kanamycin, streptomycin and ampicillin binding to HEWL, QEWL, DEWL and PEWL. It became a valuable tool for biological systems (Arrondo and Goñi, 1999; Siebert, 1995; Jackson and Mantsch, 1995; Oberg et al., 2004; Haris and Chapman, 1992; Reinstädler et al., 1999; Fabian and Mäntele, 2002). The presence of secondary structure elements in protein structures after the binding of antibiotics is well elaborated and studied and is in accordance with that of typical proteins with conformations pertaining to alpha helix, beta sheet and random coils (Chirgadze and Nevskaya, 1976; Torii and Tasumi, 1992; Kubelka and Keiderling, 2001; Khurana and Fink, 2000; Susi and Byler, 1987). The presence of random coils in the protein may be attributed to the role of intermediates in lysozyme folding mechanisms. The IR spectra peaks in cm^{-1} for HEWL, QEWL, DEWL, PEWL and its antibody complex are shown in Figure 1.

Conclusion

C-type lysozymes from avian sources: hen (HEWL), quail (QEWL), duck (DEWL) and pigeon (PEWL) were extracted, isolated and purified to the best standards. The concentration of protein was determined both for the crude extract as well as purified sample and was found to be in synchronization with literature values. The molecular weight of the purified samples was judged by their mobility in SDS-PAGE and validated from the *in silico* predictions. The characterization of lysozyme-antibiotic complex was well-understood and analyzed using UV spectroscopy and partial validation done by IR spectroscopy. The computational methods will be employed in this investigation as a future perspective to obtain novel inhibitors of lysozyme to combat amyloid aggregation.

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HEWL-PURE	HEWL-AC	HEWL-SC	HEWL-KC	QEWL-PURE	QEWL-AC	QEWL-SC	QEWL-KC	DEWL-PURE	DEWL-AC	DEWL-SC	DEWL-KC	PEWL-PURE	PEWL-AC	PEWL-SC	PEWL-KC
3436	3435	3436	3437	3435	3436	3435	3445	3444	3435	3435	3436	3435	3446	3437	3437
2083	2082	2091	2118	2080	2922	2944	2918	2919	2920	2944	2919	2924	2919	3230	2918
1637	1637	1738	1738	1637	2094	2116	2299	2850	2116	2097	2300	2091	2850	2921	1736
1365	1365	1637	1638	1060	1738	1738	1738	1738	1737	1736	2116	1623	1620	2599	1619
1217	1217	1365	1365	703	1637	1638	1619	1620	1636	1620	1738	1523	1531	2094	1372
1057	1052	1228	1228		1374	1365	1366	1374	1365	1519	1619	1466	1156	1741	1230
714	706	1217	1217		1230	1228	1229	1235	1229	1466	1518	1058	1112	1628	1155
		1052	1052		1156	1217	1216	1157	1217	1372	1462	720	1051	1460	1051
		711	720		1051	1051	1155	1051	1052	1229	1365		884	1403	760
					720	706	1052	907	704	1055	1229		592	1293	
							764	764		893	1217		515	1216	
							662	617		712	906			1154	
							528				740			1036	
											617			905	
											527			703	

Figure 1. IR spectra peaks in cm^{-1} for lysozyme-antibody complexes.

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